Sugammadex for the reversal of rocuronium induced deep neuromuscular blockade in patients with severe renal impairment:

Incorporation of findings from a phase IIIb, multicentre, parallel group, comparative clinical trial evaluating the efficacy, pharmacokinetics and safety of sugammadex 4.0mg/kg administered at 1-2 post tetanic count in subjects with normal or severely impaired renal function

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Abbreviations

ACh	acetylcholine
AChE	acetylcholinesterase
AE	adverse event
ASA	American Society of Anesthesiologists
ASE	all subjects enrolled
ASPE	all subjects pharmacokinetically evaluable
AST	all subject treated
AUC	area under curve
Ca^{2+}	calcium
CD	cyclodextrin
CIAC	Central independent adjudication committee
CKD	chronic kidney disease
Cl	clearance
CrCl	creatinine clearance
DBS	double burst stimulation
GFR	glomerular filtration rate
iAChE	inhibitor of acetylcholinesterase
ICH GCP	International Conference on Harmonisation guidelines, Good Clinical Practice
IMP	Investigational medicinal product (sugammadex)
ITT	intent to treat
IV	intravenous
K^+	potassium
LLOQ	Lower limit of quantification
MSD	Merck Sharpe Dohme
MRT	mean residence time
Na^+	sodium
nAChR	nicotinic acetylcholine receptor
NIBP	non invasive blood pressure
NMBA	neuromuscular blocking agent
NMJ	neuromuscular junction
NYHA	New York Heart Association
PACU	post anaesthetic care units
PK	pharmacokinetic

PORC	post operative residual curarisation
PP	per protocol
PTC	post tetanic count
RSI	rapid sequence induction
SAE	serious adverse event
SD	standard deviation
SmPC	Summary of product characteristics
SRBA	Selective Relaxant Binding Agent
ST	single twitch
T1,2,3,4	1^{st}, 2^{nd}, 3^{rd}, 4^{th}, twitch of train of four
$t_{1/2}$	half life
$t_{1/2}$ eff	effective half life
TOF	train of four
V_d	volume of distribution
V_{ss}	volume of distribution at steady state
Wn	weight-normalised

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Title: Sugammadex for the reversal of rocuronium induced deep neuromuscular blockade in patients with severe renal impairment.

Background: Sugammadex is a selective relaxant binding agent which can encapsulate and thus rapidly reverse the action of the neuromuscular blocking agent rocuronium. Sugammadex and the sugammadex-rocuronium complex are excreted via renal pathways therefore patients with renal impairment may experience differences in drug action compared to controls. To investigate any differences, this study was designed to evaluate the efficacy, safety and pharmacokinetics of sugammadex given for reversal deep neuromuscular blockade, in patients with and without severe renal impairment.

Methods: Adult patients received intravenous (IV) anaesthesia with remifentanil and propofol. Neuromuscular function monitoring was then implemented, using acceleromyography at the adductor pollicis muscle using the TOF-Watch® SX, V1.6. Rocuronium 0.6mg/kg iv was given to facilitate tracheal intubation and further doses of rocuronium 0.1-0.2mg/kg iv were given to ensure deep neuromuscular blockade as measured by a post tetanic count (PTC) of 1-2. When surgery was completed and the PTC measured 1-2, a single IV bolus of sugammadex 4.0mg/kg was administered. The primary efficacy data collected was time to recovery of the train of four (TOF) ratio≥0.9. Blood samples were taken for safety, pharmacokinetic and dialysis data from the day of surgery to the 28 day assessment window. Serious/adverse event data were collected according to ICHGCP guidelines. Further safety data were collected on signs of recurrence of neuromuscular blockade, and vital signs throughout the trial period.

Results: Our study centre treated 16 patients out of 68 treated in the entire study. In our study centre the geometric mean time from start of administration of sugammadex to recovery of the TOF ratio≥0.9 was 176 sec (95% confidence interval (CI):112-278) for the renal group and 50 sec (95% CI: 30-83) for the control group. Non-parametric analysis indicated that equivalence in efficacy of sugammadex could not be claimed. Post hoc Wilcoxon rank sum test demonstrated statistically significant differences between the control and renal groups for time of start of sugammadex administration to time of TOF ratio≥ 0.9 ($p=0.004$). Results of the efficacy analysis were similar in the entire study population. Comparison of pharmacokinetic parameters of sugammadex showed statistically significant differences (Student t-test $p<0.001$) between the groups with increased exposure to sugammadex in the renal group; geometric mean AUC $0\text{-}\infty$ *μg.min/ml* (coefficient of variation*)* control group 3985 (20.7), renal group 28569 (27.8) $p<0.001$.There were no incidences of recurrence of neuromuscular blockade in either group and there appeared to be no difference in the safety profile between the groups.

Conclusions: Reversal of rocuronium induced neuromuscular blockade by sugammadex is likely to be slower and exposure to sugammadex longer in patients with severe renal impairment when compared to healthy controls. In patients with severe renal impairment, sugammadex has been shown to reverse deep neuromuscular blockade efficaciously i.e. no recurrence of neuromuscular blockade, and in a clinically useful timeframe when considering other methods of reversal of blockade. Further work, comparing the use of sugammadex in patients and controls, both with renal failure, will allow more valid pharmacokinetic and safety comparisons to be made.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

Acknowledgments

Merck Sharpe Dohme have given approval for this thesis to be submitted for a higher degree, with the provision that the thesis will remain closed access for 18 months or until the results for the main study have been submitted to and published in peer review journal.

This thesis is based upon and incorporates the results from one study centre of a phase IIIb, multicentre, parallel group, comparative clinical trial evaluating the efficacy, pharmacokinetics and safety of sugammadex 4.0mg/kg administered at 1-2 post tetanic count in subjects with normal or severely impaired renal function. This study was sponsored by Merck Sharpe Dohme. The study protocol was written by the team at Merck Sharpe Dohme and the co-investigators at all the study centres. As co-investigators, Dr Nigel Harper and I were involved in study protocol revisions before I submitted the application to the Regional Ethics Committee for ethical approval.

I would like to acknowledge the help, assistance and guidance of Dr Nigel J N Harper (Educational supervisor) and Dr Clare Austin (University advisor) in the completion of this thesis. I would also like to acknowledge the assistance of Dr Sandeep Mitra for his advice on the interpretation of the dialysis results.

I would like to acknowledge the assistance and help of the co-investigators, Dr N J N Harper, Dr W R Macnab, Dr R Wadsworth and Mr D Glover in completing this study. To enable completion of the complex study protocol procedures detailed in Section 3.7-3.9 at least two trained co-investigators were needed in theatre, in addition to the anaesthetic team. All of the co-investigators had received study protocol training from MSD.

Identification of potential participants was undertaken entirely by me. Tasks during the Screening Period such as taking of informed consent, medical and drug history, clinical examination and blood sampling were carried out almost entirely by me, with only occasional assistance from the study team. For example, 16 out of 19 times informed consent was taken by me.

During the Peri-anaesthetic Period, my responsibility was to oversee, direct or undertake the complex and time-specific procedures such as data entry/collection, TOF-Watch calibration (detailed in Section 3.8), blood sampling, drug dosing etc. It was my responsibility that these procedures were carried out exactly as specified in the study protocol. I was present throughout the Peri-anaesthetic Period on all but one occasion. The processing of approximately 270 blood samples (detailed in section 3.9) was carried out by me in a vast majority of occasions with only occasional assistance from study team members.

Procedures during the Post-Anaesthetic and Follow-up Periods were carried out by me on all but a very few rare occasions.

With regards to data analysis, the following were carried out by employees of MSD, in Oss, The Netherlands; sample size calculations, the Hodges-Lehman, Moses non-parametric analysis of efficacy, calculation of pharmacokinetic and dialysis variables. I would like to take this opportunity to acknowledge the help and assistance of members of staff at MSD, Oss, The Netherlands in particular Martine Prins: Senior Clinical Project Manager, Michiel van den Heuvel: Pharmacokineticist and Marion Kaspers: Statistician.

The following data analysis was carried out by me using Microsoft Excel 2003 and an online statistical calculator at www.socr.ucla.edu; descriptive statistics for patient characteristics, Wilcoxon rank sum analyses of time to train of four ≥ 0.9, graphical, descriptive and comparative statistical analyses of rocuronium and sugammadex concentration:time data, graphical and statistical analyses of blood pressure and HR data, descriptive and comparative statistical analyses of pharmacokinetic variables, statistical analyses of potential influence on efficacy of duration of anaesthesia, BMI and age.

Section 1: Background and Study Rationale

1.1 A brief history of anaesthesia and neuromuscular blockade

"Every body wants to have a hand in a great discovery. All I will do is to give you a hint or two as to names—or the name—to be applied to the state produced and the agent. The state should, I think, be called 'Anaesthesia' [from the Greek word *anaisthēsia,* "lack of sensation"]. This signifies insensibility..."

1846 O. W. HOLMES *Letter to W. T. G. Morton* 21 Nov. in E. Warren *Letheon* (ed. 2, 1847)

The History of Anaesthesia is interspersed with pioneers and trailblazers. These physicians championed new drugs and techniques, conducted countless experiments, and attempted to further their fledgling art to the level of parity it now enjoys with other medical specialties.

Throughout the development of anaesthesia there has been a continued search for 'ideal' anaesthetic agents which were efficacious, safe, easy to use, and devoid of unwanted side effects [1]. However, with each new drug or technique there also seemed to be an associated danger or caveat to its use, and it is still true today, that each drug is only given with a necessary knowledge of the risks and benefits attributed to it.

On the 16th October 1846, ether was introduced to the World by Dr W.T.G Morton, which heralded a significant change in the medical landscape [2]. Ether was able to render patients "insensible, without any excitement or struggling…" and therefore allow surgery to take place under controlled conditions [3]. This was the 'start of the end' of the horror that was surgery without anaesthesia. Prior to this, surgery would only have been performed as an excruciatingly painful, lifesaving or pre-terminal event, needing strong men or sturdy strapping to ensure the patient did not escape. Ether now gave the surgeon more time to complete their operations more diligently and in turn more safely [4].

In 1847 another inhalational agent, Chloroform, was introduced by Dr James Simpson. Unfortunately, it was not long after that when the first death attributed to anaesthesia was reported [5]. Dr John Snow, an innovator in the field of anaesthesia, felt that it was the

lack of control of the concentration of chloroform which led to the fatalities, (it was often given via a handkerchief to the face.) Dr Snow was an advocate of delivering a measured dose of agent and conducted many experiments to support his beliefs [6]. It should be mentioned here that each of the inhalational agents used were at the very least inflammable and at the worst explosive. These properties themselves had lead to catastrophe on the operating table on many an occasion [7].

Other problems with inhalational anaesthesia included patients stopping breathing altogether, or losing control of their upper airways, leading to potentially dire consequences. In 1925 Dr Harold Griffith, a Canadian anaesthetist witnessed the death of an obese patient which occurred after the patient's larynx went into spasm on induction of anaesthesia [8]. Griffith became an advocate of 'airway control' and by 1929, had an array of tracheal tubes and techniques to enable to intubation of the trachea and thus control the airway. This provided the added benefit of facilitating better control of the patients breathing and furthermore improved safety under anaesthesia. However, this was always balanced with the dangers and pitfalls of intubating the trachea itself [9, 10].

There was continued experimentation with anaesthetic techniques. Some doctors advocated the use of intravenous anaesthetics, stating in 1913 that, "ether by inhalation, is but an 'anaesthetic veneer' " [11]. These doctors used intravenous drugs which were mainly barbiturates, such as sodium pentothal (Thiopentone) [12, 13] and Avertin, (Tribromoethanol – still used as an anaesthetic agent for mice.) The use of intravenous anaesthetics was thought to be more favourable in "the feeble elderly person…the kind of patient who sometimes failed to recover after inhalational anaesthesia " [14]. However, probably due to their own poor safety record [15], they did not supersede inhalational techniques, and it was only towards the end of the 20th century that interest re-emerged in total intravenous anaesthesia. Intravenous agents did gain popularity in the 1930's, when they were used to induce anaesthesia after which anaesthesia could be maintained with inhalational agents [4]. The aim was to avoid the sometimes calamitous and problematic inhalational induction, as with this combined method the patient would pass through the stages of anaesthesia [16] much more smoothly and with less cardiac instability [4], which was less stressful for both the patient and the attending physicians.

To provide the patient conditions to enable tracheal intubation or exploration into the body cavities, the amount of anaesthesia used had to be increased, i.e. a deeper anaesthetic was required. Deeper anaesthesia was achievable with the drugs available, however there was a dose associated risk of cardiac and respiratory complications with these (and many future) anaesthetic agents. A major leap in anaesthesia came with the taming of a poison, namely Curare. This poison was extracted from the South American plant, Chondrodendron Tomentosum, which had been used for centuries by indigenous Indians on their hunting arrows, to paralyze their prey [17]. Dr Harold Griffith was at the forefront of this anaesthetic revolution and in Montreal in 1942 demonstrated the use of Intracostin (an extract of curare) to provide muscle relaxation for surgery [18]. Within a few years d-tubocurarine (another potentially more stable extract of curare) was being used by Drs Gray and Halton in Liverpool and in 1946 they presented their findings to the Royal Society of Medicine, heralding the introduction of muscle relaxation into UK practice [19]. With increased availability and understanding of the potential uses of d-tubocurarine, doctors were now able to facilitate tracheal intubation more safely and surgeons could explore deep into the body's cavities with the patient in a state of controlled flaccid paralysis during what was hoped to be a safer anaesthetic.

However, in 1954, a study of deaths relating to anaesthesia and surgery in America from 1948-52 [20] attributed a six-fold increase in mortality with the use of curare. This led to many American institutions abandoning the drug and suggestions in the UK to reduce the dose used to avoid fatalities [21] . However, the findings of the American study were questioned and later modified as it was thought that the increase in mortality arose due to the acceptance of sicker patients for surgery [22]. d-tubocurarine continued to be used for many years to come and vied for use amongst a growing battery of neuromuscular blocking agents (NMBAs).

Aligned with the use of NMBAs was the development of antidotes or 'reversal agents' [23]. Inhibitors of acetylcholinesterase, previously used to treat myasthenia gravis, would antagonize the action of the NMBA but only at the cost of activating the parasympathetic nervous system leading to significant slowing of the heart rate and on occasion, death [24]. To counteract this effect the reversal agents had to be given with anticholinergic drugs, such as atropine, i.e. an antidote to the antidote.

During the first half of the 20th century there was such a developmental deluge of new anaesthetic drugs that it led to a prominent anaesthetist, Macintosh, to state;

‘‘patients would be better off if research on new anaesthetic drugs was halted for five years and attention directed more into training young anaesthetists in the care of the unconscious patient and in the correct administration of the time-proved anaesthetics readily to hand in any hospital” [25].

Although this warning was stark indeed, there continued to be considerable pharmacological development of anaesthetic drugs and the endeavours to find ideal anaesthetic agents has resulted in the pharmacological armoury available to the modern anaesthetist. It is with cautious use of the drugs and techniques available now, that a modern day anaesthetic is thankfully incomparable to the proceedings in Boston on 16th October 1846.

As we entered a new millennium, a new type of anaesthetic drug was developed as a reversal agent to certain NMBAs. It is this drug, sugammadex, which is being tested in this clinical trial. This section of the thesis will explore the need for this drug, its development, clinical introduction and finally the justification of this clinical trial,

The major anaesthetic drug developments of the 20th century are summarised in Table 1.1 below with the main advantages and disadvantages in their use.

Table 1.1: Major anaesthetic drug developments of the 20th century

Drug	Development/ Introduction	Specific Advantages	Specific Disadvantages
Inhalational Agents			
Halothane [26-29]	1953-1956	Smooth induction Non-Irritant Increased potency Non-Inflammable	CVS instability Arrhythmias Halothane Hepatitis
Isoflurane [30]	1965-1980	Fast induction	Respiratory irritation Vasodilatation, hypotension
Enflurane [31]	1966-1980	Fast, smooth induction Non-irritant	Epileptiform activity Myocardial depressant
Sevoflurane [32, 33]	1966-1995	Fast, smooth induction and emergence Non-irritant	'Compound A' with sodalime
Desflurane [33]	1966-1995	Rapid emergence Stable with sodalime	Irritant to airways
Intravenous Agents			
Thiopentone [12]	1932-1934	Smooth and rapid induction in one arm-brain circulation	'Groggy' wake up Hypotension in hypovolaemic patients Hazardous intra-arterial injection
Methohexitone [34]	1957-1976	Improved recovery characteristics	Pain on injection Involuntary muscle movements
Ketamine [35]	1957-1969	Dissociative anaesthesia Bronchodilatation	Emergence delirium Raised intracranial pressure
Etomidate [36, 37]	1964-1973	Cardiovascular stability	Inhibition of steroid synthesis Unsuitable for infusion
Propofol [38]	1977-1986	Smooth induction and clear emergence Depression of laryngeal reflexes Anti-emetic Useful for induction, maintenance and sedation	Hypotension on induction Pain on injection Supports bacterial growth

Table 1.1 Cont.: Major anaesthetic drug developments of the 20th century

Neuromuscular Blocking Agents			
Suxamethonium [39]	(1906) 1949-1951	Fastest spontaneous onset and offset of all types of muscle relaxants	Prolonged paralysis (genetic variability) Hyperkalaemia Muscle Pains Histamine release Trigger for Malignant hyperpyrexia
d-tubocurarine [18]	1942	First available NMBA	Slow onset and offset
Pancuronium [40]	1964-1967	Faster onset than tubocurarine	Tachycardia Prolonged action in patients with renal failure
Vecuronium [41, 42]	1980-1983	No direct cardiovascular effects No histamine release	Associated with bradycardia (not a direct effect)
Mivacurium [43]	1988-1993	Rapid offset	Histamine release Prolonged action in susceptible individuals
Atracurium [44]	1974-1980	No direct cardiovascular effects Elimination independent of renal or hepatic function	Histamine release
Rocuronium [45, 46]	1992-1994	Fastest onset of non-depolarising agents No histamine release	Prolonged action in patients with renal failure
Cisatracurium [47]	1996-2001	Single isomer of Atracurium leading to avoidance of histamine release	Slower onset time than Atracurium
Rapacuronium [48]	1993-2000	Fast onset neuromuscular blockade	Withdrawn in 2001 due to reported cases of bronchospasm [49]
Reversal Agents			
Neostigmine [23, 50]	1931-1952	Reversal of moderate neuromuscular blockade	Parasympathetic activation Possible neuromuscular blockade if given in the absence of NMBA
Edrophonium [51]	1950-1954	Faster onset of reversal	Parasympathetic activation
Pyridostigmine [52]	1954-1969	Long duration of action	Parasympathetic activation
Sugammadex [53, 54]	2001-2008	Rapid reversal of NMB induced by rocuronium or vecuronium	Not recommended for use in patients with severe renal impairment

1.2 The neuromuscular junction (NMJ)

The NMJ describes the interface between motor neurones and skeletal muscle. This occurs through the interaction of a terminal motor neurone and a single muscle cell across a synaptic cleft, using acetylcholine (ACh) to transmit the action potential. We can describe the NMJ in terms of the presynaptic neuron, the synaptic cleft and the postsynaptic membrane.

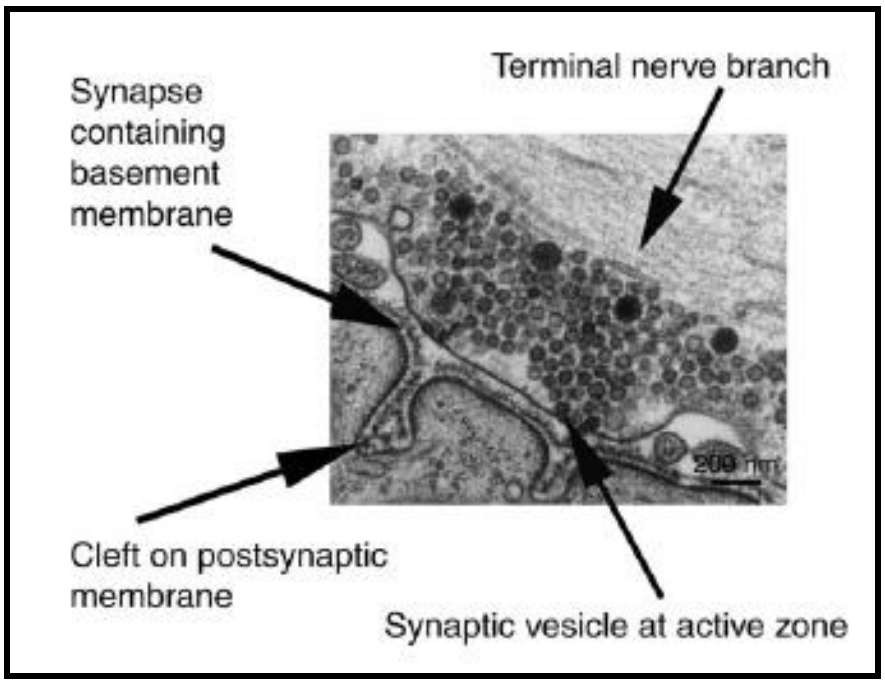


Figure 1.1: Transmission electron micrograph of the NMJ

Reproduced from reference [55] with permission from Oxford Uni Press

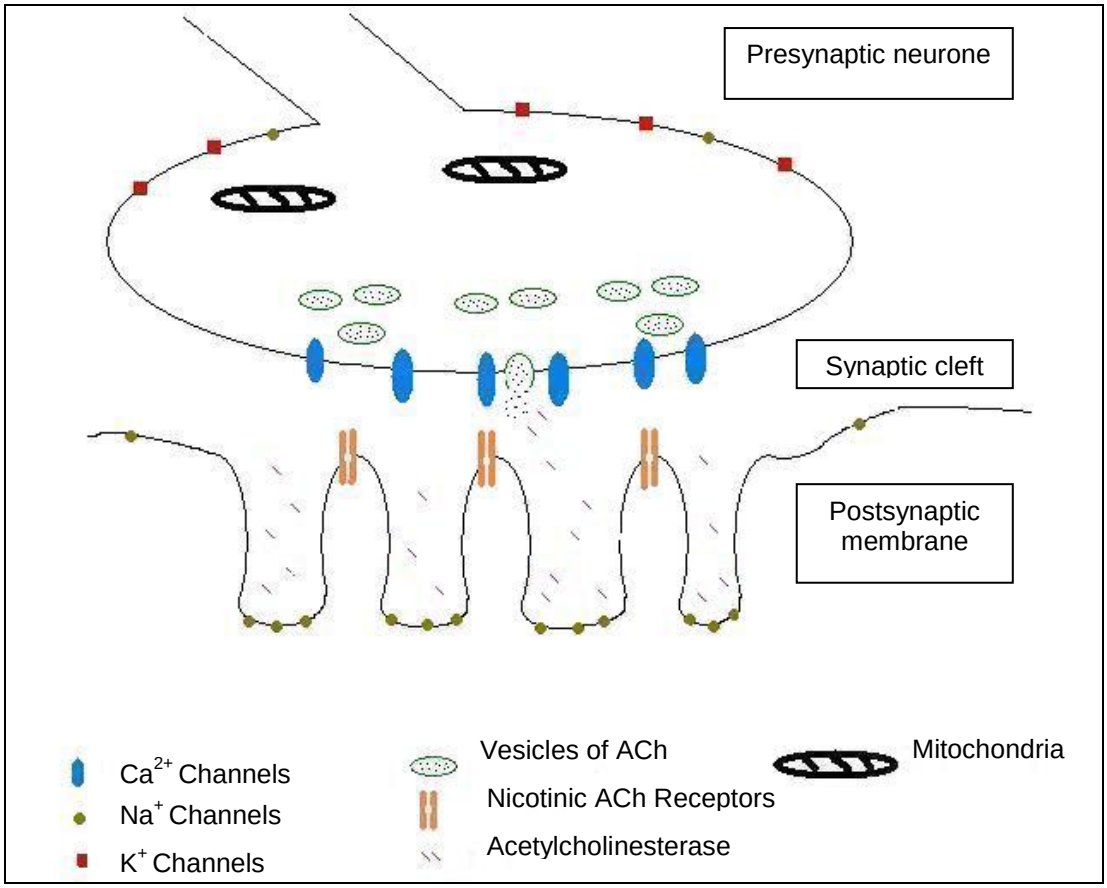


Figure 1.2 schematic of the NMJ

Presynaptic neurone

When considering voluntary movement, an action potential originating in the cerebral cortex, descends via a first order neurone and synapses in the anterior horn of the spinal cord. This action potential is then rapidly propagated (>50ms^{-1}) along a large myelinated motor neurone by saltatory conduction [56]. At the final destination, the neurone has split into 20-100 unmyelinated nerve branches (Fig.1.1). Each of these terminal branches are aligned opposite specialised areas of postsynaptic membrane, known as the motor end plate, of one muscle cell. [57]. All of the muscle fibres innervated from one motor axon represent a motor unit.

The nerve terminal membrane contains sodium (Na^+)and potassium (K^+)channels which control the amplitude and duration of the action potential, they also contain ACh vesicles in readily releasable and reserve pools and mitochondria to provide the energy required[57].

When the action potential reaches the nerve terminal, voltage gated P/Q type and N-type Calcium ($Ca^{2+)}$ channels are activated leading to Ca^{2+} influx [55]. Ca^{2+} concentration rises to 100-1000μm at the active zones, where Ca^{2+} channels are concentrated, and where vesicles containing up to 12,000 molecules of ACh are situated ready for immediate release [58]. The rise in Ca^{2+} triggers a series of events which leads to exocytosis of 50 to 300 ACh vesicles from the readily releasable pool into the synaptic cleft [59]. Positive feedback via prejunctional nicotinic ACh receptors leads to mobilization of ACh vesicles from the reserve pool to the readily releasable pool, ready for the next action potential to arrive [60].

Synaptic cleft

ACh molecules must traverse the narrow synaptic cleft between the nerve terminal and the postsynaptic membrane to reach their target, the postsynaptic nicotinic acetylcholine receptor (nAChR). The journey is rapid due to high concentration gradients and that only a small 20- 50nm gap must be breached [61] . The amount of ACh released is approximately 10 times the amount required to activate the nAChRs on the postsynaptic membrane and depolarise the cell, however less than 50% of ACh reaches the receptor. This is a result of rapid hydrolysis by acetylcholinesterase, (hydrolysis of one molecule takes 80-100μs) and also, of diffusion of ACh out of the synaptic cleft [57, 60].

Acetylcholinesterase is present in high concentrations both in the synaptic cleft and on the postsynaptic membrane. This ensures that each evoked release of ACh will result in a controlled single activation of the postsynaptic nAChRs , i.e. the ACh molecules are prevented from activating the receptor more than once [57].

Postsynaptic membrane

On the postsynaptic membrane, opposite the areas of highest ACh release, (the active zones), are densely populated (20,000 μm^{-2}) pockets of transmembrane nAChRs atop secondary clefts of the motor end plate (fig.1) [62]. The nAChR has a pentameric structure consisting of 5 protein sub-units, denoted; α(x2), β, δ and ε in mature receptors. Immature receptors, with slower channel conductance and receptor half life, are found in neonates and in certain disease states. Immature receptors have a γ subunit in place of the ε subunit [63].

ACh binding sites are located on the extracellular surface, at the junction of the subunits α:ε and α:δ [64] . When 2 molecules of ACh bind with both α subunits, a central ionopore (tapering from 40Å to 7Å) [65] opens allowing ions to briefly flow along their electrochemical gradients. The prevalent ion flux is Na^+ entering the muscle cell membrane taking the cell to threshold potential. This triggers voltage gated Na^+ channels deep in the clefts of the postsynaptic membrane to open, resulting in depolarisation of the cell [57]. The transmitted action potential, now known as an end plate potential, spreads throughout the targeted muscle, resulting in Ca^{2+} release, actin and myosin interaction and ultimately, muscle contraction.

Safety factor for neuromuscular transmission

A safety factor for neuromuscular transmission exists which can be thought of as the ratio of the amount of ACh released to the amount required to depolarise the muscle cell membrane [66]. As stated previously, more ACh is released than is required to depolarise the cell. *In vitro* experiments have shown that the percentage of post-synaptic nAChRs required to be activated for cell depolarisation will vary from 5% to 25% depending on muscle type and animal studied [67, 68]. Studies on human single muscle fibres have shown the variability is also due to ongoing or previous activity such as the forces that are placed on the fibre [66].

1.3 Neuromuscular blocking agents (NMBA)

The introduction of curare (tubocurarine) in 1942 [18] was the first of many attempts to provide an ideal NMBA which would provide rapid onset, and rapid or controllable/predictable offset, of muscle relaxation. Whilst development of NMBAs has continued anaesthetists have had to balance the disadvantages of each NMBA with the advantages they provide with regards to facilitation of tracheal intubation [69], improving surgical conditions [70] and the possibility of reducing the amount of anaesthetic required [71].

Clinically useful neuromuscular blockade is provided by drugs which exert either an agonist or antagonist effect at the NMJ on the postsynaptic nAChR. Presently there are 2 types of NMBA available for clinical use in the UK, namely *depolarizing* NMBAs which are agonists at the nAChR, and *non-depolarizing* NMBAs which are antagonists.

Depolarizing NMBA

Suxamethonium (developed in 1949-51[39]) is the only depolarizing NMBA in regular clinical use. When suxamethonium, which is essentially 2 molecules of ACh joined together, (table 1.3) binds to both α sub-units of the postsynaptic nAChR an agonist effect occurs leading to opening of the central ionopore of the nAChR and resultant depolarisation of the muscle membrane. The channel remains in an open state and the membrane is unable to repolarize until the drug is metabolized by plasma cholinesterase which, unlike acetylcholinesterase, is not present in high concentrations at the NMJ, (acetylcholinesterase has no effect on suxamethonium metabolism)[72]. When a sufficient dose of depolarizing NMBA is given to occupy more than approximately 25% of the nAChRs, a clinical effect is seen as a short period of muscle fasciculation followed by a rapid onset of short term paralysis [60].

The speed of onset and offset of suxamethonium is a central advantage to its use. For this reason it is commonly used in a 'rapid sequence induction' (RSI); a standardized technique to anaesthetise the patient and intubate the trachea as quickly and safely as possible [73, 74]. This is employed when difficulty in tracheal intubation is expected or when rapid control of the airway is required to protect against aspiration of gastric contents. The fast onset allows for early tracheal intubation and the fast offset gives the chance, if attempts at airway control fail, for a pre-oxygenated patient to return to spontaneous breathing before fatal hypoxia occurs. However, due to its side effect profile (Table 1.2) and the fact that the offset is not always fast enough or predictable, there is still a need for a safer way to provide rapid, controllable muscle relaxation [75].

Table 1.2 : Side effects of Suxamethonium

Prolonged Paralysis	**Hyperkalaemia**	**Cardiovascular effects**
May last from minutes to hours due to a decrease in plasma cholinesterase activity *Congenital*- 4% of population affected *Acquired* – Liver disease, pregnancy, old age [72, 76].	A potassium rise of 0.5mmol/L is expected in patients with normal NMJ function. Patients with burns or nerve injuries can experience potentially fatal rises of up to 6mmolL^{-1} [77].	Agonist effect at muscarinic ACh receptors of the parasympathetic nervous system can lead to a significant bradycardia, especially in children or with a large dose [78].
Muscle Fasciculation	**Adverse Drug interaction**	**Miscellanea**
Due to muscle depolarisation and often leading to post operative muscle pains (myalgia)[79], raised intraocular pressure and raised intragastric pressure [72].	Anaphylactic reactions are more common than with other NMBAs [80].	Trigger for Malignant Hyperpyrexia [81]. Variability of action in patients with muscle disorders [76]. Masseter spasm [82, 83]. Phase II block [84].

Non-Depolarizing NMBA

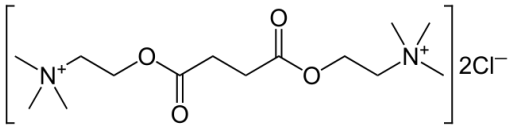
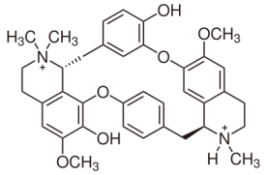
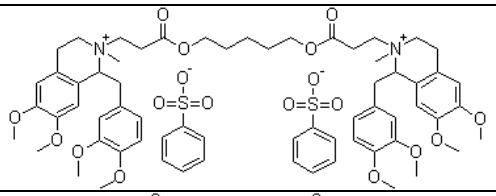
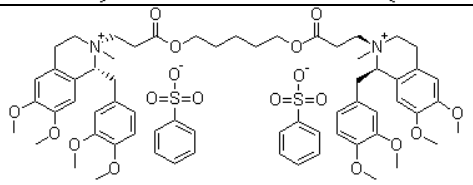
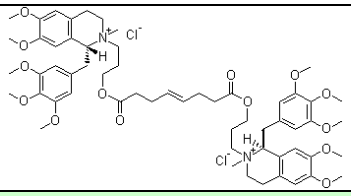
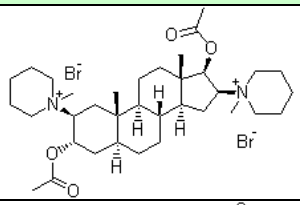
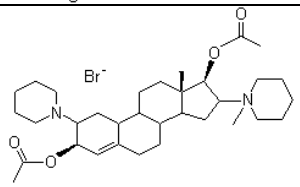
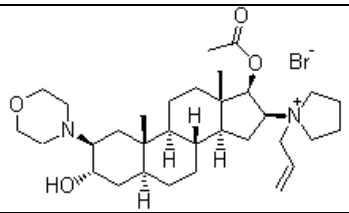
Non-depolarizing NMBAs are antagonists to ACh at the NMJ which competitively bind with the α sub-unit of the postsynaptic nAChR. When a non-depolarizing NMBA is present there is a constant association and dissociation of ACh and non-depolarizing NMBA at the NMJ. Whichever molecule has a higher concentration at the NMJ its effect will usually prevail as a higher concentration will proffer a higher likelihood of receptor occupancy i.e. if an non-depolarizing NMBA is given in sufficient quantities the effects of neuromuscular blockade will be measurable [85]. As the plasma concentration decreases, the rate of which is determined the drugs' pharmacokinetic characteristics, the likelihood of blockade will decrease as the relative concentration of ACh molecules increases, i.e. muscular function will start to return.

There have been many non-depolarizing NMBAs developed since tubocurarine in the search for the 'ideal', (table 1.3). *Pancuronium*, a steroid based NdNMB introduced in 1967 [40], had a faster onset than tubocurarine and a long duration of action. However, it has effects on the autonomic nervous system leading to tachycardia and increased blood

pressure. It also has a prolonged offset in patients with renal disease. *Vecuronium*, another steroidal non-depolarizing NMBA introduced in 1983, had a slightly faster onset time than pancuronium but did not have cardiovascular effects. *Atracurium*, a benzylisoquinilone compound (as is tubocurarine), also introduced in the early 1980's had a fast onset and a short and more predictable offset than previous drugs. It also has a route of elimination independent of renal or hepatic function, however its use is associated with histamine release [42]. *Cisatracurium* is one of the isomers of atracurium which does not release as much histamine but has a slower onset and offset [47]. *Mivacurium*, introduced in 1988 has a slow onset but a rapid offset. It often leads to a large histamine release and as it is metabolized by plasma cholinesterase, it can have a prolonged action in susceptible individuals (similar to suxamethonium) [43]. *Rocuronium*, introduced in 1996, has the fastest onset of NdNMBs [45], has no cardiovascular effects or histamine release [86]. However, it has a less predictable offset in patients with renal failure [87]. The most recently introduced non-depolarizing NMBA, *Rapacuronium*, promised a fast onset and offset but was withdrawn in 2001 due to reported cases of bronchospasm, possibly related to the effects of the drug on parasympathetic muscarinic ACh receptors in the lung [49].

The above mentioned armoury of non-depolarizing NMBAs is capable of servicing most of the needs of modern anaesthesia, providing safe and reliable neuromuscular blockade for routine use. However when very rapid onset and offset of neuromuscular blockade is required, such as when rapid sequence induction is concerned, the available drugs can be found lacking the requisite properties which prompted some to ask if the issue lay with reversal of the blockade rather than its provision [75].

Table 1.3 : Molecular structures of neuromuscular blocking agents

NMBA	Chemical formula, Molecular weight	Molecular Structure
Depolarising NMBA		
Suxamethonium chloride [88, 89]	$C_{14}H_{30}N_2O_4^{+2}$ 290.399 [g/mol]	
Benzylisoquinilone Compounds		
Tubocurarine [90, 91]	$C_{37}H_{41}N_2O_6^{+}$ 609.73124 [g/mol]	
Atracurium besylate [92, 93]	$C_{65}H_{82}N_2O_{18}S_2$ 1243.47918 [g/mol]	
Cisatracurium [93, 94]	$C_{65}H_{82}N_2O_{18}S_2$ 1243.47918 [g/mol]	
Mivacurium chloride [93, 95]	$C_{58}H_{80}N_2O_{14}^{+2}$ 1029.2608 [g/mol]	
Aminosteroidal Compounds		
Pancuronium bromide [93, 96] (*Bis-quaternary*)	$C_{35}H_{60}Br_2N_2O_4$ 732.6699 [g/mol]	
Vecuronium bromide [93, 97] (*Mono-Quaternary*)	$C_{34}H_{57}BrN_2O_4$ 637.73138 [g/mol]	
Rocuronium bromide [93, 98] (*Mono-Quaternary*)	$C_{32}H_{53}N_2O_4^{+}$ 529.77422 [g/mol]	

1.4 Monitoring of neuromuscular blockade

Neuromuscular blockade can be monitored via purely clinical methods, such as the ability to sustain head lift or hand grip for more than 5 seconds [99, 100]. However, clinical methods are limited to the post operative period and are reliant on patient cooperation, understanding and consciousness [101]. The use of peripheral nerve stimulation adds to the clinical evaluation of the patient and can be used during anaesthesia. Common sites used are the ulnar nerve proximal to the wrist, leading to thumb movement (twitch) via the adductor pollicis muscle, and the facial nerve near the eye, resulting in eyebrow movement via the corrugator supercilii muscle. Adductor pollicis is useful for monitoring return of neuromuscular function due to its ease of access and that it is generally one of the last muscle groups to recover [101]. Under blockade, corrugator supercilii is thought to behave similarly to the laryngeal muscles and along with its accessibility this makes it useful to monitor during the onset of NMBAs [101, 102].

Peripheral nerve stimulators can be used to evoke visual or tactile responses to particular patterns of stimulation, giving subjective information on neuromuscular function (see below). These subjective methods, although useful in skilled hands, can be inaccurate compared to objective techniques [103]. The objective methods available use the same patterns but employ different means to measure the evoked responses. Mechanomyography is based directly on the force of muscle contraction, electromyography on the electrical activity of the muscle stimulated. Mechanomyography is difficult to use in the clinical setting but has been used as a gold standard for research [104]. Acceleromyography measures the acceleration of the muscle stimulated and the machines are small and easier to use. They rely on Newton's second law ($f=ma$) such that if the mass (m) remains constant, acceleration (a) is directly proportional to force (f). Although employing an indirect measure of force, acceleromyography is commonly used in clinical research with the understanding that it may overestimate recovery from blockade when compared with mechanomyography [105, 106].

This study used the TOF-Watch® SX which uses accelerometry to measure the muscle contraction following stimulation of a motor nerve. Figure 1.3 below shows how the TOF-Watch® SX is used. The stimulating electrodes are placed over the ulnar nerve to evoke a twitch at the thumb. The accelerometer probe is carefully secured to the thumb in order

that it moves in the same plane as the thumb. A skin temperature sensor helps ensure that skin temperature remains above 32°C. See Section 3: Methods for more details of TOF watch setup.

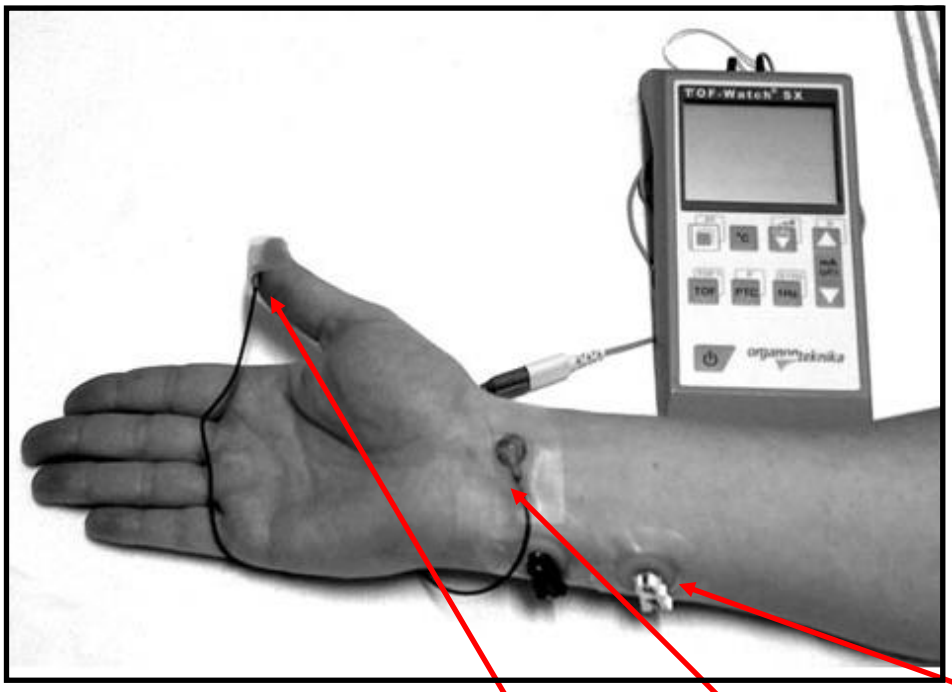


Figure 1.3: TOF-Watch® SX : accelerometer, skin temperature sensor, electrodes
Reproduced from reference [101] with permission from Canadian Journal of Anesthesia

Patterns of stimulation most widely used:

Train of Four (TOF)

This was first described by Ali and Utting in 1970 [107]. A peripheral motor nerve is stimulated by a current of up to 60mA in a 2Hz pattern over 2 seconds. This elicits four evoked 'twitches' in the muscle group served by the nerve. When non-depolarizing NMBAs are used fade in twitch height is seen from the 1st to the 4th twitch. This is due to a decrease in the presynaptic release of ACh resulting from non-depolarizing NMBA blockade of prejunctional ACh receptors [107]. The magnitude of the fourth twitch divided by the first gives the TOF ratio which is useful in evaluating neuromuscular blockade [107]. When the T4:T1 ratio is ≥0.9 this corresponds to a sufficient recovery from blockade to allow airway protection and safe removal of an endotracheal tube [108, 109]. The ratio of T4:T1 ≥0.9 is clinically useful but subjective evaluation of the ratio has limited sensitivity above ratios of 0.4 [101].

At higher doses, the magnitude of all twitches decreases until the 4th to the 1st twitch disappear sequentially. On recovery from blockade there is sequential reappearance of the twitches from 1st to 4th, with a gradual increase in height. The reappearance of the 2nd twitch (T2) is the recommended time to safely give acetylcholinesterase inhibitors for reversal of neuromuscular blockade [110].

Double burst stimulation (DBS)

Two bursts of 50Hz stimulation, 750msec apart will deliver two twitches. Each burst is comprised of 3 impulses, 20msec apart [111]. Using DBS, the ratio between the magnitude of the 1st and 2nd twitches correlates with the TOF ratio (T4:T1) when non-depolarizing NMBA are used. When neuromuscular blockade is rescinding, the fade with DBS has been thought to be more easily detected clinically than TOF ratio [111] although this has been questioned [112]. Hemmerling et al. suggest that the proposed clinical utility of DBS is not a substitute for objective quantative methods of neuromuscular monitoring [101].

Post tetanic count (PTC)

A 5 seconds tetanic stimulation of 50 Hz is applied, there is a 3 second delay and then single twitches at 1Hz are applied. The tetany mobilizes ACh stores and briefly facilitates transmission at the NMJ, hence this method is used to assess deep blockade when other patterns of stimulation, i.e. TOF, will not elicit a response. The number of post tetanic twitches corresponds to the depth of blockade. A PTC of 1-2 denotes deep blockade and that time to return of TOF could take from 10-50 minutes [113].

Single twitch stimulation (ST)

A single square wave stimulus lasting 0.2msec delivered at up to 80mA can deliver a twitch of which the twitch height amplitude can be measured. This twitch amplitude can be compared to a control taken pre delivery of NMBA. This method can be used to develop and compare dose response curves for NMBAs as well as evaluating onset times of NMBAs, which can be useful in the research setting. However, the need for a pre NMBA control and the preference for clinicians to use tactile responses means that the utility of this pattern of stimulation is limited [101].

The routine use of objective neuromuscular monitoring is a contentious issue as although some believe it must be used whenever NMBAs are used [100, 114], the evidence that it decreases the risk of post operative residual neuromuscular blockade is not absolute [115].

1.5 Reversal of neuromuscular blockade

It is common practice in the UK, and widely recommended, to use reversal agents to optimize and speed up the recovery of neuromuscular function after the use of non-depolarizing NMBAs. [100] (dNMBAs cannot be reversed.) The aim of reversal of muscular blockade being to enable the patient to breathe spontaneously, protect their airway and have full control of their musculoskeletal system when they wake

Until recently, only 2 classes of reversal agent were available, both inhibitors of acetylcholinesterase (iAChE):

- *Neostigmine* and *pyridostigmine* form carbamylated compounds with acetylcholinesterase (AChE), slowing its effect on ACh and therefore increasing the concentration of ACh at the NMJ.
- *Edrophonium* competitively inhibits AChE and has the same net effect.

The increased concentration of ACh increases the probability of ACh rather than NMBA occupying the postsynaptic nAChR, leading to an increased probability of reaching threshold potential and muscle cell depolarisation i.e. reversal of neuromuscular blockade. However, these drugs have some drawbacks.

The resultant increase in ACh also affects the autonomic nervous system, in which ACh has agonist effects at the muscarinic ACh receptors of the parasympathetic nervous system. An unchecked increase in ACh will result in bradycardia, increased gut motility and bladder contractility, bronchospasm, salivation, and sweating [116]. For this reason, iAChEs, when used as reversal agents, are given with anticholinergic drugs such as atropine or glycopyrronium, which can themselves lead to tachycardia, dry mouth and confusion in the elderly [117].

If iAChE are given accidentally or after recovery from neuromuscular blockade, a degree of muscle weakness, especially in the upper airways can be observed. The findings in animal experiments are convincing although the experience in humans is more equivocal [50, 118, 119].

A key drawback of these reversal agents is their inability to reverse deep neuromuscular blockade [120]. This is not a problem in routine practice as anaesthesia can continue until there is evidence of return of neuromuscular function, for example reappearance of T2 (of the train of four), at which point iAChEs can be given [110]. However, when fast offset of profound blockade is required, as in the situation of a patient who cannot be intubated or ventilated after the administration of an intubating dose of non-depolarizing NMBA, the above mentioned reversal agents will be ineffective [116].

1.6 Post operative residual curarisation

Post operative residual curarisation (PORC) can be defined as clinical signs of muscle weakness associated with objective evidence of weakness from neuromuscular monitoring, i.e TOF ratio <0.9, older studies used the TOF ratio <0.7 [104, 121]. PORC is also known as residual neuromuscular blockade.

PORC is most often observed in the post anaesthetic care units (PACU) when patients wake from anaesthesia and is associated with impaired airway reflexes, decreased respiratory function, decreased oxygen saturation of the blood, retention of carbon dioxide, small airway collapse (atelectasis), the distress of muscle weakness and a prolonged recovery period [122-124].

An incidence of PORC has been found of between 6% to 64% in the PACU and this varies depending on the NMBA used, if reversal agents were given and if blockade was objectively monitored [104, 125].

NMBAs with a long duration of action, such as pancuronium, are associated with increased risk of PORC, however one study showed that shorter acting drugs; atracurium, vecuronium, rocuronium, can still be associated with incidences of PORC of up to 41% despite two thirds of these patients receiving iAChEs [126]. In a carefully designed study,

patients were given iAChE at the end of surgery when at least T2 was present, then a protocol was used to determine the timing of extubation based on clinical signs and *subjective* neuromuscular monitoring. This resulted in 88% of patients having an *objectively* measured TOF ratio<0.9 at the time of extubation [127].

To decrease the chance of encountering PORC, anaesthetists can use short acting NMBAs, subjectively or objectively monitor the block and routinely using iAChEs. Despite this, the problem of PORC remains. One answer may be to avoid using NMBAs altogether. Tracheal intubation and acceptable surgical conditions can be achieved with the use of strong narcotics in association with anaesthetic agents; however intubating and surgical conditions can be improved with NMBAs [69, 70]. In addition, there are certain surgical procedures where the risk of patient movement make profound blockade imperative, such as in intracranial and ocular surgery.

As mentioned above, the routine use of objective neuromuscular monitoring may not be able to decrease the risk of PORC [115], which begs the question once more; does the answer lie in the reversal agents themselves [75] ?

1.7 The use of NMBAs in patients with chronic renal disease

Chronic renal disease can be classified in relation to glomerular filtration rate (GFR), normal function being indicated by a GFR > 90 ml/min/1.73m^2 ,severe renal impairment <30ml/min/1.73m^2 and renal failure <15 mL/min/1.73m^2 [128].

Patients with renal disease tend to experience large variability in response to medications, NMBAs are no exception. The factors involved are; decreases in renal clearance of the drug, larger volume of distribution of drugs,(V_d : the theoretical volume in which a drug would need to be diluted to achieve the plasma concentration) and metabolic or fluid status disturbances. The NMBAs which rely to some extent on renal excretion can have prolonged effects and it is recommended to use the smallest possible dose of drug and to closely monitor neuromuscular function in these patients [110]. With rocuronium approximately 20-30% is renally excreted and the duration of action is significantly prolonged in patients with renal failure [129]. The duration of action of iAChEs are also prolonged in patients with renal failure [116].

In combination with the problems associated with potentially prolonged blockade, renal patients can often have symptoms of gastro-intestinal acid reflux, especially if uraemic, and require rapid sequence induction to protect from aspiration of gastric contents [130]. However, the use of suxamethonium can be contraindicated due to high plasma potassium concentration in patients with renal failure and the dangers of potassium release when suxamethonium is used [77]. Rocuronium can be used in a modified rapid sequence induction, which provides safe conditions for intubation but as mentioned before, its action will be prolonged [131]. The complexities and challenges of patients with renal failure further illustrate the lack of 'ideal' agents to provide neuromuscular blockade.

1.8 An alternative method of reversing neuromuscular blockade

In 2001, after preliminary animal studies, a patent was issued for "the use of chemical chelators as reversal agents for drug induced neuromuscular blockade" [132]. This was a novel method for reversal of neuromuscular blockade. Bom et al. [132] postulated that chemical encapsulation of a NMBA would reduce the concentration of unbound drug in the plasma. This would result in diffusion of free drug away from the NMJ i.e. decreasing the effector site concentration, allowing the endogenous ACh the upper hand in its competitive battle with the NMBA leading to the return of neuromuscular transmission (see figure 1.4 below) [133, 134] .

There were many proposed benefits of such a method of reversal. Firstly, by circumnavigating the need to manipulate the cholinergic system this would avoid both the side effects which were currently experienced when using conventional reversal agents and the need to co-administer anticholinergic drugs. This method would also enable the reversal of deep neuromuscular blockade, adding a new dimension to the anaesthetic formulary and opening up new clinical uses and applications for NMBAs [132].

A: Describes the mechanism action of NMBAs as they bind to the nicotinic acetylcholine receptors of the NMJ, blocking ACh induced neurotransmission.

B: Describes the mechanism of standard reversal agents such as neostigmine which inhibit the action of acetylcholinesterase thereby increasing the ACh available at the NMJ and favouring neurotransmission.

C: Show encapsulation of the NMBA (in the plasma) which decreases the amount of NMBA able to block the action of ACh, thereby favouring neurotransmission.

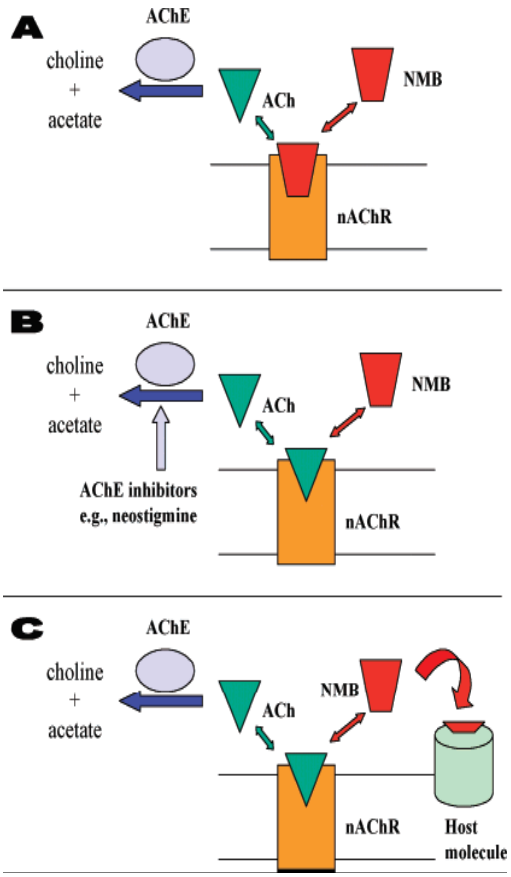


Figure 1.4

Schematic describing the mechanism of neuromuscular blockade and reversal

Reproduced in part from reference [133] with permission from American Chemical Society

Drug Development

Bom et al. studied a group of cyclic oligosaccharides, known as Cyclodextrins (CDs), which had many of the properties needed to encapsulate a positively charged biologically active molecule such as a NMBA.

CDs, discovered in the Nineteenth century as a by-product of starch degradation by bacteria [135], are small host molecules in the shape of a truncated cone, with an axial cavity, (they can also be thought of as resembling a basketball net.) They have an hydrophilic outer surface (allowing water solubility) and a lipophillic cavity which can attract and encapsulate non-polar molecules with the appropriate dimensions, thus forming

of a host-guest complex. The size and shape of the cavity varies with the number of glucose units. α-CDs have a cavity diameter of 0.57nm, β-CDs 0.78 and γ-CDs 0.98nm which corresponds to 6, 7 and 8 glucose units respectively [136] .

CDs are generally biologically well tolerated [137] and had previously been used as pharmacological excipients, making lipophillic drugs more stable, water soluble or increasing their bioavailability [138, 139]. However, of greatest interest was the fact that they had also been shown to encapsulate steroids, which are lipophillic molecules [140]. With this in mind, a number of CDs were designed and synthesised, with the purpose of investigating their ability to encapsulate, and render biologically inactive, the steroidal NMBAs rocuronium and vecuronium [133]. I have only discussed rocuronium in further sections as that is the NMBA used in the clinical trial upon which the thesis is based.

Certain properties of CDs were found to be important in the formation of stable CD-Guest complexes, namely Van der Waals and hydrophobic interactions [141]. Van der Waals interactions were known to be dependant on the interconnecting characteristics of CD and guest. Manipulation of the size and shape of the CD cavity varied the ability of the guest (rocuronium) to enter and be held by Van der Waals forces. The hydrophobic portion of the CD could also be manipulated to determine the effect of hydrophobic cavity area on the formation of the CD-Rocuronium complex. In addition to varying the cavity size and hydrophobic cavity area, the effect of placing negatively charged functional groups at the cavity rim was studied.

A select array of synthesized CDs were developed varying in cavity size, hydrophobic cavity area and position and number of functional groups. Their ability to reverse the action of rocuronium (their potency) was tested in vitro on isolated mouse hemidiaphragm and in vivo on anaesthetised guinea pigs [133].

γ-CDs have the largest cavity size and, formed the strongest bonds with rocuronium, i.e. they were the most potent. The width of the rocuronium molecule is approximately 7.5 nm which corresponds favourably with the cavity size of γ-CDs. Manipulation of the hydrophobic cavity area was achieved by substitution of hydroxyl groups with lipophillic chains. CDs which had had all eight hydroxyl groups substituted with lipophillic chains were more potent than their analogues with a single substitution. This supported the idea

that hydrophobic cavity area influenced complex formation. The inclusion of negatively charged carboxyl groups made significant differences to the structure-activity relationships exhibited by the CDs. The electrostatic interactions with the positively charged rocuronium molecule led to greater potencies in comparison to corresponding CDs with neutrally charged hydroxyl groups. One of the most potent carboxylated γCDs was compared with its hydroylated analogue. Using isothermal titration calorimetry, it was found that the carboxylated γCD formed more stable bonds with rocuronium, (K_A = 1.8 x 10_7 M^{-1} compared to K_A = 2.0 x 10_5 M^{-1}) [133].

Furthermore, X-Ray crystallography was used to compare the structure of CDs with and without negatively charged groups. It was shown that the CDs with negatively charged groups had a cavity which was more open, this being attributed to electrostatic repulsion. The more open structure was thought to confer a more suitable dynamic for encapsulation. This is demonstrated in the computer generated models in figure 1.5 below.

The most potent γCD derivative was coded Org 25969, Chemical formula $C_{72}H_{104}O_{48}S_8Na_8$, and was to be the first commercially available drug in the new class of drug known as 'Selective Relaxant Binding Agents'.

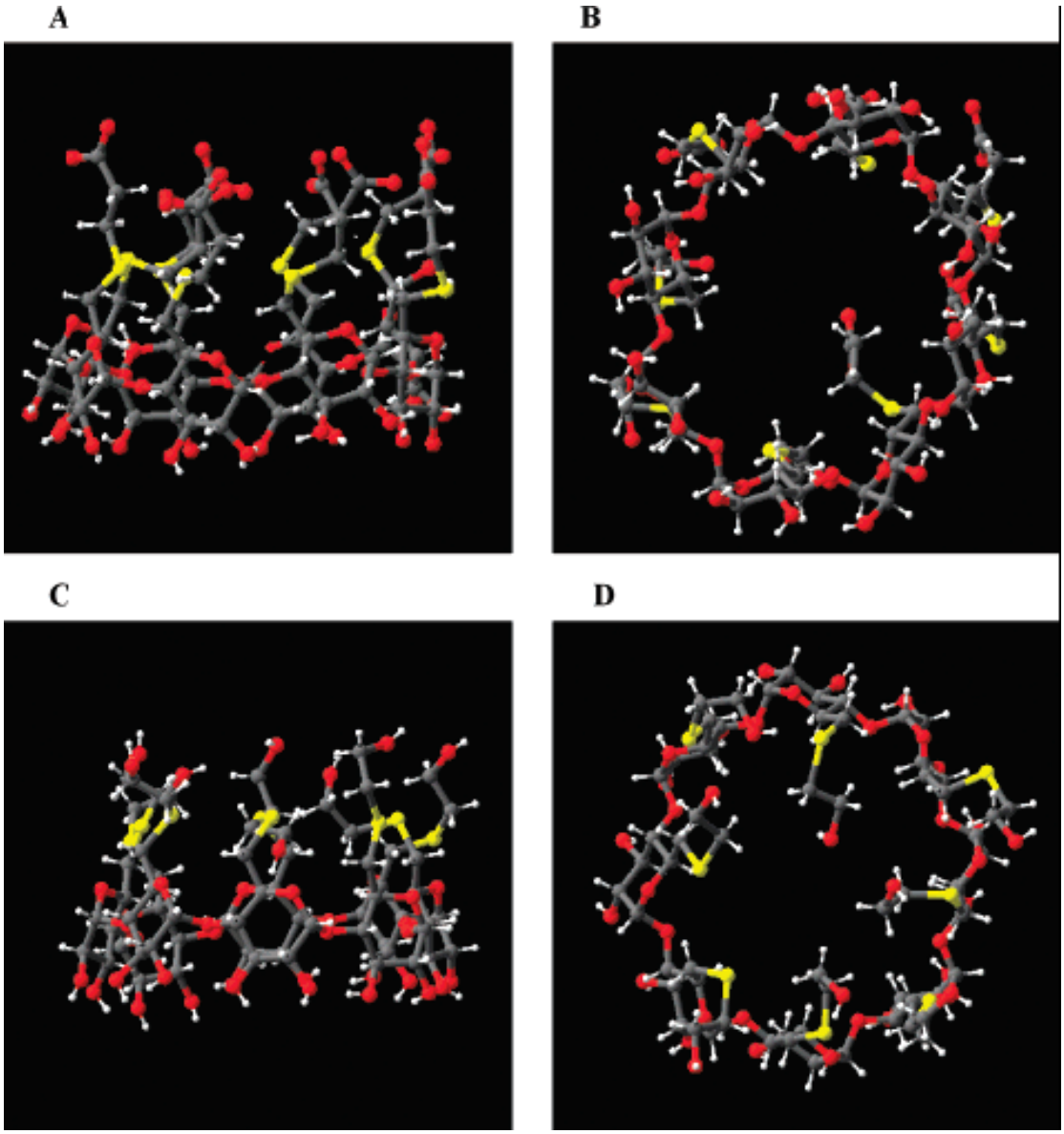


Figure 1.5: X-Ray crystallography representations of Cyclodextrins.

Figures A and B represent side and top views respectively of the carboxylated γCD
One side chain can be seen in the cavity in figure B which tends to 'pucker' the ring
Figures C and D are the same views of its hydroxylated analogue.
Reproduced from reference [133] with permission from American Medical Society

Animal Studies

Efficacy

Org 25969 was shown to be efficacious in reversing the NMBA rocuronium, both in vitro and in vivo [53, 133]. It was shown to reverse blockade twice as fast as neostigmine 40 μg/kg with atropine 15 μg/kg in anaesthetized Rhesus monkeys, [142] and to reverse profound blockade in Rhesus monkeys which had previously been not possible to achieve with standard reversal agents [143].

Safety

In vivo experiments with Org 25969 on anaesthetised cats showed no significant changes in blood pressure, heart rate, left ventricular pressure or left ventricular contractility. In addition, there was stability with regards to direct right vagal nerve stimulation and heart rate [133]. In experiments with guinea pigs and Rhesus monkeys, there were no significant changes in heart rate or blood pressure with the administration of Org 25969 [133, 142, 143]. Further in vitro experiments on mouse vas deferens, mouse hemidiaphragm and rat aortic ring showed Org 25969 to be devoid of intrinsic biological activity [133].

Pharmacokinetics

Pharmacokinetic experiments in guinea pigs have helped to conclude that after intravenous injection, Org 25969 occupies a low volume of distribution representing the extracellular water compartment. Clearance is predominantly renal, excretion approaching glomerular filtration rate, hence metabolism is not thought to contribute significantly [134, 144].

Human Studies

Efficacy

There have been many studies exploring the clinical possibilities of sugammadex, the name given to Org 25969. In the first human study, 29 healthy male subjects were given doses of sugammadex from 0.5 to 8.0mg/kg, with or without 0.6mg/kg rocuronium. The drug was reported to be efficacious in a dose dependent manner at doses above 1.0mg/kg [145]. Further studies in anaesthetised surgical patients found that a dose of 2.0-4.0mg/kg of sugammadex, given at the reappearance of T2 (using TOF stimulation) would reverse the neuromuscular blockade established with rocuronium 0.6mg/kg, within 3 minutes to a

TOF ratio ≥0.9 [146]. It has been shown that sugammadex will reverse deep neuromuscular blockade (as demonstrated by a PTC 1-2) significantly faster than neostigmine,(geometric mean time to TOF ratio≥0.9; sugammadex vs neostigmine with gylcopyrrolate: 2.9 mins vs 50.4 mins for, $p<0.0001$) [147].

Safety

Sugammadex acquired its European license on 29th July 2008. Prior to this, over 1700 patients and 120 volunteers had received sugammadex. At high doses (32mg/kg) 10% of non anaesthetised volunteers experienced dysguesia, a bad or metallic taste in their mouth. Allergic reactions, such as rash, are rare and have been confirmed in one volunteer. Other side effects such as nausea, coughing, hypotension, movement, parasomia and dry mouth have all been reported. One study showed prolongation of QT_C interval in both placebo (5 volunteers) and sugammadex (3 volunteers) groups, further studies are planned to investigate this [148]. Two analyses of bleeding complications have been investigated by the European Medicines Agency which has recently issued an update to the product characteristics stating that 'the evidence currently available indicates an effect of sugammadex on haemostasis parameters'[149].

There are two types of drug interactions which could be of concern with sugammadex; *Displacement interactions*, where a drug displaces rocuronium from sugammadex leading to a possibility of reoccurrence of neuromuscular blockade and *capturing interactions* when sugammadex could bind another drug, therefore reducing the free plasma concentrations and/or efficacy of the other drug. Modeling work has identified flucloxacillin, fusidic acid and toremifene as drugs which could potentially displace rocuronium from sugammadex, in addition, the prostagenic compound in hormonal contraceptives may be encapsulated by sugammadex [150].

Pharmacokinetics

The pharmacokinetics of sugammadex is discussed in detail in Section 2.1 and 2.4.

Table 1.4: Summary of clinical trials using sugammadex including special populations

Study	n	Treatment groups	Time to TOF ratio≥0.9
Gijsenberg et al [145] Sugammadex 3 min after 0.6mg/kg rocuronium	29	Healthy volunteers Sugammadex 0.1-8mg/kg	1mg/kg:23min 2mg/kg:13min 4mg/kg:2.6min 8mg/kg:1.2min
Suy et al [151] Sugammadex at reappearance of T2, 0.6mg/kg rocuronium given	80	Adult patients ASA I-II Sugammadex 0.5-4mg/kg Placebo	1mg/kg:2.3min 2mg/kg:1.7min 4mg/kg:1.1min Placebo:31.8min
Puhringer et al [152] Sugammadex at 3 after 1.0 or 1.2mg/kg rocuronium	88	Adult patients ASA I-III Sugammadex 2-16mg/kg Placebo	Roc 1.0mg/kg Sug 16mg/kg: 1.6min Placebo:111.1min Roc 1.2mg/kg Sug 16mg/kg :1.3min Placebo: 124.3min
Puhringer et al [152] Sugammadex at 15 after 1.0 or 1.2mg/kg rocuronium	88	Adult patients ASA I-III Sugammadex 2-16mg/kg Placebo	Roc 1.0mg/kg Sug 16mg/kg: 0.9min Placebo:91min Roc 1.2mg/kg Sug 16mg/kg :1.9min Placebo: 94.2min
Jones et al [147] Sugammadex or neostigmine at after 0.6 mg/kg rocuronium with maintenance dose 0.15mg/kg rocuronium to maintain PTC 1-2	74	Adult patients ASA I-IV Sugammadex 4mg/kg Neostigmine 70µg/kg with glyc. 14µg/kg	Sugammadex:2.9min Neo/glycol: 50.4min
Special populations	n	Treatment groups	Outcome
Staals et al [54] Sugammadex 2mg/kg at reappearance of T2 after 0.6mg/kg rocuronium	30	Adult patients severe renal impairment 15 with CrCl<30 15 Controls with CrCl>80	Rapid and effective reversal of neuromuscular blockade in both groups
Dahl et al [153] Sugammadex 2 or 4mg/kg at reappearance of T2 after 0.6mg/kg rocuronium	116	Adult patients NYHA Class II-III, ASA II-IV Non cardiac surgery	Effective reversal of blockade No association with prolonged QT_c
Amao et al [154] Sugammadex 2or 4mg/kg at reappearance of T2 after 0.6mg/kg rocuronium	77	Adult patients with pulmonary disease, ASA II-III	Effective reversal of blockade 2 cases of bronchospasm in asthmatics
McDonagh et al [155] Sugammadex 2mg/kg at reappearance of T2 after 0.6mk/kg rocuronium with maintenance dose 0.15mg/kg	150	Adult patients ASA I-III	Mean recovery times: <65 yrs: 2.3min 65-74 yrs: 2.6min >75 yrs:3.6min
Monk et al [156] Pooled data from 18 clinical trials. End point TOF ratio≥0.9	662	Adult patients BMI<30kg/m^2 BMI≥30kg/m^2	No significant difference in recovery times between groups

Possible clinical applications

Rocuronium, along with sugammadex, can be used to provide control of neuromuscular blockade, allowing deep or moderate blockade to be maintained throughout a procedure and then reversed within two to three minutes [150].

An area of particular interest is the possibility for rocuronium and sugammadex to be able to replace the use of suxamethonium for rapid sequence induction. As previously mentioned suxamethonium has a fast onset and offset time which gives the opportunity for early tracheal intubation and gives the chance, if attempts at airway control fail, for a pre-oxygenated patient to return to spontaneous breathing before fatal hypoxia occurs. The onset time of 1.2mg/kg rocuronium has been found to be 60s (range 30-120) compared to 71s (range 40-120) with 1.0mg/kg suxamethonium [157, 158]. A Cochrane review in 2007 showed no statistically significant differences in intubating conditions when these drugs were used at these doses [74]. When comparing the spontaneous recovery of suxamethonium 1.0mg/kg to rocuronium 1.2mg/kg followed 3 minutes later with sugammadex 16mg/kg, recovery to 10% twitch height of T1 was 7.1 min(SD 1.6) and 4.4min(SD 0.7) respectively, $p<0.0001$. Time to 90% twitch height of T1 was found to also be significantly slower in the suxamethonium group, 10.9 min(2.4) compared to 6.2 min(1.8), $p<0.0001$ [159].

The introduction of SRBAs may lead to significant changes in practice. There is a possibility that an intubating dose of rocuronium1-1.2mg/kg will be used in a modified rapid sequence induction with the knowledge that if needed sugammadex 16mg/kg 3 minutes later will reverse the blockade. This may lead to a decrease in the routine use of suxamethonium, in addition, there is the possibility of decreasing the occurrence of PORC with the correct use of a SRBA and neuromuscular monitoring. As with many new drugs, the use of sugammadex may well be decided on pharmaco-economic factors, alongside the pharmacodynamic and pharmacokinetic [160].

1.9 Rationale for clinical trial

There remains a question with regards to use of this drug in patients with renal failure. Since sugammadex and the sugammadex-rocuronium complex are renally excreted, there has been concern that these drugs may behave differently in this group of patients due to the prolonged exposure expected [145, 161] [162]. The prolonged exposure could, in theory, lead to the dissociation of rocuronium from sugammadex, thereby leading to recurarization. However there is evidence that the complexed drug is very stable. Using isothermal titration calorimetry, Bom et al have measured the association constant of the sugammadex-rocuronium complex as approximately $10^7 M^{-1}$ [142] This represents a high level of host-guest affinity and suggests that it is unlikely that recurarization secondary to disassociation would occur despite prolonged exposure [142].

To investigate sugammadex in patients with renal failure Staals et al. undertook a clinical trial in which sugammadex 2mg/kg was given to reverse rocuronium induced neuromuscular block at the reappearance of T2 in patients with severe renal impairment (CrCl <30ml/min) and controls (CrCl ≥80ml/min) [54]. The results of this trial showed equivalence in safety between the two groups and there was no evidence of recurarization in either group. Mean (SD) recovery to TOF ratio 0.9 was 2.0 (0.72) min in the renal group and 1.65 (0.63)min in controls. The trial demonstrated a rapid recovery for both groups, although equivalence was demonstrated on post hoc statistical analysis when study centre was removed as a variable. The limitations of this study were firstly, that data were only collected on 15 patients with, as such there is still a need for more evidence of the efficacy and safety of this drug at different doses and at different levels of neuromuscular blockade to allow its widespread use in this group of patients. Secondly, although there was a dense sampling scheme for pharmacokinetic analysis during the first 24 hours, there was no sampling after that. Renal patients were assessed clinically for signs of recurarization for 48 hours however there remains a question as to the pharmacokinetics of sugammadex over a longer timeframe.

The clinical trial which forms the basis of this thesis aims to further investigate the use of this drug in patients with renal failure, to better understand and inform the use of sugammadex in a group of patients that may benefit greatly from the safe application of this drug.

Section 2: Pharmacokinetics of Sugammadex

2.1 Pharmacokinetics of rocuronium and sugammadex alone and combined

Pharmacokinetics: 'What the body does to the drug'
Pharmacodynamics: 'What the drug does to the body'[163]

Pharmacokinetics is the study of how drugs are handled by the body. The time course of drugs' absorption, distribution, metabolism and excretion are studied in an attempt to understand and model the drug handling alongside gaining insight into mechanisms of action.

The pharmacokinetics of sugammadex have been evaluated in adult patients and healthy volunteers in a number of clinical studies: Sorgenfrei et al, 27 male surgical patients aged 18-64 [146], Suy et al , 80 patients aged ≥18 [151], Gijsenbergh et al, 29 healthy volunteers with and without anaesthesia [145] and Sparr et al, 98 male patient aged 18-64 [164].

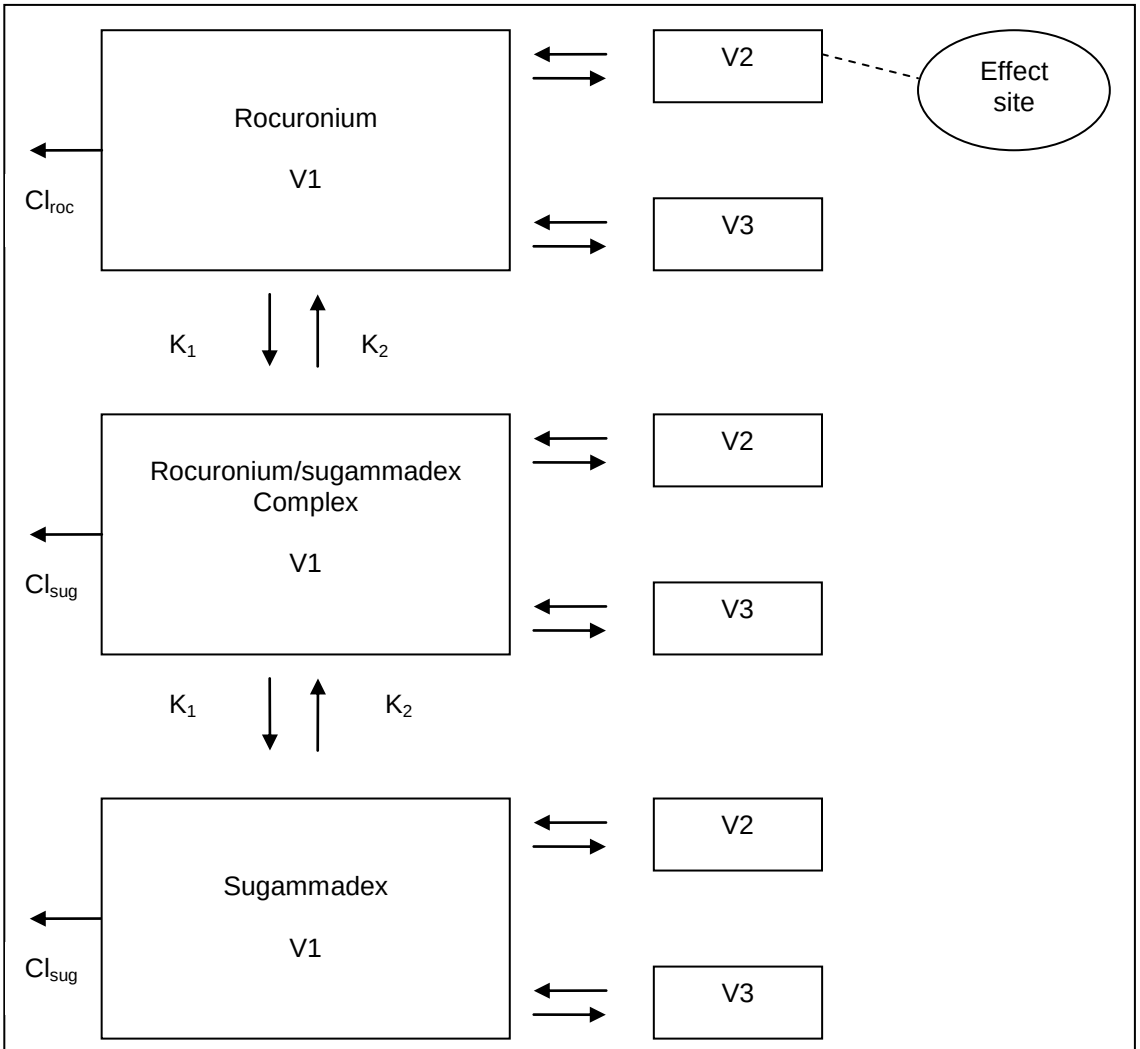
Sugammadex is given as an intravenous injection and therefore absorption is treated as being instantaneous. From the above mentioned studies the volume of distribution at steady state (V_{ss}) was found to range between 11 and 14 L, which corresponds to the approximate volume of extracellular water in the body. The V_{ss} is consistent with the physiochemical properties of sugammadex and that sugammadex does not bind with plasma proteins or erythrocytes [165]. Metabolism of sugammadex is thought to be very limited as in pre-clinical and clinical studies no metabolites have been found whilst greater than 90% of the drug has been collected in the urine [145]. The reported rate of clearance of sugammadex ranges from 88ml/min to 120ml/min which, being in the range of normal glomerular filtration rate, supports the renal route as the main mechanism of elimination (faecal and respiratory elimination <0.02%) [165]. The terminal elimination half life ($t_{1/2}$) is approximately 110 minutes with over 90% of the drug eliminated via the urine in the first 24 hours [145, 165]. When considering all pharmacokinetic parameters and body weight was adjusted for, there were no gender differences observed [165].

Rocuronium has a V_{ss} of 14-16 L, $t_{1/2}$ is approximately 70 minutes and the clearance is 3-400 ml/min [166]. Route of elimination is primarily via hepatic uptake and excretion of the unchanged drug in the bile and ultimately the faeces, approximately a quarter of excretion is via the renal route [167].

When rocuronium is given and followed 3 minutes later by administration of sugammadex at a dose greater than 2mg/kg the distribution and elimination of rocuronium are significantly altered. V_{ss} of rocuronium approaches that of sugammadex and clearance decreases by approximately two thirds, to a value close to the clearance of sugammadex [145]. Sparr et al confirmed these findings in addition to confirming an increase in the renal excretion of rocuronium after sugammadex administration [164]. These results support the encapsulation theory of the mechanism of action of sugammadex as encapsulated rocuronium appears to be cleared mainly by the sugammadex pathway i.e. renal route.

Pharmacokinetic model of interaction between sugammadex and rocuronium

There is a validated PK model developed by Ploeger at al.[168] into which the data generated in this trial will be inputted at a latter date with the intention of increasing the fidelity of the model, especially with regards to renal function. The published PK model is described in Figure 2.1 below [168].



- Sugammadex and rocuronium are both described by three compartment models with first order elimination (Cl_{drug}) occurring from the central compartment:V1.
    - As discussed above the clearance of rocuronium/sugammadex complex can be represented by clearance of sugammadex.
- It is hypothesised that complexation/encapsulation occurs in the central compartment, K_1 and K_2 representing the respective association and disassociation constants.
- V2 represents the first tissue compartment within which the effect site (the NMJ) is theoretically situated. V3 represents the second tissue compartment.
- When encapsulation occurs, the concentration of free rocuronium in V1 decreases creating a concentration gradient with V2.
    - This results in free rocuronium molecules returning to V1 where they are encapsulated.
    - This decreases the rocuronium concentration at the effect site leading to reversal of neuromuscular blockade
- There is no assay method which can differentiate between free rocuronium and the encapsulated form hence this proposed mechanism of action is very difficult to prove. However, Ploeger et al. have used validated mechanism based modelling techniques which can reliable predict the reversal of rocuronium induced blockade by sugammadex thereby supporting the hypothesised mechanism of action.

Figure 2.1: Pharmacokinetic model of rocuronium/sugammadex interaction [168]

The PK analysis in this study has been based on non-compartmental methods to enable a more quantitative description of any differences in PK between renal subjects and controls in this study. Sugammadex was given as a single dose for reversal and as such, this analysis is appropriate in this case. Rocuronium was given as a single dose for intubation followed by a variable number of maintenance doses and as such, reliable non-compartmental PK analysis could not easily be performed for rocuronium. The following PK parameters were calculated: (The calculation methods used appear in the section 3: Methods)

Clearance (Cl): $mlmin^{-1}$
Volume of plasma from which a drug is completely removed per unit time

Volume of distribution at steady state (V_{ss}): *L*
The apparent volume into which a drug distributes at a steady state.

Half life ($t_{1/2}$): *min*
Time taken for plasma concentration of drug to reduce by 50%.

Area under curve(AUC): $\mu g.min/ml$
Integral of plasma concentration versus time.

Mean residence time (MRT): *hours*
Mean time that the drug spends in the body.

2.2 The effect of Chronic Renal Failure on pharmacokinetics

Chronic renal failure, also known as chronic kidney disease (CKD), is a worldwide public health problem [128]. CKD can be defined as either kidney damage or a renal glomerular filtration rate (GFR) of less than 60 ml/min/1.73m^2 present for 3 or more months. CKD can further be classified as follows[128, 169]:

- Stage 1: Kidney damage with normal or increased GFR (>90 ml/min/1.73 m^2)
- Stage 2: Mild reduction in GFR (60-89 ml/min/1.73 m^2)
- Stage 3: Moderate reduction in GFR (30-59 ml/min/1.73 m^2)
- Stage 4: Severe reduction in GFR (15-29 ml/min/1.73 m^2)
- Stage 5: Kidney failure (GFR <15 ml/min/1.73 m^2 or dialysis)

In England in 2007, amongst adults registered with a General Practitioner, the prevalence of CKD was estimated at 8.8% with 3.73% classified as Stage 3 to 5 [170]. In the United States the prevalence was estimated at 11%, 4.7% were classified as Stage 3 to 5 [171].

As can be appreciated by the prevalence of renal disease, understanding its influence on drug handing and drug effects (the pharmacokinetics and pharmacodynamics) is key to the development of new therapeutic agents. Ideally the effects of renal disease should be investigated through special population studies during Phase III clinical drug trials such as the trial described in this thesis [172].

Changes in drug distribution

Drug distribution can be thought of as the movement of the drug to and from the site of measurement and it is dependant on physicochemical properties of the drug, regional blood flow, protein binding and sequestration mechanisms.

CKD can affect the protein binding of acidic drugs to albumin. The proportion of unbound phenytoin in patients with normal renal function is approximately 8% whereas in patients with CKD stage 4-5 the unbound portion can be 16% or higher [173]. The reduction in binding in these patients may be due to reduced albumin concentration, changes in the structure of albumin binding sites, or displacement of drugs from these sites by organic molecules that accumulate in CKD.

There is little or no reduction in protein binding of neutral or basic drugs in patients with stage 4-5 CKD. Basic and neutral drugs will bind to α_1-acid glycoprotein which tends to have an increased concentration in these patients which may explain the limited changes in protein binding [173].

The volume of distribution of drugs can be affected in patients with CKD by protein binding itself or by other mechanisms. The relationship between changes in volume of distribution and stage of CKD cannot always be understood [173].

Changes in drug elimination

Drug elimination is the removal of drug from the site of measurement by either excretion of unchanged drug from the body or by metabolism [174]. Excretion can be via renal or extra-renal routes such as biliary excretion. Renal excretion is a factor of glomerular filtration, active drug secretion at the proximal tubule and active or passive drug reabsorbtion along the renal tubule [173].

When there is renal damage and renal function is reduced, any drug which relies upon the renal route of excretion, either completely or in part, will have a reduced rate of excretion. The effect this has on the patient will depend upon the characteristics of the drug and its metabolites. For example, rocuronium, elimination is 20- 30% via the renal route[76] and Robertson et al demonstrated a significantly increased duration of action of rocuronium (0.6mg/kg) in patients with Stage 5 CKD [129].

Changes in drug metabolism

Drug metabolism is the conversion of a chemical species to another chemical species which may be inactivated, activated or made more soluble to allow removal from the plasma [174]. The liver is the primary site for drug metabolism in the body however metabolism also takes place in the gastrointestinal tract, the lungs and the kidneys.

Certain metabolic pathways can be affected by CKD, depending on the degree of reduction of GFR, which may in turn affect the metabolism of drugs which rely on those pathways [173].

Sun at al. state that renal disease can alter the non-renal metabolism of drugs by reducing metabolic enzyme activity and adversely affects transporter systems in tissues of the intestine, the liver and in the kidney itself [175]. The exact mechanisms are not known, although uraemic toxins, which are not cleared in renal failure, are thought to have both direct and indirect effects.

2.3 Pharmacokinetics of rocuronium in renal failure

Robertson et al. investigated the pharmacokinetics of rocuronium (0.6mk/kg) in 17 patients with stage 5 CKD and 17 healthy controls. The time to onset of neuromuscular block was similar between groups however the duration of action and time to recovery of TOF ratio 0.7 were both increased in the renal failure group (statistically significant). There were also differences between the PK parameters measured listed in table 2.1 below. The authors suggested that the pharmacodynamic differences between the groups may be explained (although not conclusively) by the decreased clearance of rocuronium in the renal failure group, the disease processes occurring in renal failure or the medications taken by the renal failure group [129].

Table 2.1: Pharmacokinetics of rocuronium in patients with renal failure and healthy controls reproduced with permission from Robertson et al.[129]

Pharmacokinetic parameter	Patients with normal renal function	Renal failure patients: CrCl<15 ml/min
Cl *ml/kg/min*	4.5 $^{+}_{-}$ 1.2 (3.1-6.7)*	2.7 $^{+}_{-}$ 0.7 (1.6-4.3)*
V_{ss} *ml/kg*	194 $^{+}_{-}$ 45(121-297)	220 $^{+}_{-}$ 77 (113-380)
$t_{1/2\ (1)}$ *min*	7.8 $^{+}_{-}$ 1.8 (4.8-10.8)	6.5 $^{+}_{-}$ 3(2.2-12)
$t_{1/2(2)}$ *min*	57 $^{+}_{-}$ 17 (38-89)	70 $^{+}_{-}$ 23 (43-122)
MRT *min*	45 $^{+}_{-}$ 11 (30-68)*	83 $^{+}_{-}$ 26 (50-142)*
Data presented as mean $^{+}_{-}$ SD (range) *statistically significant difference between groups p<0.0001		
Cl= plasma clearance, V_{ss}= volume of distribution at steady state, $t_{1/2\ (1)}$ and $t_{1/2(2)}$= half lives of first and second exponential phases, MRT= mean residence time [129]		

2.4 Pharmacokinetics of sugammadex in renal failure

Staals et al. investigated the pharmacokinetics of sugammadex 2mg/kg given at the reappearance of T2 after rocuronium 0.6mg/kg was given in 30 anaesthetised adult patients, 15 with severe renal impairment (ASA II-III) and 15 healthy controls (ASA I-II) [162]. Pharmacokinetic data were collected up to 72 hours after administration of sugammadex. The efficacy and safety results of this trial were reported in an earlier paper by Staals et al. showing rapid and efficacious recovery of neuromuscular function in both groups in addition to equivalence in safety, with no occurrence of recurarization in either group [54]. The pharmacokinetic data showed some significant differences between the groups as described in table 2.2 below.

Table 2.2: Pharmacokinetics of sugammadex 2mg/kg in patients with renal failure and healthy controls, reproduced with permission from Staals 2010 [162]

Pharmacokinetic parameter	Patients with normal renal function CrCl>80ml/min	Severe renal failure patients CrCl<30ml/min
AUC 0-∞ *µg.min/ml* range	1730 (34.8)* 1060-3330	27500 (114) 6480-147000
Cl *ml/min* range	95.2 (22.1)* 58.3-138	5.5 (108) 1.15-18.1
V_{ss} *litre* range	13.8 (20.5) 10.0-19.7	16.0 (35.5) 9.3-31.8
$t_{1/2\ \beta}$ *hour* range	2.3 (44.4)* 1.6-7.5	35.7 (121) 10.7-282
MRT *hour* range	2.4 (25.5)* 1.8-4.0	48.2 (132) 13.2-399
Data shown as geometric mean (coefficient of variation) *=statistical significant (Student's t-test)P<0.05		
AUC=Area under curve, Cl= plasma clearance, V_{ss}= volume of distribution at steady state, $t_{1/2\ \beta}$= terminal elimination half life, MRT= mean residence time [162]		

Despite no clinical difference shown in this trial, the fact that the sugammadex-rocuronium complex remains in the body for longer in patients with severe renal failure prompted the authors to suggest that further trials were needed with longer follow up periods to evaluate whether the prolonged exposure may have an adverse effect in patients with severe renal failure.

Section 3: Methods

3.1 Summary of clinical trial

This was an open-label, multi-centre, parallel-group, comparative study evaluating the efficacy, pharmacokinetics and safety of sugammadex 4.0mg/kg administered at a target depth of blockade of 1-2 PTC after rocuronium administration in subjects with normal or severely impaired renal function. Our hospital was one of eight study centres in four countries: Austria (2 sites), France (1 site), The Netherlands (3 sites) and United Kingdom (2 sites).

Hypothesis

There is no difference in the efficacy of sugammadex 4.0mk/kg administered at deep neuromuscular blockade in patients with normal or severely impaired renal function.

Aims of the study

Primary : To investigate whether sugammadex has equivalent efficacy in subjects with normal or severely impaired renal function.

Secondary : To collect more data on the safety of sugammadex in subjects with normal or severely impaired renal function.

: To collect more data on the pharmacokinetics of sugammadex in subjects with normal or severely impaired renal function.

: To collect more data on the dialysability of sugammadex in patients with severely impaired renal function.

3.2 Clinical trial medication

Formulation

Treatments were manufactured, packaged, labelled, shipped, stored, and administered according to the protocols of MSD.

- Sugammadex was supplied in vials containing 500 mg active entity in 5 ml of sugammadex i.e. 100mg/ml

- Rocuronium was supplied in colourless vials containing 100 mg in 10 ml of rocuronium bromide i.e. 10mg/ml

Dose

At stable anaesthesia, an intubating dose of rocuronium (0.6mg/kg) was given through a fast running drip over less than 10 seconds. Post tetanic count (PTC) was measured by the TOF-Watch® SX to ensure that deep neuromuscular block was maintained (i.e. 1-2 PTC) and further doses of rocuronium (0.1-0.2 mg/kg) were administered as required. When surgery was completed and the PTC measured 1-2, a single intravenous bolus of sugammadex 4.0mk/kg was administered via a fast running drip over less than 10 seconds. All doses were based on actual body weight.

Randomisation and Blinding

This was an open label trial with all subjects receiving the same dose of sugammadex hence randomisation and blinding were not applied.

3.3 Ethical considerations

This study was designed by NV Organon a Dutch drug company which is a subsidiary of the MSD Corporation. In the case of our trial site, ethical approval was obtained from the Central Manchester Research Ethics Committee, in July 2008. In March 2009 a substantial amendment relating to exclusion criteria was submitted and further approval was given May 2009 (REC ref: 08/H1008/103, EudraCT 2007-006-935-29). Ethical approval was obtained from local independent medical ethics committees in each other centre. Written informed consent was given by all participating patients. The study was conducted in accordance with the current revision of the Declaration of Helsinki, the International Conference on Harmonisation guidelines, Good Clinical Practice and current regulatory guidelines [176].

3.4 Patient Selection

Eligible patients (see inclusion/exclusion criteria below) were approached at their pre-operative visit or at a minimum of 24 hours prior to surgery, and the study explained to them. A patient information leaflet was given for them to read. The patient was then invited to contact the anaesthetic department if they wished to be included in the trial.

Written informed consent was taken on the day of surgery, prior to any study procedures taking place.

Inclusion criteria

A subject was eligible for participation in the study based on the following inclusion criteria:

- Scheduled for a surgical procedure under general anesthesia with propofol requiring neuromuscular relaxation with the use of rocuronium.
- Scheduled for a surgical procedure in supine position.
- At least 18 years of age.
- American Society of Anesthesiologists (ASA) class I-III
- Creatinine clearance < 30 ml/min with no anticipated clinical indication for high flux haemodialysis during the first 24 hours after sugammadex administration (for renal group) or Creatinine clearance ≥ 80 ml/min (for control group).
- Written informed consent

Exclusion criteria

A subject was excluded from participation in the study based on the following exclusion criteria:

- Subjects known or suspected to have neuromuscular disorders impairing neuromuscular blockade and/or significant hepatic dysfunction.
    - Due to potentially variable neuromuscular blockade
- Subjects scheduled for renal transplant surgery.
    - Due to the complex nature of the surgery
- Subjects known or suspected to have a (family) history of malignant hyperthermia.
    - Due to the possibility of precipitation of the condition by use of neuromuscular blocking agents
- Subjects known or suspected to have an allergy to narcotics, muscle relaxants or other medication used during general anesthesia.
    - Due to the possibility of development of allergic reactions
- Subjects receiving fusidic acid, toremifene and/or flucloxacillin.
    - Due to the possibility of displacement reactions relating to sugammadex as discussed in section 1.8
- Subjects who had already participated in a sugammadex trial.

- Subjects who had participated in another clinical trial, not pre-approved by the sponsor, within 30 days of entering into this trial.
- Female subjects who were breast-feeding.
- Female subjects who were pregnant.
    - In female subjects pregnancy was excluded from medical history and by a urine or blood hCG test within 24 hours before surgery. This was not done in females who were not of childbearing potential

Removal of patients from the study

A patient had the right to withdraw their consent to participate in the study at any point and without the need to give a reason. As investigators, we had the right to remove any patient from the trial if we felt that continuation in the study could threaten the patient's health or wellbeing.

If a patient was discontinued prior to sugammadex administration, no further analysis took place. If the patient was discontinued after sugammadex administration, all data collected up to that point was used for analysis and no further data was collected from the patient.

3.5 Subject data sets

The following subject data sets were defined:

- The All-Subjects-Enrolled (ASE) group consists of all subjects who were enrolled into the trial (ie, signed informed consent).
- The All-Subjects-Treated (AST) group consists of all subjects from the ASE group who received a dose of sugammadex.
- The Intent-to-Treat (ITT) group consists of all subjects from the AST group who had at least one efficacy measurement.
- The Per Protocol (PP) group consists of all subjects from the ITT group without any major protocol violation. In addition, subjects with multiple minor protocol violations leading to the exclusion of all efficacy data from the PP analysis are excluded from the PP group.
- The All-Subjects-Pharmacokinetically-Evaluable (ASPE) group consists of all subjects from the AST group who provided at least one measurable sugammadex or rocuronium concentration for which the related dosing and

sampling times have been documented according to the protocol, and who did not have any protocol violations interfering with pharmacokinetics.

The data from the patients in the AST group were included in the safety analysis. The data from the patients in the PP group were included in the efficacy analysis. The data collected from the ASPE group were included in the pharmacokinetic analysis.

3.6 Protocol Violations

Major protocol violations were described as follows:

- Inclusion criteria not met (see above)
- Exclusion criteria (which could influence the efficacy data) met (see above)
- Dose of sugammadex administered deviated more than 10% from the dose of 4.0mk/kg
- Sugammadex administered at a PTC measurement of greater then 5 or equal to 0
- Sugammadex administered more than 2 minutes after the determination of PTC of 1-2
- Administration of any medication expected to interfere with rocuronium, based on the dose and/or time point of administration (eg, use of another neuromuscular blocking agent, use of reversal agents other than sugammadex or a second dose of sugammadex) before scoring any efficacy variable
- Use of a measurement device to assess the degree of neuromuscular blockade other than the TOF-Watch® SX.
- The time of 1-2 PTC occurrence is considered unknown or unreliable.

Minor protocol violations were described as follows:

- Administration of any medication expected to interfere with rocuronium, based on the dose and/or time point of administration (eg, use of another neuromuscular blocking agent, use of reversal agents other than sugammadex or a second dose of sugammadex) after recording some but not all of the efficacy variables.

3.7 Study procedures

The trial was split into 4 distinct periods:

Screening period

The screening period began once eligible patients had given written informed consent and they were then deemed enrolled in the study. At this point a full medical history was obtained and all medication taken for the 7 days prior to surgery was recorded. Height, weight, heart rate and blood pressure were recorded. Venous blood was taken for serum creatinine and creatinine clearance calculated using the Cockroft & Gault formula [177]. Pregnancy was excluded by medical history and urinary or blood βhCG when applicable.

Peri-anaesthetic period

This period began when the patient entered the theatre suite. Prior to induction of anaesthesia, standard monitoring was placed on the patient; NIBP, ECG, SaO_2. An intravenous cannula was placed for the delivery of anaesthetic drugs and maintenance fluids. Anaesthesia was induced with propofol (Target Controlled Infusion: Marsh model) and remifentanil infusion (mcg/kg/min). Once anaesthetised the patient was manually ventilated via a facemask with oxygen or an air oxygen mix (depending on the needs of the patient) and a second cannula was placed to obtain blood samples throughout the procedure.

Neuromuscular function monitoring was then implemented, using acceleromyography at the adductor pollicis muscle using the TOF-Watch® SX, V1.6 (MSD, Dublin, Ireland.) The details of neuromuscular monitoring are included in section 3.8 below.

At stable anaesthesia, an intubating dose of rocuronium (0.6mg/kg) was given through a fast running drip over less than 10 seconds. When full neuromuscular blockade had occurred, indicated by no evoked twitches of the TOF, the trachea was intubated with a cuffed endotracheal tube and mechanical ventilation started. When the tube was secured, surgery was allowed to commence.

Post tetanic count (PTC) was measured throughout the procedure by the TOF-Watch® SX to ensure that deep neuromuscular block was maintained (i.e. PTC of 1-2) and further doses of rocuronium (0.1-0.2 mg/kg) were administered as required. When surgery was

completed and the PTC measured 1-2, a single intravenous bolus of sugammadex 4.0mk/kg was administered via a fast running drip over less than 10 seconds. TOF measurements were continued to be taken every 15 seconds and when TOF ratio T4:T1 was ≥0.9, intravenous anaesthesia was ceased, the patient allowed to wake up, the endotracheal tube removed and the patient taken to the recovery room.

Standard monitoring (NIBP, ECG, SaO_2) continued in the recovery room, in addition, the patient was monitored for clinical signs of recurarization. Vital signs were recorded and blood samples were taken at the points stipulated in table 3.1 below.

Post anaesthetic period

Approximately 24 hours after sugammadex administration a post anaesthetic visit took place when vital signs were recorded. In addition, a physical examination was carried out, concomitant medication was recorded and any (serious) adverse events were recorded. Blood samples were taken at the points stipulated in table 3.1 below. Patients in the renal group were visited at 48 hours to obtain a sample for PK analysis. If haemodialysis was performed within the first 48 hours post sugammadex administration, blood was taken for PK analysis immediately prior to and following dialysis.

Follow up period

On the 7^{th} and 28^{th} post operative day, patients were visited/attended hospital. Vital signs were recorded and blood samples taken at the points stipulated in table 3.1 below. In addition, a physical examination was carried out, concomitant medication was recorded and any (serious) adverse events were recorded. The pregnancy status of the patient or their partners was also ascertained.

Table 3.1: Timing of blood samples and vital sign measurement

Time point	Blood sample for PK analysis	Blood sample for safety analysis	Vital signs
Pre Roc	x	x	x
2 mins post roc	x		
15 mins post roc	x		
Pre sugammadex	x		x
2 mins			x
5 mins	x		x
10 mins			x
20 mins	x	x	
30 mins			x
5 hours	x	x	
10 hours	x		
24 hours	x	x	x
48 hours	x		
7 days	x	x	x
28 days	x	x	x
Pre and post Dialysis	x		
	PK samples at 48 hours, 7days and 28 days were only taken from patients in the renal group		

3.8 Neuromuscular monitoring and calibration

After the induction of anaesthesia one arm was placed, protected and immobilised on an arm board. Neuromuscular function monitoring was then implemented, using acceleromyography at the adductor pollicis muscle of that arm using the TOF-Watch® SX V1.6. Neuromuscular data were collected and transferred via an interface to a laptop computer by means of the TOF-Watch® SX Monitoring Program, Version 2.3. The program also allowed recording of contemporaneous events.

Surface paediatric ECG-electrodes (1720 Neotrode®) were placed over the ulnar nerve, proximal to the wrist, after the area had been properly cleansed using skin disinfectant wipes. Electrodes were placed between 3 and 6cm apart with the negative electrode placed distally. A temperature sensor was attached to the thenar eminence to ensure that peripheral skin temperature was maintained above 32°C. The acceleromyography transducer was then carefully placed and secured to the distal phalanx of the thumb perpendicular to the plane of movement.

Based on the work of Kopmann et al [178] a 5 second tetanic stimulation at 50Hz was performed to reduce the time required to stabilize the response to subsequent TOF stimulation and to decrease the possibility of repeated evoked stimulation of the nerve leading to an increased evoked response of the muscle i.e. the Staircase Phenomenon. To determine the supramaximal stimulus, the "CAL 2" protocol of the TOF-Watch® SX was selected. This was followed by TOF pulses, of 200μs pulse width at 2Hz, repeated every 15 s, until the TOF ratio stabilized and a final calibration could take place. TOF ratio was said to have stabilised when;

- The TOF ratio (T4:T1) was 100% (+/- 10%)
- There was less than a 10% deviation in the percentage heights of the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} twitches.
- The stimulation required for a maximal response was less than 60mA i.e supramaximal stimulation could then be maintained throughout the monitoring period
- Surface skin temperature was ≥ 32°C
- The transducer sensitivity (i.e the gain) was at an appropriate level set by the manufacturers which was less than 180 (unit-less parameter).
    - The gain is automatically set by the TOF-Watch® SX using the first 7 twitches of a 10 single twitch cycle. If the response is within the acceptable range, the last 3 twitches are used to check the stability of the signal. If the response is out of range and error signal would be displayed and the gain would increase or decrease accordingly until a stable signal at the appropriate level of gain was achieved.

The data recorded from the set up and calibration of one of our control patients is displayed in figure 3.1 below.

Time 08/09/2009	?!*	Mode	Tw1 %	Tw2 %	Tw3 %	Tw4 %	TOF %	CNT	Temp °C	Stim mA	T µs	Sens.	CAL [C - [mA]
08:13:10	*	Comment	"Time synchronized at: 07:56:00, radio clock clock shows 08:13:10"										
08:22:26	*	Comment	"start remifentanil"										
08:23:22	*	Comment	"start propofol"										
08:24:51	*	Comment	"cannula inserted for anaesthesia at 08:18:00"										
08:58:42		TET 50Hz							29.4	60.00	200	157	
09:00:54	*	Comment	"CAL2"										
09:00:54		TOF	100	102	102	103	103		30.5	33.00	200	100	2 [30]
09:01:09		TOF	100	102	103	104	104		30.5	33.00	200	100	2 [30]
09:01:24		TOF	101	102	104	104	102		30.6	33.00	200	100	2 [30]
09:01:39		TOF	100	101	103	101	101		30.8	33.00	200	100	2 [30]
09:01:54		TOF	100	101	100	100	100		31.5	33.00	200	100	2 [30]
09:02:09		TOF	99	100	102	101	102		32.0	33.00	200	100	2 [30]
09:02:24		TOF	98	100	101	101	103		32.4	33.00	200	100	2 [30]
09:02:39		TOF	99	100	102	101	102		32.8	33.00	200	100	2 [30]
09:02:54		TOF	98	100	101	101	103		33.0	33.00	200	100	2 [30]
09:03:09		TOF	98	100	101	101	103		33.2	33.00	200	100	2 [30]
09:03:24		TOF	99	100	101	102	103		33.3	33.00	200	100	2 [30]
09:03:39		TOF	99	100	101	102	103		33.4	33.00	200	100	2 [30]
09:03:54		TOF	98	100	101	101	103		33.5	33.00	200	100	2 [30]
09:04:09		TOF	98	101	101	101	103		33.6	33.00	200	100	2 [30]
09:04:32	*	Comment	"Not calibrated"										
09:04:38	*	Comment	"CAL2"										
09:04:38		TOF	99	100	102	102	103		33.7	33.00	200	97	2 [30]
09:04:53		TOF	99	101	102	102	103		33.7	33.00	200	97	2 [30]
09:05:08		TOF	99	100	102	102	103		33.7	33.00	200	97	2 [30]
09:05:23		TOF	98	100	100	100	102		33.7	33.00	200	97	2 [30]
09:05:38		TOF	97	100	100	100	103		33.7	33.00	200	97	2 [30]
09:05:53		TOF	97	99	100	100	103		33.7	33.00	200	97	2 [30]
09:06:08		TOF	96	99	100	100	104		33.7	33.00	200	97	2 [30]
09:06:23		TOF	96	99	100	100	104		33.7	33.00	200	97	2 [30]
09:06:38		TOF	96	99	100	100	104		33.7	33.00	200	97	2 [30]
09:06:53		TOF	97	99	100	100	103		33.7	33.00	200	97	2 [30]
09:07:08		TOF	96	98	99	99	103		33.7	33.00	200	97	2 [30]
09:07:23		TOF	96	99	99	99	103		33.7	33.00	200	97	2 [30]
09:07:38		TOF	96	98	98	99	103		33.6	33.00	200	97	2 [30]
09:07:53		TOF	96	98	99	99	103		33.6	33.00	200	97	2 [30]
09:08:08		TOF	96	99	99	99	103		33.6	33.00	200	97	2 [30]
09:08:16	*	Comment	"blood sample for pk pre roc"										
09:08:19	*	Comment	"blood sample for safety pre roc"										
09:08:23	*	Comment	"bp 110/70 hr 53"										
09:08:23		TOF	96	99	99	99	103		33.5	33.00	200	97	2 [30]
09:08:38		TOF	96	99	99	100	104		33.4	33.00	200	97	2 [30]
09:08:53		TOF	96	99	99	100	104		33.4	33.00	200	97	2 [30]
09:09:08		TOF	96	99	99	100	104		33.4	33.00	200	97	2 [30]
09:09:23		TOF	96	100	99	99	103		33.4	33.00	200	97	2 [30]
09:09:32	*	Comment	"start roc"										
09:09:34	*	Comment	"stop roc"										
09:09:38		TOF	93	96	97	96	103		33.5	33.00	200	97	2 [30]
09:09:53		TOF	93	96	96	96	103		33.4	33.00	200	97	2 [30]
09:10:08		TOF	91	93	92	91	100		33.4	33.00	200	97	2 [30]
09:10:23		TOF	55	53	50	49	89		33.4	33.00	200	97	2 [30]
09:10:38		TOF	25	21	19	18	72		33.4	33.00	200	97	2 [30]
09:10:53		TOF	8	6	5	5		4	33.4	33.00	200	97	2 [30]
09:11:08		TOF	3	0	0	0		1	33.5	33.00	200	97	2 [30]
09:11:23		TOF	0	0	0	0		0	33.6	33.00	200	97	2 [30]

Figure 3.1: Sceenshot of calibration of Neuromuscular Monitoring: subject 015 (renal group) produced using TOF Watch SX® V1.6 and TOF-Watch® SX Monitoring Program, Version 2.3.

TOF=Train of Four, TET 50 HZ= tetanic stimulation at 50Hz, TW1-4%= Percentage height of 1st-4th twitch, TOF%= Train of four ratio T4:T1 as a percentage, CNT= count of twitches, Temp°C= skin temperature, Stim mA= supramaximal stimulation in milleamperes, Tµs= pulse width in µs, Sens.= sensitivity setting (gain), CAL C[mA]= calibration mode [stimulation used in milleamperes], comment= commentary on study procedures e.g "start roc" denotes start of administration of IV bolus of rocuronium.

Once the calibration and stabilisation had occurred, 200μs TOF pulses, at 2Hz were repeated every 15 s throughout the monitoring period.

The calibration procedure detailed above was used with the intention of decreasing any variability in the quality of TOF traces between study centres. Comprehensive training was provided to ensure good TOF technique and test traces had to be approved by the Trace Team at MSD before a centre was allowed to recruit patients. The Trace Team also had the responsibility to review and where needed, ask for clarification of the TOF traces to ensure adherence to the study protocols. A central independent adjudication committee (CIAC) was made available for consultation on any TOF traces in which there were deviations from the prescribed guidelines set up by MSD.

Figure 3.2 below shows a graphical summary of a TOF trace from a patient in the renal group.

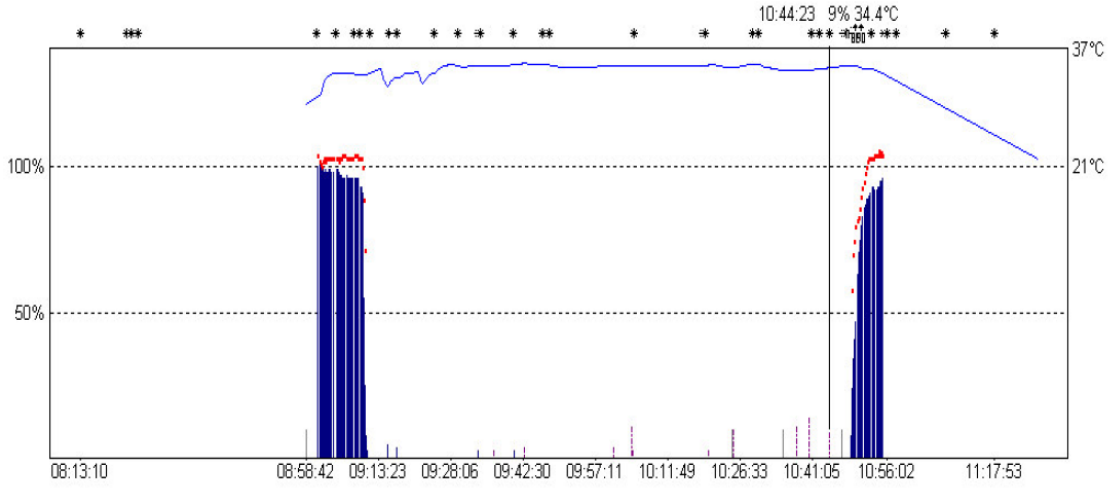


Figure 3.2: Graphical summary of TOF trace: subject 015 (renal group) produced using TOF Watch SX® V1.6 and TOF-Watch® SX Monitoring Program, Version 2.3.
Red dot = TOF ratio in percentage, vertical navy line = twitch height as percentage from baseline, continuous horizontal blue line = skin temperature, small vertical black lines = graphical representations of post tetanic count (PTC), long vertical solid black line = time of administration of sugammadex

3.9 Efficacy/Pharmacokinetic/safety assessments

Efficacy assessments

The efficacy of sugammadex was monitored using TOF-Watch® SX V1.6. Neuromuscular data were collected via a transducer (accelerometer) affixed to the top of the thumb and transferred on-line via an interface to a laptop computer by means of the TOF-Watch®SX Monitoring Program, Version 2.3. The primary neuromuscular efficacy parameter assessed by the TOF-Watch SX was time to recovery of the T4/T1 ratio ≥ 0.9. Data were also collected on time to recovery of the T4/T1 ratio ≥ 0.7 and 0.8.

Pharmacokinetic assessments

Blood samples were collected at stipulated time points for pharmacokinetic analysis of sugammadex and rocuronium, (minimum 8, maximum 14 samples) see table 3.1.

Approximately 3ml of blood was taken and placed in a green top hard plastic heparin Vacutainer collection tube. The tubes were then placed in a Capricorn Table-top centrifuge for 15 minutes at 2000-3000g, the plasma was carefully removed by pipette and stored upright in hard plastic tubes in a -20°freezer until ready for dispatch under 'dry ice' to BARC Europe NV central laboratory in Belgium for analysis. If the samples could not be centrifuged within 15 minutes, they were placed in an insulated box on ice at 0-4°C to avoid haemolysis until they could be processed.

The concentrations of sugammadex and rocuronium in the plasma of the samples sent to the lab were determined using validated liquid chromatographic assay methods with mass spectrometric detection under the responsibility of the Department of Bioanalytics-Waltrop, MSD Research Laboratories, Essex Pharma Development GmBH, Waltrop, Germany. The assays were carried out in full compliance with Good Laboratory Practice regulations[179]. Further details of the assay methods for sugammadex are included in the appendix.

Safety assessments

Safety during the study was assessed via the following parameters;

- Detailed serious/adverse event (S/AE) reporting mechanisms
    - including pregnancy follow up
- Physical examination
    - Any changes or abnormalities were recorded as S/AEs
- Vital signs
    - Non-invasive blood pressure, heart rate
- Laboratory blood tests
    - Haematology
        - Basophils, Eosiniphils, Erythrocyte count, Haematocrit, Haemoglobin, Leukocyte count, Lymphocytes, Monocytes, Neutrophils, Platelet count.
    - Biochemistry
        - Alanine aminotransferase, Albumin, Alkaline phosphotase, Aspartate aminotransferase, Bilirubin total, Calcium, Chloride, Cholesterol total, Creatine kinase, Creatinine, Gamma glutamyl transferase, Glucose, Glucose fasting, Haptoglobin, Lactate dehydrogenase, Magnesium, Potassium, Protein total, Sodium, triglycerides, Urea nitrogen.
- Recurrence of neuromuscular blockade or residual neuromuscular blockade was assessed clinically and by observation of the TOF trace and recorded as an S/AE.

Adverse events (AE) were defined according to ICH GCP guidelines as:
"Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product" [176]

The details of all AEs were recorded with reference to start/stop times, intensity of AE (mild, moderate, severe,) action taken on IMP, relationship to IMP, subject outcome and whether an AE led onto an SAE.
Serious adverse events were defined according to ICH GCP guidelines as follows:
"Any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires inpatient hospitalisation or prolongation of existing hospitilisation
- Results in persistent or significant disablity/incapacity
- Is a congenital anomaly/birth defect " [176]

The details of all SAEs were recorded and in addition to start/stop times, intensity of AE (mild, moderate, severe,) action taken on IMP, relationship to IMP, subject outcome, the criteria of SAE (listed above) was noted. An SAE form was completed with all the relevant details surrounding the event and sent to the Drug Safety Surveillance Department of MSD within 24 hours.

For the laboratory safety blood tests, six samples were taken at stipulated time points (see table 3.1)for the measurement of biochemical, haematological and glucose safety parameters. Approximately 5ml of blood was placed in a gold top hard plastic 'clotted' BD Vacutainer collection tube and was stood upright for 30 minutes to ensure blood clotting. The tube was then placed in a Capricorn Table-top centrifuge for 10 minutes at 1300-2000g after which the plasma was pipetted into a clear hard plastic tube. A further 3-4 ml of blood was placed in a lavender top EDTA BD Vacutainer tube and 1-2ml of blood in a grey top sodium fluoride, oxalate, EDTA BD Vacutainer tube. All three tubes were then sent within 24 hours via courier to the BARC Europe NV central laboratory in Belgium for analysis of biochemical and haematological safety parameters. The lab results were then sent to our centre by fax within 24 hours (except at weekends) for review by me.

3.10 Pharmacokinetic parameter calculations

I have collated the plasma rocuronium and plasma sugammadex concentrations and provided numerical data and graphical comparisons between the groups (table 4.13 to 4.15 and figures 4.9 to 4.15). Medians are presented as the concentrations were not expected to be normally distributed and also to guard against outliers. I have carried out statistical analyses (detailed in section 3.11) on rocuronium concentrations at the 'pre-sugammadex' baseline timepoint, when the PTC was 1-2. This sampling timepoint was chosen as it is the time immediately prior to sugammadex administration and as such intergroup comparisons of the rocuronium concentration are of interest. Data are presented up to the 24 hour post-sugammadex sampling timepoint as no further pharmacokinetic samples were taken from the control group after then.

I have carried out statistical analyses (detailed in section 3.11) on sugammadex concentrations at sampling timepoints up to 10 hours and group pharmacokinetic parameters. Comparisons between the groups are made up to the 10 hours post sugammadex sampling point as the entire control group had plasma sugammadex concentrations below the lower level of quantification (0.1mcg/ml) at 24 hours, which was also the last timepoint that sugammadex and rocuronium plasma concentrations were measured in the control group.

Group pharmacokinetic calculations detailed below, were carried out by staff working for the trial sponsors at MSD in Oss, The Netherlands. The plasma concentrations of sugammadex and rocuronium were obtained from the samples sent to the central laboratory (BARC Europe NV), the assay method is detailed in the appendix. Due to time and resource constraints the pharmacokinetecists working in Oss decided during data collection that the more sophisticated pharmacokinetic modelling methods for calculating pharmacokinetic parameters for subjects in this trial were not feasible at that time. Pharmacokinetic calculations were therefore restricted to calculation of parameters for sugammadex using simple non-compartmental analysis methods (although the possible drawbacks from the sparse sampling scheme were recognized). To enable more sophisticated modelling methods to be used, the data from this study would have to be combined with data from other studies with more intensive sampling schedules.

As rocuronium was given in a multiple dosing scheme, (i.e. intubating dose of rocuronium followed by a variable number of maintenance doses) the use of non-compartmental methods to calculate the rocuronium parameters in this study would most likely be flawed. In addition the pharmacokinetics of rocuronium had been studied before in depth both in patients with renal failure and with normal renal function [129]. It would be possible to calculate the rocuronium PK parameters with an adjustment to the dosing schedule using more advanced software and methods but this non-compartmental analysis was not carried out by the pharmacokinetecists again due to time and resource constraints.

The data for sugammadex were inputted to a computer running Statistical Analysis Software version 9.1 (SAS V9.1) which calculated the parameters described immediately below. The description of these calculations was provided by Michiel Van den Heuvel from MSD DMPK-Oss.

- The terminal elimination rate constant **(λz)** and Half time **(t½)**

  The slope (β) of the terminal log-linear phase of the concentration-versus-time curve was determined by linear regression. The data were fitted to the function

  $$\log_e C_i = \log_e C_{interc} + \beta \cdot t_i$$

  Starting with the last 3 concentration:time (C_i, t_i) data pairs for which C_i was greater than or equal to 0.1 mcg/ml i.e. the 'Lower limit of quantification' (LLOQ);
  C_{interc} is the intercept with the concentration axis at t=0. The procedure continued adding preceding data points one at a time and fitting the regression equation. The terminal log-linear portion was defined by the data yielding the smallest mean square error (MSE) term in the regression analysis. The terminal elimination rate constant (λz) was defined as -β from which the elimination half-life (t½) was calculated as $\log_e 2/\lambda z$.
  Concentrations lower than LLOQ in the elimination phase were ignored.

- AUClast

  The area under the concentration-versus-time curve (AUC) from zero to tlast (AUClast) was calculated by means of the linear trapezoidal rule,

where tlast represents the last time point with a measurable concentration above the LLOQ.

- AUC0-∞

  The AUC from zero to infinity was calculated as AUClast + AUClast–∞. For AUClast see above and AUClast–∞ = Ctlast/λz, where Ctlast is the fitted concentration at time tlast using the regression line from which λz was calculated.

- AUC%extrap

  The percentage AUC extrapolated from tlast to infinity was calculated as (AUCtlast–∞/AUC0-∞)·100%.

- Clearance (Cl) and Wn-Cl

  The total plasma clearance (Cl) was calculated as Dose/AUC0-∞. The weight-normalized clearance (wn-Cl) was calculated as Cl divided by body weight (kg).

- Mean residence time (MRT)

  The mean residence time was calculated as MRT = (AUMC/AUC0-∞), where AUMC is the area-under-the-moment-curve calculated as AUMC = AUMClast + AUMCtlast–∞. AUMClast was calculated from the product of concentration and time ($C_i \cdot t_i$) by means of the linear trapezoidal rule and AUMCtlast–∞ = (Ctlast·tlast/λz) + (Ctlast/λz^2).

- Volume of Distribution at steady state (V_{ss}) and Wn-V_{ss}

  The apparent volume of distribution at steady state was calculated as V_{ss} = CL·MRT. The weight-normalized apparent volume of distribution (Wn-V_{ss}) was calculated as V_{ss} divided by body weight (kg).

- Effective half-life (t½,eff)

  The effective half-life was calculated as MRT·$\log_e 2$.

In addition to the above PK calculations, all patients who underwent haemodialysis in the first 48 hours after sugammadex administration had blood taken for PK analysis immediately prior to (C_{pre}) and after (C_{post}) dialysis took place. At our study site only low

flux filters i.e. small pore size, were used. Other study sites used high flux, large pore size, filters. The intention was to collect more data on the in vivo dialysability of sugammadex and any difference between dialysis membranes used. To enable comparisons about the adequacy of dialysis to be made the 'dialysis half-life' and 'reduction ratio' were calculated by scientists at MSD, Oss. The calculations for reduction ratio are based on the kinetics of urea during dialysis and the dialysis half life is a general formula for half-life assuming exponential decline i.e. linear decline in log-scale. A description of the calculations provided by Michiel van den Heuvel appears in the clinical trial protocol and is provided below. If a patient underwent dialysis, all PK data after dialysis were calculated by extrapolation of the sugammadex concentration data from the pre-dialysis sample. All measured plasma concentrations after dialysis were disregarded.

- Dialysis half-life ($t_{½,dialysis}$)

  The rate constant during dialysis was initially calculated as

  $$k_{dialysis} = (\log_e Cpre - \log_e Cpost) / \text{duration of dialysis}$$

  from which the half-life during dialysis was calculated as $\log_e 2/k_{dialysis}$.

  The $t_{½,dialysis}$ calculated this way is the result of endogenous elimination in addition to dialysis. In order to calculate the $t_{½}$ dialysis without the endogenous elimination a correction is applied according to the following formula:

  $k_{dialysis,corrected} = k_{dialysis} - \lambda z$.

- Reduction ratio

  The reduction ratio was calculated as

  reduction ratio = (1-Cpre/Cpost)*100%.

3.11 Statistical Analysis

Microsoft Excel 2003 and an online statistical calculator at www.socr.ucla.edu were used for all mathematical and statistical calculations performed by me. Where the calculations were undertaken by representatives of MSD, this is described in the text.

Sample size considerations

To evaluate the sample size for the trial, data were collected by representatives of MSD, Oss, The Netherlands from four clinical trials, in which a dose of sugammadex 4.0 mg/kg was administered at a PTC of 1-2. From this data, a standard deviation of 1.0 min was calculated. The sample size was then calculated using a standard deviation of 1.2 min in order to safeguard against outlying results. This resulted in a projection of 32 subjects per group (Renal and Control) to be able to show equivalence at a power of 80%. Taking into account that about 5% - 7% of the subjects might drop out, the sample size was calculated to be 35 subjects per subject group, ie, 70 subjects in total.

The data I am presenting has been collected from our study centre (one of the eight study centres) therefore any inference from the data is significantly limited in its relevance to the wider population.

Patient characteristics

The mean, median, standard deviation and range were calculated for continuous data relating to the AST group. For categorical variables, frequency counts and percentages were calculated.

Efficacy

To compare efficacy between the groups, times from start of administration for sugammadex to TOF ratio≥ 0.7, TOF ratio≥ 0.8 and TOF ratio≥ 0.9 were analysed using Wilcoxon rank sum test for two independent samples as the data was continuous and expected to be not normally distributed. This was post hoc, independent sub group analysis carried out using an online statistical calculator at www.socr.ucla.edu.

The planned statistical analysis carried out by representatives of MSD used the confidence interval approach to investigate equivalence. Equivalence was to be claimed if the two sided 95% confidence interval for the difference in recovery times (TOF≥ 0.9) between the renal and control groups lay in the interval between -1 and +1 minutes. This is the same method used by Staals et al. when comparing the efficacy of sugammadex 2mg/kg at reappearance of T2 in renal patients and controls [54] .

The calculations were carried out by Marion Kaspers, a statistician at MSD, Oss, using the methods of Hodges-Lehman and Moses [180] on a computer running SAS V9.1. This is a non-parametric method which compares the medians between the two groups. It is used because the recovery times are expected to be not normally distributed. The Hodges-Lehmann and Moses method consists of 3 steps:

- All possible differences are formed between the groups.
- The Hodges-Lehmann estimate is the median of all these differences.
- The method of Moses is then used to create a distribution-free confidence interval.

Safety

The analysis of the safety data is limited by the small sample size both in my study centre and in the trial as a whole. The study was designed to collect more safety data to be added to the safety profile and as such no claims will be made regarding safety of the investigation medicinal product.

Descriptive data for systolic blood pressure, diastolic blood pressure and heart rate have been presented and mean values, +/- one standard deviation, have been plotted for inter group comparison. Post hoc, independent sub group statistical analyses of differences between the groups for all the vital sign parameters at all of the assessment timepoints were carried out using Student pooled t test as the data is continuous and expected to be normally distributed.

Pharmacokinetics

For inter group comparisons of plasma rocuronium concentrations, the median, mean, 95% confidence interval, standard deviation and ranges for the 2 minutes post rocuronium to the 600 minutes post sugammadex timepoints were calculated using Microsoft Excel 2003. Median rocuronium concentrations were also plotted against timepoint of assessment.

For inter group comparisons of plasma sugammadex concentrations, the median, mean, 95% confidence interval, standard deviation and range for the 5 minute to 600 minute

timepoints were calculated using Microsoft Excel 2003. Median sugammadex concentrations (+/- one standard deviation) were plotted against mean sampling time from 5 minutes to 600 minutes post sugammadex administration. Median was chosen as the concentrations were not expected to be normally distributed and also to guard against outliers.

In addition, statistical analyses of the plasma rocuronium concentrations at the pre-sugammadex sampling timepoint and plasma sugammadex concentrations at the 5 minute, 20 minute, 5 hours and 10 hours sampling timepoints were carried out between the groups using Wilcoxon rank sum test for 2 independent variables as the data was continuous and expected to be not normally distributed. This was post hoc sub group analysis carried out using an online statistical calculator at www.socr.ucla.edu. The comparisons of rocuronium concentrations were only carried out at the 'pre-sugammadex' baseline sampling timepoint, when the PTC=1-2, as this was a key comparative timepoint in the study i.e. the time when sugammadex was to be administered.

PK parameters (AUC 0-∞, $t_{1/2}$ eff, V_{ss}, MRT, Cl) were calculated via the methods described in Section 3.10 by the staff of MSD at Oss, The Netherlands as described above. The data from the ASPE group were inputted into Microsoft Excel 2003 and geometric means were calculated (as PK concentration data tends to zero.) The geometric coefficient of variation was also calculated as ($\sqrt{(\exp(SD_{log}^2 - 1))}*100$), where SD_{log} is the standard deviation of the $\log_e$ transformed PK parameter.

To compare PK parameters (AUC 0-∞, $t_{1/2}$ eff , V_{ss}, MRT, Cl) between renal and control groups, Student t test was calculated using $\log_e$ transformed values, as described by Bland and Altman [181]. This method was chosen as PK parameters are in general assumed to be log-normally distributed.

Other calculations

Using the AST group, 2 tailed Student's t-test was used to compare the duration of anaesthesia between renal and control subjects.

To investigate any relationships between rocuronium concentrations at the pre-sugammadex sampling timepoint, body mass index, age or duration of anaesthesia and the time to recovery of the TOF ratio ≥0.9 the data from the ASPE group were plotted as scatter charts, linear regression was performed and the R^2 value presented.

Section 4: Results

Our hospital was one of eight study centres in four countries involved in this clinical trial: Austria (2 sites), France (1 site), The Netherlands (3 sites) and The UK (2 sites).

The complete data from the study will be published in 2011 and until then, the data from the entire clinical trial remains confidential. I have permission from the trial sponsors to publish the data from my trial site as this thesis will remain closed access for 18 months. I have permission to refer to general trends in data between the entire study population and my study centre where the trend is relevant.

4.1 Disposition of subjects

In our trial centre 19 patients had signed a form for informed consent and were enrolled in the clinical trial. The disposition of these patients is described in Table 4.1and figure 4.1 below**.** A description of allocation into subject data sets appears in Section 3.5. Figure 4.1 also gives the reasons for patient withdrawal.

Table 4.1 Disposition of subjects

	Control	Renal	Total
All-Subjects-Enrolled group (ASE)	9	10	19
All-Subjects-Treated group (AST)	8	8	16
Intent-to-Treat group (ITT)	8	8	16
Per Protocol group (PP)	5	7	12
All-Subjects-Pharmacokinetically-Evaluable group (ASPE)	6	8	14

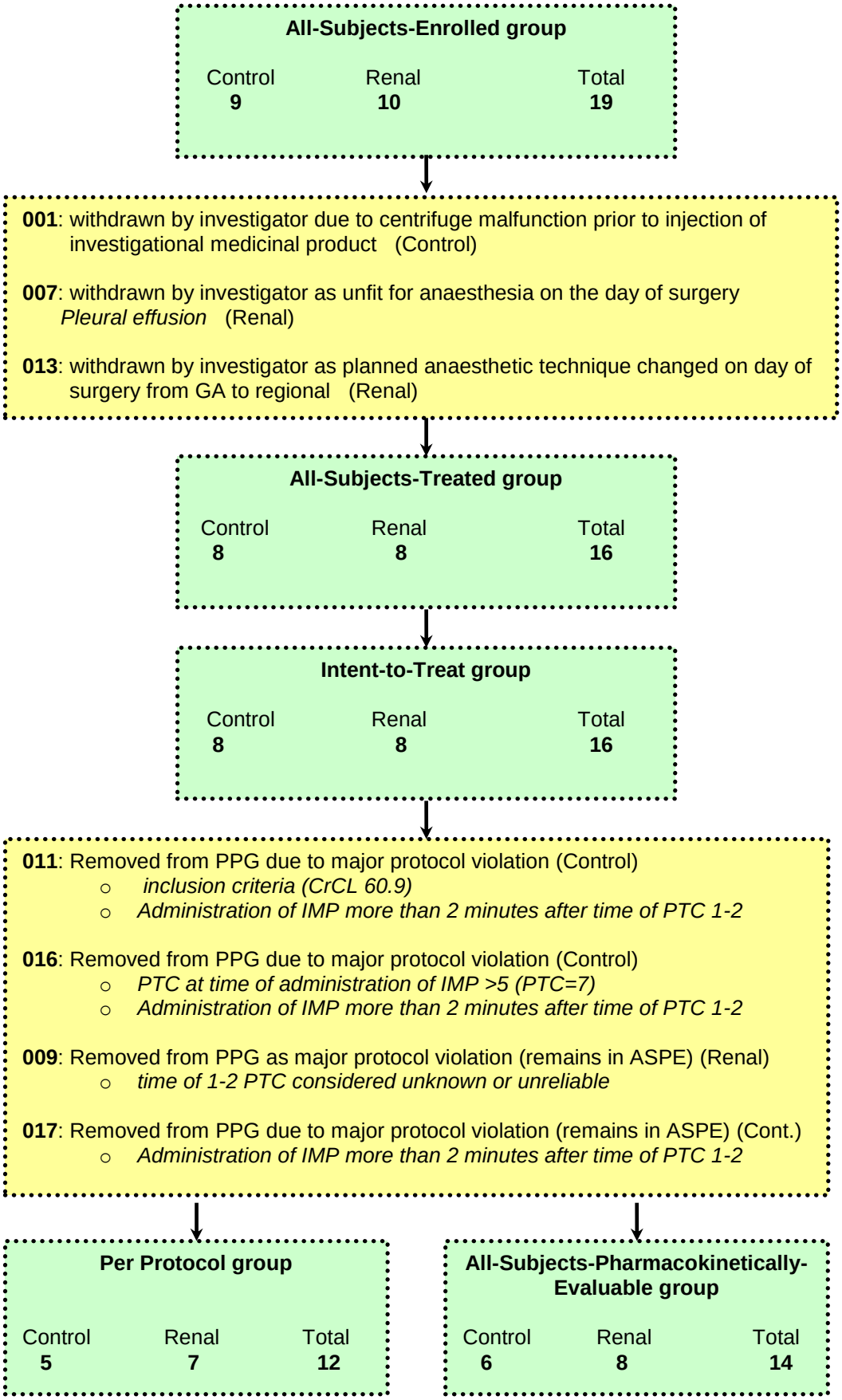


Figure 4.1: Disposition of subjects

4.2 Patient characteristics

Table 4.2 below provides information on the patient characteristics of the patients in the AST group. Keeping in mind that this is a presentation of data from one study centre I must state that any comments on this data are not necessarily generalisable to the entire study data.

The renal group has a higher mean age and a lower mean height and weight than the control group. The renal group were all in ASA class III whereas the control group were from ASA class I-II, that is to say that the renal group are all classified as having severe systemic disease whereas the control group is not. There were a higher proportion of female subjects in the renal group. There did not appear to be any noticeable differences in race or ethnicity between groups. As to be expected, the mean creatinine clearance was much lower in the renal group.

Due to an error relating to the inclusion criteria, subject 011 was included in the study despite having a creatinine clearance of 60.9 ml/min which explains why the range of the creatinine clearance in the control group is 61ml/min rather than ≥80ml/min as required in the inclusion criteria. Subject 011 was excluded from all efficacy and pharmacokinetic evaluation in this thesis.

Table 4.2: Patient characteristic data for All Subjects Treated group

		Subject Group		Combined (N=16)
		Control (N=8)	Renal (N=8)	
Age (years)	n	8	8	16
	Mean (SD)	37(16)	52(14)	45(17)
	Median	38	54	45
	Range	18-64	27-73	18-73
Weight (kg)	N	8	8	16
	Mean (SD)	76(12)	61(20)	68(18)
	Median	81	58	68
	Range	54-89	41-98	41-98
Height (cm)	n	8	8	16
	Mean (SD)	174(8)	160(10)	167(11)
	Median	175	160	169
	Range	160-185	145-175	145-185
Gender (n (%))	Female	4(50)	6 (75)	10(63)
	Male	4(50)	2(25)	6(38)
Race (n(%))	Black /Afro-Caribbean	0(0)	0(0)	0(0)
	White	8(100)	7(88)	15(94)
	Other	0(0)	1(13)	1(6)
Ethnicity (n(%))	Hispanic or Latino	0(0)	0(0)	0(0)
	Non Hispanic or Latino	8(100)	8(100)	16(100)
ASA class (n (%))	1	6(75)	0(0)	6(38)
	2	2(25)	0(0)	2(13)
	3	0(0)	8(100)	8(50)
Creatinine clearance (ml/min)	N	8	8	16
	Mean (SD)	120(31)	14(6)	67(59)
	Median	123	14	43
	Range	61-167	6-24	6-167

4.2.1 Duration of anaesthesia (AST group)

In our study centre there was a statistically significant difference in the duration of anaesthesia between the groups with the control group tending to have longer surgery (p=0.02).

Table 4.3: Duration of anaesthesia for All Subjects Treated Group

		Subject Group		Combined (N=16)
		Control (N=8)	Renal (N=8)	
Duration of anaesthesia (min)	N	8	8	16
	Mean (SD)	193* (51)	127 (44)	160 (57)
	Median	173	108	165
	Range	139-293	76-192	76-293
		*Statistically significant Student pooled t-test p=0.02		

As can be seen in table 4.4 below, the dose of sugammadex given was uniform at 4.0mk/kg. In addition, almost all subjects received sugammadex at a PTC of 1-2. Subject 017 received sugammadex at PTC=7 due to a TOF-Watch malfunction.

Table 4.4: Individual durations of anaesthesia, sugammadex dose and number of PTC at time of administration of sugammadex for the All Subjects Treated group

Subject	Group	Duration of anaesthesia (*min*)	Dose of Sug. (*mg*)	Weight (*kg*)	Dose of sug. per kilo (*mg/kg*)	PTC at time of admin of sugammadex
002	Control	208	216	54	4.0	2
005	Control	293	356	89	4.0	1
006	Control	153	252	63	4.0	1
010	Control	181	340	85	4.0	1
011	Control	139	273	68.3	4.0	1
016	Control	238	328	82	4.0	7
017	Control	164	320	80	4.0	2
018	Control	165	332	83	4.0	2
003	Renal	192	173	43.2	4.0	1
004	Renal	98	163	40.7	4.0	1
008	Renal	171	256	64	4.0	1
009	Renal	118	188	47	4.0	*2*
012	Renal	76	308	77	4.0	1
014	Renal	94	207	51.7	4.0	1
015	Renal	172	272	68	4.0	1
019	Renal	96	394	98.4	4.0	1

4.2.2 Pre-existing medical conditions

By definition, all patients in the renal group had a significant pre-existing medical condition. Table 4.5 below gives group by group comparison of pre-existing medical conditions. Further details on pre-existing medical conditions, indication for surgery and surgery performed appear in the appendix table A1 and A2.

Table 4.5: The numbers of patients with clinically significant pre-existing medical conditions across subject group for the AST group.

	Subject Group		Combined (N=16)
	Control (N=8)	Renal (N=8)	
Pre-existing medical conditions present	5	8	13
Pre-existing medical conditions NOT present	3	0	3

4.3 Concomitant medication (AST group)

- All subjects treated received propofol and remifentanil infusions for induction and maintenance of anaesthesia.
- Analgesia was given as IV paracetamol, morphine, fentanyl and paracoxib (injectable non-steroidal anti inflammatory) at the discretion of the anaesthetic team. Local anaesthesia was given by the surgeon to the wound site if indicated.
- Antibiotics were given at the request of the surgeon.
- No subject received any medication throughout the study period which was thought to interact with the study medication i.e flucloxacillin, toremifene, fusidic acid.

4.4 Treatment compliance (AST group)

- All subjects received an intubation dose of rocuronium of 0.6mg/kg.
- All subjects received a dose of sugammadex of 4.0mk/kg for reversal

4.5 Major protocol violations

The ITT group consisted of 16 patients, 8 in each group. 4 patients were subject to major protocol violations:

- Subject 009: The time of 1-2 PTC occurrence and number of PTC were considered unknown or unreliable by the Central Independent Adjudication Committee (CIAC) due to interference in the TOF trace, probably caused by the use of surgical equipment. Therefore the recovery times were considered unreliable.
- Subject 011: The subject had a creatinine clearance outside of the pre-defined range (60.9ml/min) and administration of sugammadex was more than 2 minutes after the time of PTC=1-2.
- Subject 016: Sugammadex was given at a PTC of 7 and administration of sugammadex was more than 2 minutes after the time of PTC=1-2.
- Subject 017: Sugammadex was given more than 2 mins after PTC=1-2

All the subjects above were excluded from efficacy analysis. Subject 009 and 017 were excluded from efficacy analysis but were included in the pharmacokinetic analysis as the deviations from the protocol were not thought to have affected the pharmacokinetic behaviour of sugammadex.

4.6 Analysis of efficacy

All patients in the Per Protocol group (PP) were included in the efficacy evaluation, renal n=7, control n=5. Peripheral skin temperature remained above 32°C throughout evaluation.

Time in seconds to TOF ratio≥ 0.7, TOF ratio≥ 0.8 and TOF ratio≥ 0.9 for individual subjects in the PP group are presented in Table 4.6. Post-hoc analysis using Wilcoxon rank sum test for two independent variables demonstrated statistically significant differences between the control and renal groups for time of start of sugammadex administration to time of TOF ratio≥ 0.7 (p=0.009), TOF ratio≥ 0.8 (p=0.007) and TOF ratio≥ 0.9 (p=0.004).

The originally planned analysis of efficacy used the confidence interval approach of Hodges, Lehman and Moses. The geometric mean time from start of administration of sugammadex to recovery of the TOF ratio≥ 0.9 was 50 sec (SD 20.4, 95% CI: 30-83) for the control group and 176 sec (SD 95.6, 95% confidence interval (CI):112-278) for the renal group. Using the methods of Hodges, Lehman and Moses, the statistical evaluation of the time from start of administration of sugammadex to recovery of the TOF ratio ≥ 0.9 showed the estimated treatment difference in median recovery time to be 126 sec with corresponding 95% CI ranging from 51-248 sec. This figure is not within the pre-defined range of -60 sec to +60 sec and as such no equivalence in efficacy between the groups can be claimed.

The results of the Hodges, Lehman and Moses statistical analysis from my study centre were similar to those from the entire (all sites) study population in that the estimated treatment difference in median recovery time was not within -60 sec to +60 sec and equivalence in efficacy could not be claimed. I did not have access to the efficacy data for the entire study group hence Wilcoxon rank sum analyses were not carried out on the entire study population.

Table 4.7 presents a summary of the time from start of administration of sugammadex to recovery of the TOF ratio ≥ 0.7, ≥ 0.8 and ≥ 0.9 in the PP group for control and renal groups.

Table 4.6: Presentation of individual efficacy data

Subject No.	Group	Time (s) to TOF ratio≥ 0.7	Time (s) to TOF ratio≥ 0.8	Time (s) to TOF ratio≥ 0.9
002	Control	60	60	60
005	Control	67	82	82
006	Control	53	53	53
010	Control	43	43	43
018	Control	27	27	27
003	Renal	270	330	360
004	Renal	200	230	275
008	Renal	60	75	90
012	Renal	103	118	133
014	Renal	102	117	117
015	Renal	80	110	185
019	Renal	163	178	208
Wilcoxon rank sum test for difference between control and renal groups		**p=0.009**	**p=0.007**	**p=0.004**

Table 4.7: Presentation of group efficacy data

		Subject Group	
		Control	Renal
Time in seconds from start of administration of sugammadex to recovery of TOF ratio to 0.7	N	5	7
	Geometric mean	48	124
	95% CI for geometric mean	31-74	76-202
	Median	53	103
	95% CI for median	27-67	60-270
	Minimum – maximum	27-67	60-270
	Estimated median of difference (95% CI) in sec	60 (20,173)	
Time in seconds from start of administration of sugammadex to recovery of TOF ratio to 0.8	N	5	7
	Geometric mean	50	148
	95% CI for geometric mean	30-83	93-236
	Median	53	118
	95% CI for median	27-82	75-330
	Minimum – maximum	27-82	75-330
	Estimated median of difference (95% CI) in sec	83 (35, 203)	
Time in seconds from start of administration of sugammadex to recovery of TOF ratio to 0.9	N	5	7
	Geometric mean	48	**176**
	95% CI for geometric mean	31-74	112-278
	Median	53	185
	95% CI for median	27-67	90-360
	Minimum – maximum	27-67	90-360
	Estimated median of difference (95% CI) in sec	**126** (51, 248)	

4.7 Analysis of safety data

Subjects in the 'All Subjects Treated' group were included in the safety evaluations, control n=8, renal n=8.

4.7.1 Recurrence of neuromuscular blockade

There were no instances recorded of recurrence of neuromuscular blockade or residual neuromuscular blockade in either study group up to and including the 28 day final follow up visit.

4.7.2 Adverse Events (AE)

Table 4.8 below summarises AEs by study group, along with some detail as to the type of AE experienced by the participating patients (AST group). There was no discernable trend or difference between the renal group and the controls with regards to AEs. The AEs reported were all deemed to be unrelated to the investigational medicinal product, this was evaluated by the investigator team. None of the AEs were of a severe intensity.

4.7.3 Serious Adverse Events (SAE)

2 patients in the renal group experienced SAEs. Neither incident was deemed to be related to the investigational medicinal product.

Subject 003 experienced potentially life-threatening narcotic intoxication secondary to treatment with methadone and palladone approximately 3 days after treatment with sugammadex. The patient received treatment with naloxone and intravenous fluids and recovered from this event with no sequelae.

Subject 009 was readmitted to hospital 4 days after treatment with sugammadex with symptoms of worsening renal failure i.e. vomiting, confusion, and was found to have a raised blood urea and creatinine. This was thought to be related to cessation of

peritoneal dialysis following surgery on an inguinal hernia. The patient received haemodialysis and recovered from this event with no sequelae.

Table 4.8: Summary of adverse event data All Subjects Treated group

	Subject Group		
	control n=8	renal n=8	Total
Subjects with at least one AE	7	6	13
Deaths during the trial period	0	0	0
Subjects with at least one SAE	0	2	2
Subjects discontinued due to an AE	0	0	0
Subject with AEs related to sugammadex in the opinion of the investigator	0	0	0
Subjects with AEs of known severe intensity	0	0	0
Details of all AEs recorded	control n=8	renal n=8	Total
Procedural pain	7	6	13
Swelling to face		1	1
Fall		1	1
Arthralgia	1		1
Lower respiratory tract infection		1	1
Upper respiratory tract infection	1		1
Narcotic intoxication		1	1
Increased neutrophil count	1		1
Pruitis		1	1
Nausea	1	2	3
Vomiting		1	1
Haematemesis		1	1
Anaemia		1	1
Uraemia		1	1
Raised creatinine		1	1
Confusion		1	1
Constipation		1	1
Phlebitis	1		1
Total number of AEs recorded	12	20	32

4.7.4 Vital signs

As a measure of safety, systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were recorded at predefined intervals throughout the study. The absolute values in addition to any increases or decreases from the baseline were recorded with reference to predetermined criteria for markedly abnormal values. Tables 4.10, 4.11 and 4.12 below show per subject data for SBP, DBP and HR with reference to abnormal values, changes from baseline and related adverse events.

Subject 004 and 012, both in the renal group, had low SBP and DBP during anaesthesia. Subject 012 required vasopressors to maintain blood pressure and this was recorded as an adverse event. It was likely that the hypotension was related to the anaesthetic agents given (propofol and remifentanil.) The AE was not thought to be related to sugammadex as it was present before sugammadex administration. Subject 014 (renal group) had high SBP and DBP after sugammadex was given, however, this also corresponded to extubation and stopping the anaesthetic drugs and for theses reasons the rise in blood pressure was not thought to be related to sugammadex, although this cannot be ruled out.

Per group mean SBP, DBP and HR were calculated and are presented in Table 4.9 and figures 4.2 to 4.8 below. Statistical analyses of differences between the groups for all the vital sign parameters at all of the assessment timepoints were carried out using Student pooled t test. At 5 and 10 minutes post administration of sugammadex, the control group had statistically significant higher mean SBP when compared to the renal group; control 5 minute mean (95% CI) SBP = 134 (115-154), renal 5 minute mean (95% CI) SBP = 105 (87-124) p=0.049, control 10 minute mean (95% CI) SBP = 137 (125-150), renal 10 minute mean (95% CI) SBP = 107 (90-124) p=0.015. The mean DBP at the 28 day assessment was lower in the control group; control 28 day mean (95% CI) DBP= 74 (71-77), renal 28 day mean(95% CI) = 83 (75-91), p=0.046. It is noted that there is overlap between the groups in the 95% confidence intervals with regards the 5 minute mean SBP and the 28 day DBP which may reduce the significance of any difference found between the groups at these timepoints.

There were no other statistically significant differences in mean SBP, DBP and HR between the groups.

The fluctuations in SBP, DBP and HR throughout the study period were consistent with those changes that would be expected to occur during anaesthesia and surgery. However, it was noticeable that the variability in all parameters was greater in the renal group.

Table 4.9: Intergroup comparisons of vital signs at all assessment timepoints

Time Of Assessment	Mean SBP Control n=8	Mean SBP Renal n=8	t-test p value	Mean DBP Control n=8	Mean DBP Renal n=8	t-test p value	Mean HR Control n=8	Mean HR Renal n=8	t-test p value
Screening	124.5 (11.0)	129.6 (20.5)	0.645	77.0 (14.8)	83.1 (14.2)	0.414	73.9 (14.2)	85.9 (13.0)	0.100
Pre NMBA	108.8 (18.4)	97.9 (17.2)	0.241	51.1 (6.6)	52.5 (13.9)	0.801	60.8 (10.6)	64.1 (11.1)	0.553
Baseline	128.1 (22.3)	110.0 (26.5)	0.162	63.4 (7.0)	59.1 (18.1)	0.641	65.9 (12.0)	62.3 (10.7)	0.537
2 mins post sugammadex	126.8 (21.1)	107.5 (27.2)	0.135	65.5 (9.4)	58.1 (17.0)	0.301	61.1 (7.2)	58.4 (9.7)	0.537
5 mins post sugammadex	**134.4*** (27.8)	105.3 (26.4)	**0.049***	66.0 (10.6)	56.0 (19.8)	0.329	60.3 (6.4)	58.1 (9.6)	0.598
10 mins post sugammadex	**137.3*** (17.9)	107.3 (24.9)	**0.015***	71.0 (15.2)	59.9 (17.0)	0.190	68.3 (10.3)	65.5 (16.7)	0.693
30 mins post sugammadex	135.8 (22.1)	134.3 (27.9)	0.917	84.4 (8.6)	81.3 (16.8)	0.749	76.9 (10.7)	74.1 (12.5)	0.648
Day 1 follow up	122.0 (16.4)	124.9 (33.0)	0.827	67.0 (9.0)	73.0 (20.0)	0.452	77.6 (10.3)	85.4 (13.4)	0.213
Day 7 follow up	123.1 (13.4)	131.3 (18.6)	0.329	75.0 (3.7)	81.4 (11.4)	0.153	82.5 (8.9)	80.9 (11.7)	0.773
Day 28 follow up	126.4 (11.4)	131.1 (19.9)	0.671	**73.9*** (4.2)	83.1 (11.1)	**0.046***	78.6 (10.6)	87.1 (16.7)	0.244

Mean SBP = mean systolic blood pressure in mmHg (SD)
Mean DBP= mean diastolic blood pressure in mmHg (SD)
Mean HR= mean heart rate in beats per minute (SD)
p value derived using Student pooled t-test: ***** denotes statistical significance

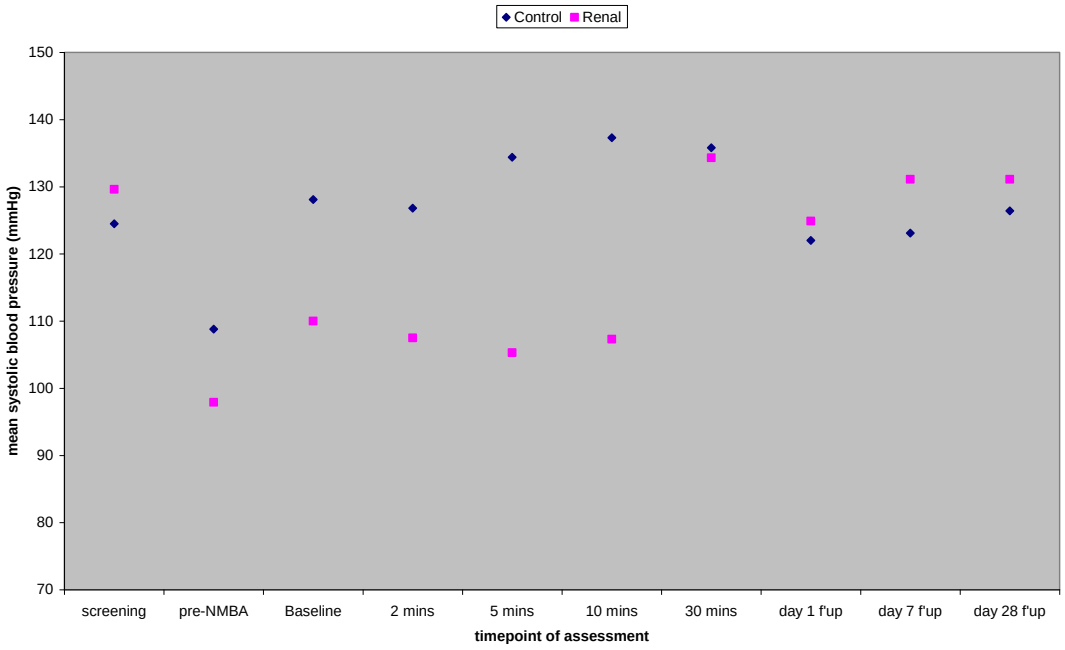


Figure 4.2 Mean systolic blood pressure for renal and control groups

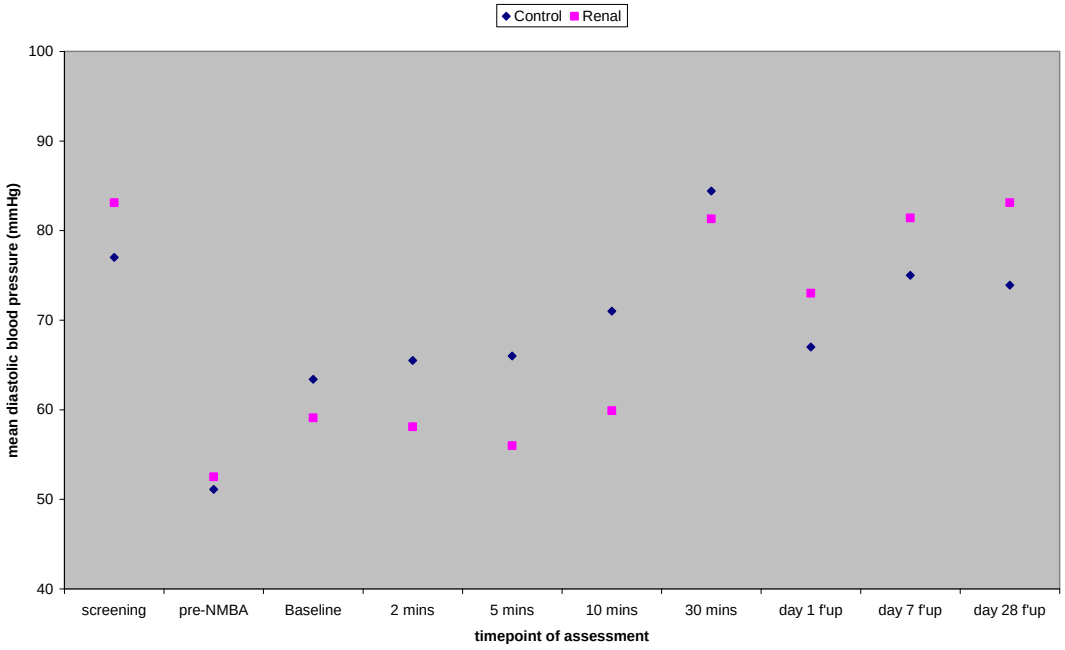


Figure 4.3 Mean diastolic blood pressure for renal and control group

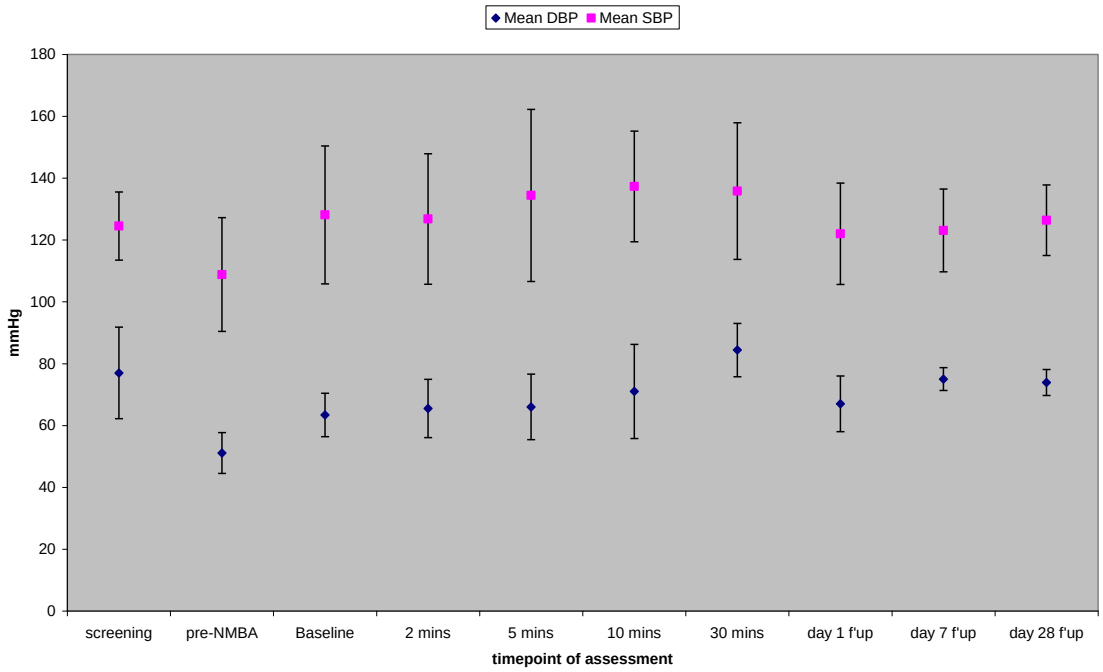


Figure 4.4: Mean SBP and DBP for control group (±1 SD)

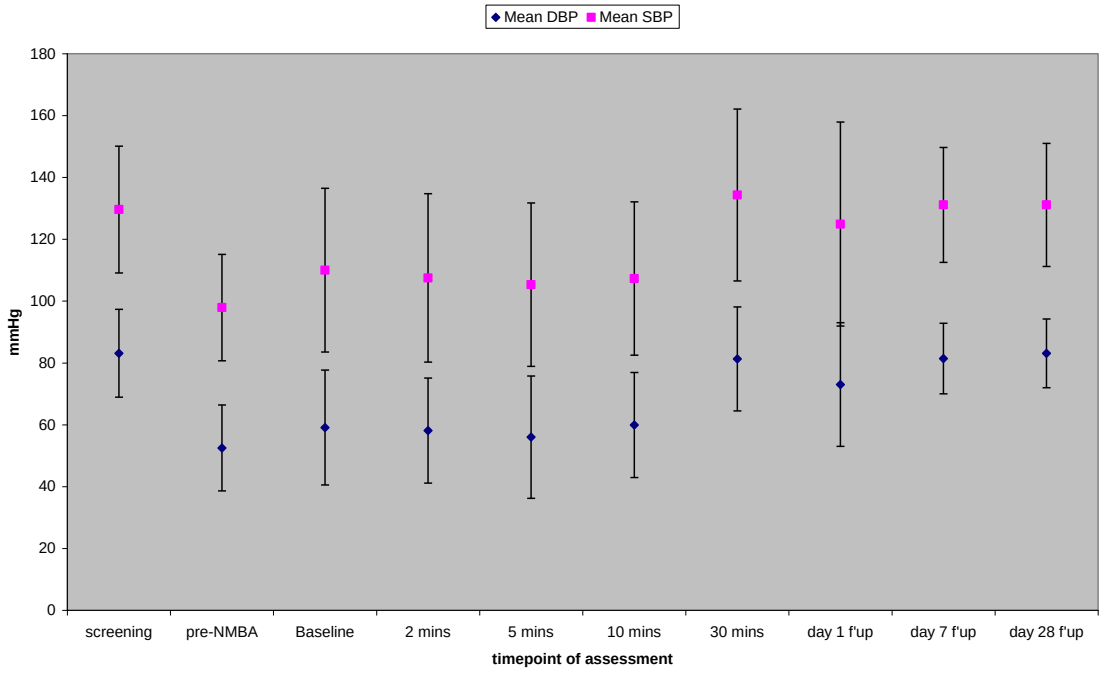


Figure 4.5: Mean SBP and DBP for renal group (±1 SD)

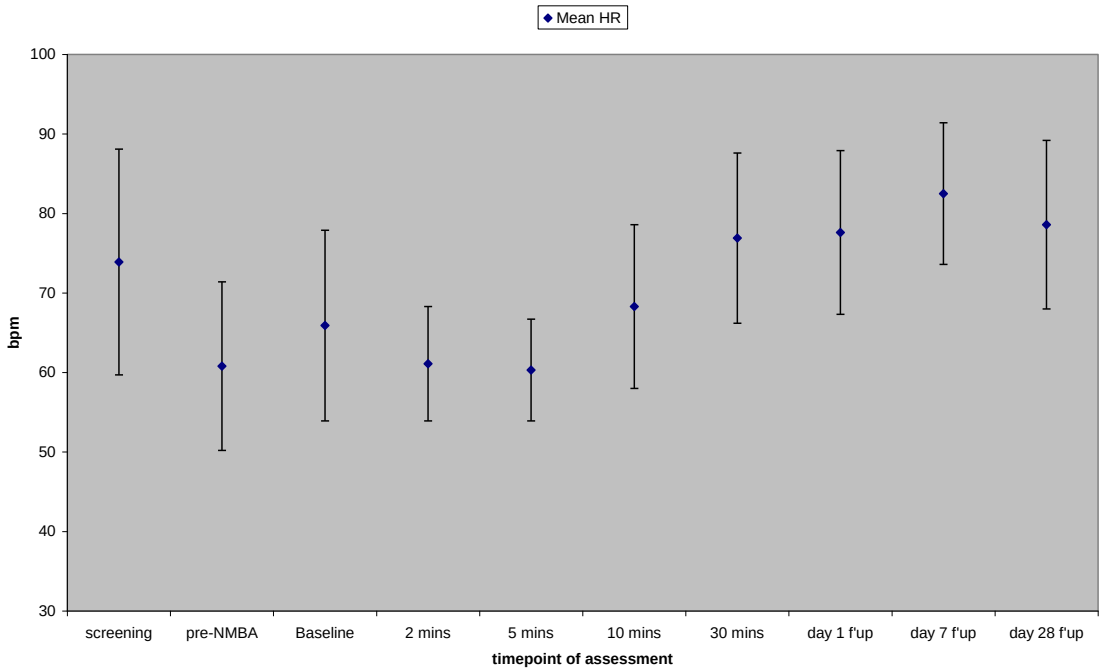


Figure 4.6: Mean HR for control group (±1 SD)

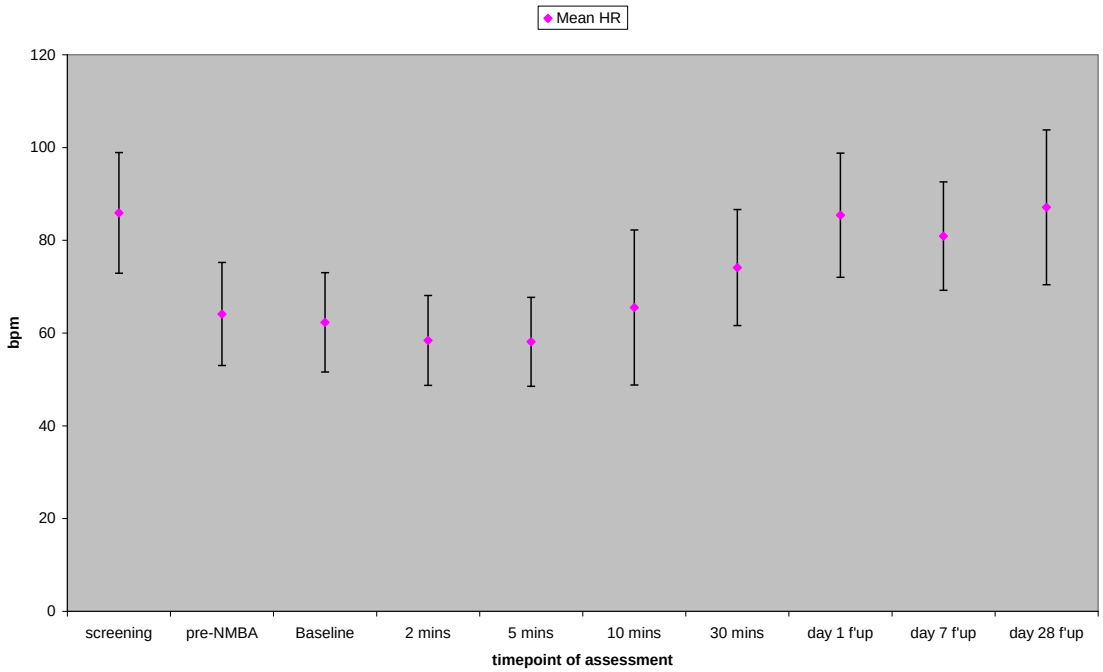


Figure 4.7: Mean HR for renal group (±1 SD)

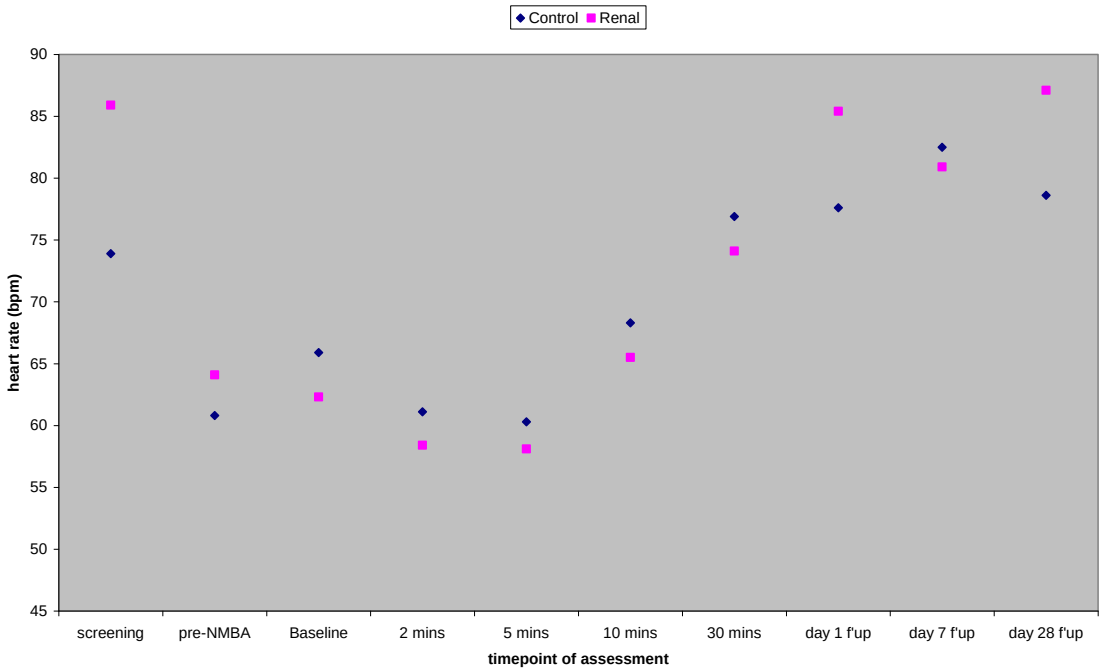


Figure 4.8: Mean HR for control and renal group

Systolic Blood Pressure data summary																
Measurement Interval	002	003	004	005	006	008	009	010	011	012	014	015	016	017	018	019
Screening																
Pre NMBA		**Y**	**Y**		**Y**					**(Y)**						
Baseline			**Y**	**X**						**(Y)**	**X**					
IMP + 2min			**Y**							**Y**	**X**					**Y**
IMP + 5 min			**Y**	**>**							**X**	**Y**	**X**			
IMP + 10 min			**Y**		**>**				**>**		**X**	**Y <**	**X >**			
IMP + 30 min	**>**		**>**	**<**			**>**		**>**	**>**	**X >**	**>**	**X >**		**>**	**>**
1 Day Follow up																
7 Day Follow up																
28 Day Follow up																

Table 4.10~~8~~: Systolic blood pressure data summary
Subjects in the shaded cells are in the renal~~SRI~~ group

Criteria for markedly abnormal values			
Variable	Unit	Abnormal range for Criterion Value	Change from baseline
Heart Rate	Bpm	>=120 <=50	Increase of >=15 Decrease of >=15
Systolic BP	mmHg	>=160 <=90	Increase of >=20 Decrease of >=20
Diastolic BP	mmHg	.>=95 <=45	Increase of >=15 Decrease of >=15

KEY
X : above normal range
Y : below normal range
> : increase from baseline
< : decrease from baseline
(?) : Clinically significant measurement i.e. Treatment given and AE reported

Diastolic Blood Pressure data summary																
Measurement Interval	002	003	004	005	006	008	009	010	011	012	014	015	016	017	018	019
Screening																
Pre NMBA		**Y**	**Y**		**Y**					**(Y)**						
Baseline			**Y**	**X**						**(Y)**	**X**					
IMP + 2min			**Y**							**Y**	**X**					**Y**
IMP + 5 min			**Y**	**>**							**X**	**Y**	**X**			
IMP + 10 min			**Y**		**>**				**>**		**X**	**Y <**	**X >**			
IMP + 30 min	**>**		**>**	**<**			**>**		**>**	**>**	**X >**	**>**	**X >**		**>**	**>**
1 Day Follow up																
7 Day Follow up																
28 Day Follow up																

Table 4.11~~9~~: Diastolic blood pressure data summary

Criteria for markedly abnormal values			
Variable	Unit	Abnormal range for Criterion Value	Change from baseline
Heart Rate	Bpm	>=120 <=50	Increase of >=15 Decrease of >=15
Systolic BP	mmHg	>=160 <=90	Increase of >=20 Decrease of >=20
Diastolic BP	mmHg	.>=95 <=45	Increase of >=15 Decrease of >=15

KEY

X : above normal range
Y : below normal range
> : increase from baseline
< : decrease from baseline
(?) : Clinically significant measurement i.e. Treatment given and AE reported

Heart Rate data summary																
Measurement Interval	002	003	004	005	006	008	009	010	011	012	014	015	016	017	018	019
Screening																
Pre NMBA														**Y**		
Baseline							**Y**									
IMP + 2min							**Y**				**Y**		**<**			**Y**
IMP + 5 min	**<**						**Y**				**Y**	**<**	**<**			**Y**
IMP + 10 min			**>**			**>**	**Y**		**>**		**Y**		**<**			
IMP + 30 min			**>**	**>**	**>**		**>**		**>**				**<**		**>**	
Post Anaesthetic visit																
7 Day Follow up																
28 Day Follow up																

Table 4.12~~0~~: Heart rate data summary

Subjects in the shaded cells are in the renal ~~SRI~~ group

KEY
X : above normal range
Y : below normal range
> : increase from baseline
< : decrease from baseline
(?) : Clinically significant measurement
i.e. Treatment given and AE reported

4.7.5 Laboratory parameters

A great deal of data was generated for each subject at each of the six time points blood was taken for safety assessment and for each laboratory test undertaken (see section 3.9 safety assessments.) Calculations based on shift from baseline and relationships to markedly abnormal values were carried out. This data was to be added to the central safety profile for sugammadex and is not reported here.

Relating to this study, the most relevant data to report was any changes in laboratory parameters that were recorded as AEs or SAEs i.e. any treatment given or action taken and any relationship to sugammadex.

Every safety blood result at each time point of measurement, for each patient was faxed to our study centre and reviewed by me. There was one AE that led to an SAE occurring in subject 009 and described in Section 4.7.2 which related to a blood result (raised serum urea and creatinine. There were no other blood results which led to AEs or SAEs.

4.8 Analysis of Pharmacokinetics

Subjects in the 'All Subjects Pharmacokinetically Evaluable' (ASPE) group were included in the PK analysis: control n=6, renal n=8.

4.8.1 Rocuronium concentration:time data

As discussed in section 2.1 there is no assay method to discriminate between complexed and non-complexed rocuronium and therefore all data relates to total rocuronium concentration in plasma in ng/ml.

Table 4.13 and figure 4.9 show the rocuronium concentration: time data for the ASPE group.

At the pre-sugammadex baseline sampling timepoint, when the PTC=1-2, there was a statistically significant different plasma concentration between the groups (Wilcoxon rank sum test for 2 independent variables p=0.05). However, there was overlap of the 95% confidence intervals at this timepoint which casts doubt as to the clinical significance of the result. In addition, the median rocuronium concentrations for the entire study group at the pre-sugammadex sampling time point are very similar; 2560 ng/ml for the control group(n=27) and 2660 ng/ml for SRI group (n=33). I therefore conclude that the difference between the groups at this sampling timepoint is likely to be an anomaly. Figure 4.9 shows a continued divergence in the rocuronium concentration profile of the control and renal groups from the pre sugammadex up to the 24 hour post sugammadex sampling timepoint. No further pharmacokinetic assessments were made on the control group after that time.

To investigate any potential link between rocuronium concentration at the pre-sugammadex sampling timepoint and time to TOF ratio ≥0.9, the data from the PP group were plotted as scatter charts and linear regression carried out using Microsoft Excel 2003. There were no strong associations found with an R^2 value calculated as 0.1058 and therefore a link between the parameters cannot be claimed.

Table 4.13: Rocuronium concentration:time data for ASPE group

Control group n=6					
sampling time (mins)	median	arithmetic mean	95% CI	SD	range
2 mins post intubation dose of rocuronium	6730	6573	5876-7270	871.4	5340-7420
15 mins post intubation dose of rocuronium	2165	2060	1666-2454	492.7	1110-2530
Pre sugammadex baseline	**2185**	**2270**	**1801-2739**	**585.6**	**1480-3180**
Sugammadex +5 mins	2280	2328	1833-2824	618.8	1520-3250
Sugammadex +20 mins	1905	2078	1520-2637	698.2	1300-3060
Sugammadex +5 hours	238	263	172-354	113.2	144-462
Sugammadex +10 hours	41	44	30-58	17.4	25-69
Sugammadex +24 hours	11	11	7-15	4.7	9-16
Renal group n=8					
sampling time (mins)	median	arithmetic mean	95% CI	SD	range
2 mins post intubation dose of rocuronium	5545	5286	4452-6120	1203.3	3920-7240
15 mins post intubation dose of rocuronium	2455	2297	1739-2855	805.5	938-3290
Pre sugammadex baseline	**2900**	**2894**	**2565-3221**	**473.4**	**2220-3540**
Sugammadex +5 mins	3570	3638	3224-4051	596.6	2790-4720
Sugammadex +20 mins	3365	3370	3151-3589	316.4	2810-3780
Sugammadex +5 hours	2105	2091	1701-2482	563.5	1000-2770
Sugammadex +10 hours	1350	1424	1032-1817	566.3	534-2250
Sugammadex +24 hours	252	348	131-566	293.7	107-921

All concentrations given as **ng**/ml

(in bold) analysis of rocuronium concentrations at Pre-Sugammadex baseline sampling timepoint using Wilcoxon rank sum test for 2 independent variables **p=0.05**

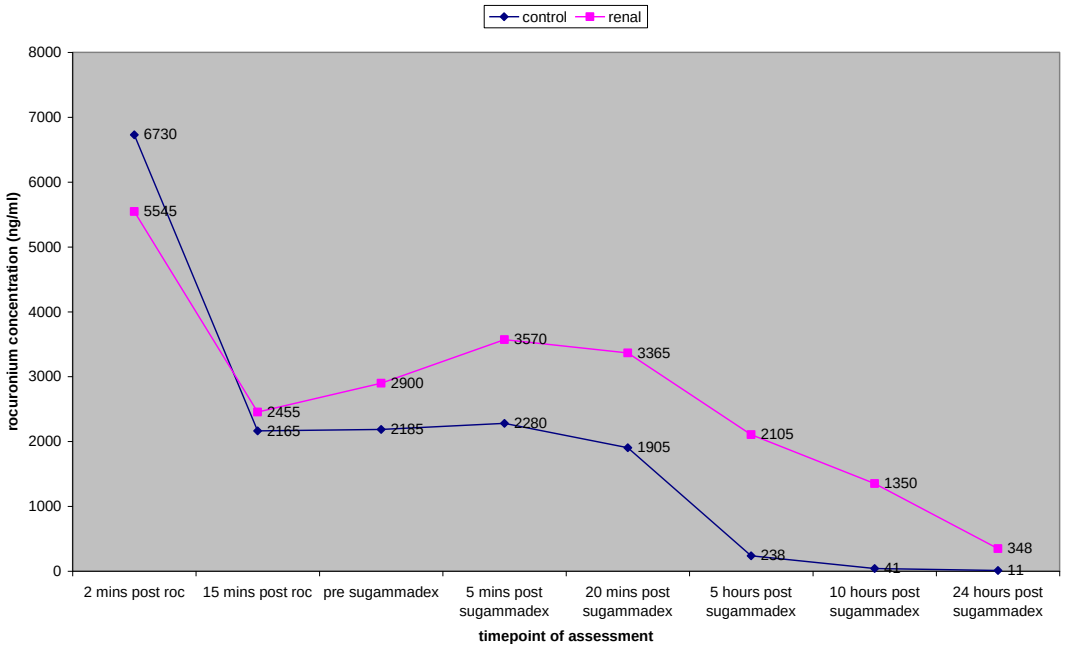


Figure 4.9: Rocuronium concentration:time plot comparing control and renal groups

4.8.2 Sugammadex concentration time data

Using the ASPE group, the following data is presented in this section:

- Table 4.14 :Statistical analysis, median, mean, 95% confidence interval and standard deviation plasma sugammadex concentration data for both groups.
- Table 4.15 :Renal group individual plasma sugammadex concentrations at sampling points from 24 hours to 28 days
- Figures 4.10 and 4.11 show the sugammadex concentration:time graphs for 2 individual cases, one renal one control.
- Figure 4.12 shows median sugammadex concentration:time graphs for the control group (±1 SD)
- Figure 4.13 shows median sugammadex concentration:time graphs for the renal group (±1 SD)
- For comparison figures 4.14 and 4.15 show median sugammadex concentration:time graphs for renal and control on the same axis.

For all data the time point t=0 represents administration of sugammadex. A list of the sampling time points is provided in Table 3.1, section 3.7.

The assay method used to determine sugammadex concentrations cannot discriminate between complexed and non-complexed sugammadex therefore all data relates to total sugammadex concentration in plasma in mcg/ml.

There were statistically significant differences between the groups with respect to plasma sugammadex concentrations at 5 and 10 hours, which can be seen in the data table 4.14 and demonstrated in figures 4.14 and 4.15. Analysis of the plasma concentrations of sugammadex at 5 minutes and 20 minutes using Wilcoxon rank sum test did not show a statistically significant difference between the groups. The fact that there is overlap of the 95% confidence intervals at these timepoints may add to the hypothesis that there is no difference between the groups.

Data is presented up to the 10 hours post sugammadex sampling point as all of the subjects in the ASPE control group had sugammadex concentrations below the lower level of quantification (LLOQ=0.1 mcg/ml) at 24 hours and the effect of dialysis on the some of the patients in the renal group would negate any further comparisons between groups.

Table 4.15 shows the individual plasma sugammadex concentrations and details of any dialysis for the renal group up to the 28 day sampling point. At the 7 day sampling point, 4/7 of the renal group had measurable sugammadex concentrations and 3/7 had sugammadex concentrations below the LLOQ. (One patient had no sample taken at the 7 day sampling point.) Of the 3 patients with concentrations below the LLOQ, 1 had haemodialysis, 1 had peritoneal dialysis and 1 had no dialysis. At 28 days all of the subjects in the ASPE renal group had sugammadex concentrations below the LLOQ, however it must be noted that 5 out of 8 subjects had undergone haemodialysis within the study period, 2 had peritoneal dialysis and 1 had no dialysis.

In general, the sugammadex concentrations at the assessment time points showed greater variability in the renal group compared with the control group.

Table 4.14: Sugammadex concentration:time data for All Subjects Pharmacokinetically Evaluable (ASPE) Group

Control group n=6					
sampling time (mins)	median	arithmetic mean	95% CI	SD	range
5	37.0	39.2	29.1-49.3	12.6	26.7-59.1
20	19.4	19.3	16.2-22.5	3.93	12..6-24.7
300*	2.09	2.17	1.56-2.78	0.76	1.34-3.25
600*	0.26	0.31	0.17-0.44	0.17	0.132-0.529
Renal group n=8					
sampling time (mins)	median	arithmetic mean	95% CI	SD	range
5	34.2	36.1	26.5-45.6	13.8	19.0-66.1
20	23.9	23.9	17.8-26.5	6.3	13.1-32.3
300	15.4	17.1	12.5-21.7	6.7	10.3-29.7
600	11.3	11.4	9.7-13.1	2.4	8.07-14.8

All concentrations given as mcg/ml

* denotes statistically significant difference between the groups using 2 independent sample Wilcoxon rank sum test

5 mins p=0.699 **20 mins** p=0.401

300 mins* p=0.002 **600 mins*** p=0.002

Table 4.15: Renal group individual plasma sugammadex concentrations at sampling points from 24 hours to 28 days

Subject No.	24 hours	48 hours	7 days	28 days	Dialysis details
003	No result as undergoing haemodialysis during sampling time	2.32	0.775	<LLOQ	Haemodialysis at 25 hours post sugammadex and every 2-3 days within study period
004	4.98	2.91	1.62	<LLOQ	Haemodialysis at 18 hours post sugammadex and every 2-3 days within study period
008	5.06	1.4	<LLOQ	<LLOQ	No dialysis during study period
009	5.74	1.79	0.201	<LLOQ	Haemodialysis between days 7 and 28
012	5.62	1.6	Sample not taken	<LLOQ	Peritoneal dialysis between 48 hours and day 28
014	7.39	3.84	0.337	<LLOQ	Haemodialysis at 26 hours post sugammadex and every 2-3 days within study period
015	8.05	4.6	<LLOQ	<LLOQ	Haemodialysis at 31 hours post sugammadex and every 2-3 days within study period
019	7.03	2.57	<LLOQ	<LLOQ	Peritoneal dialysis between 48 hours and day 28
	All concentrations given as mcg/ml <LLOQ = below lower limit of quantification of 0.1mcg/ml				

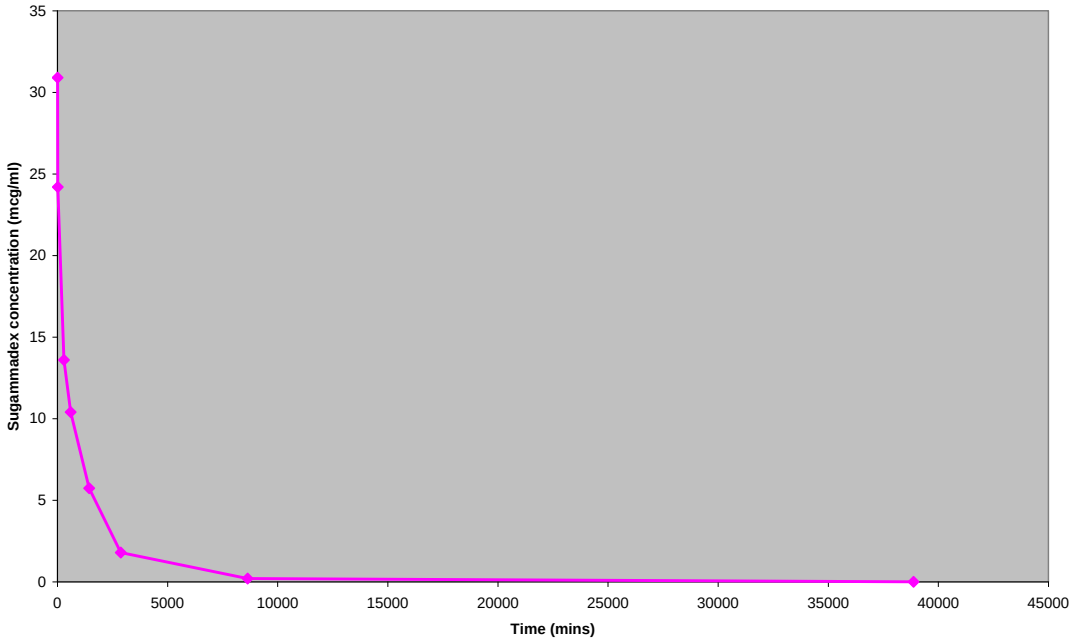


Figure 4.10: Concentration:time plot for renal subject 009 : 0-45000 mins

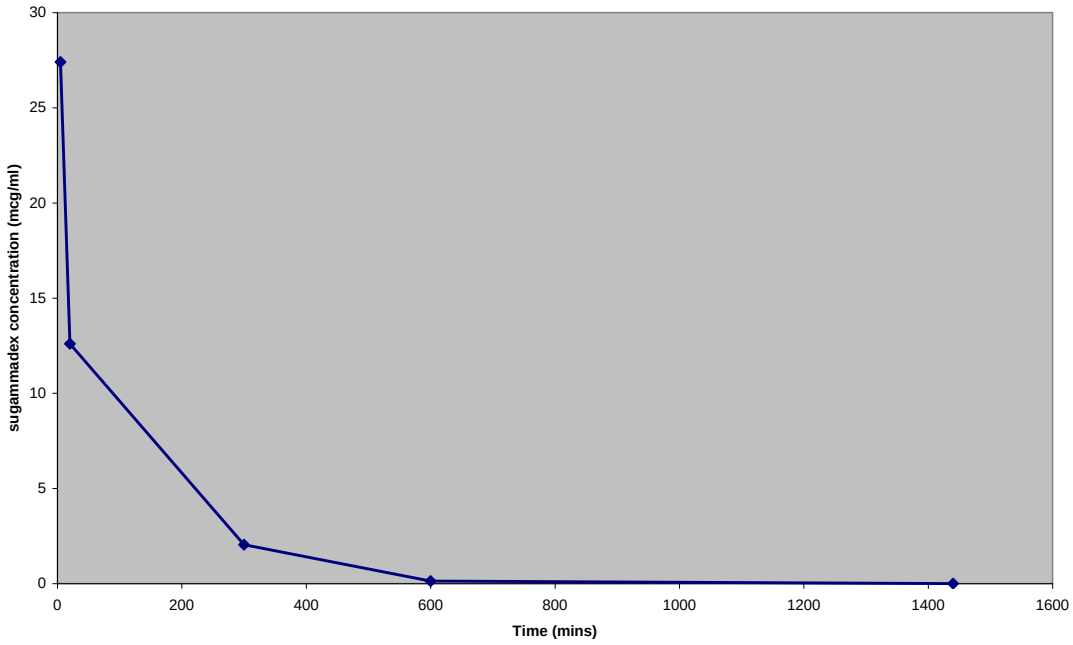


Figure 4.11: Concentration:time plot for control subject 002 : 0-1600mins

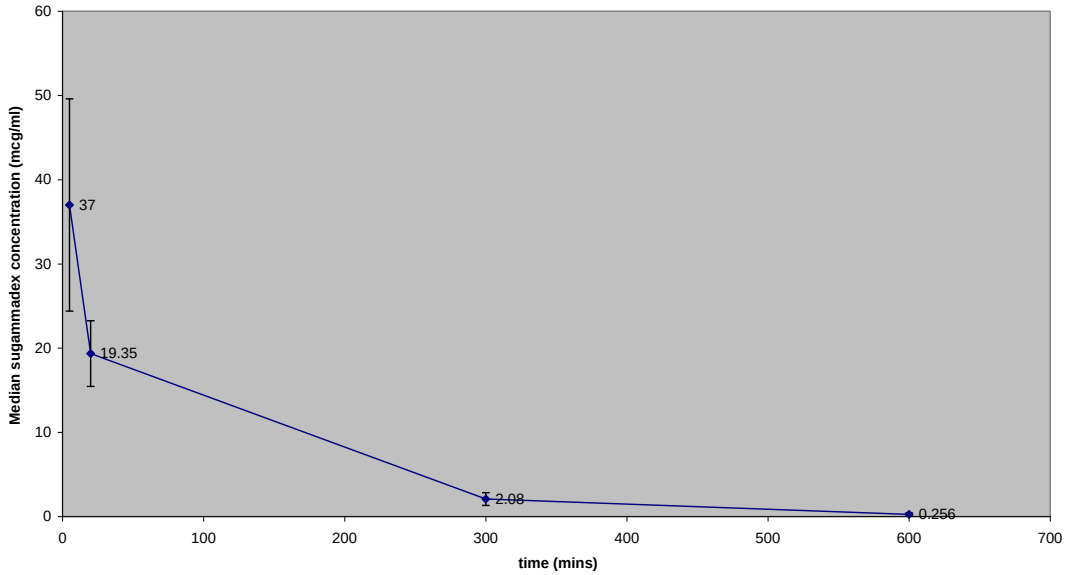


Figure 4.12: Median concentration:time plot ± 1 SD for the control group n=6

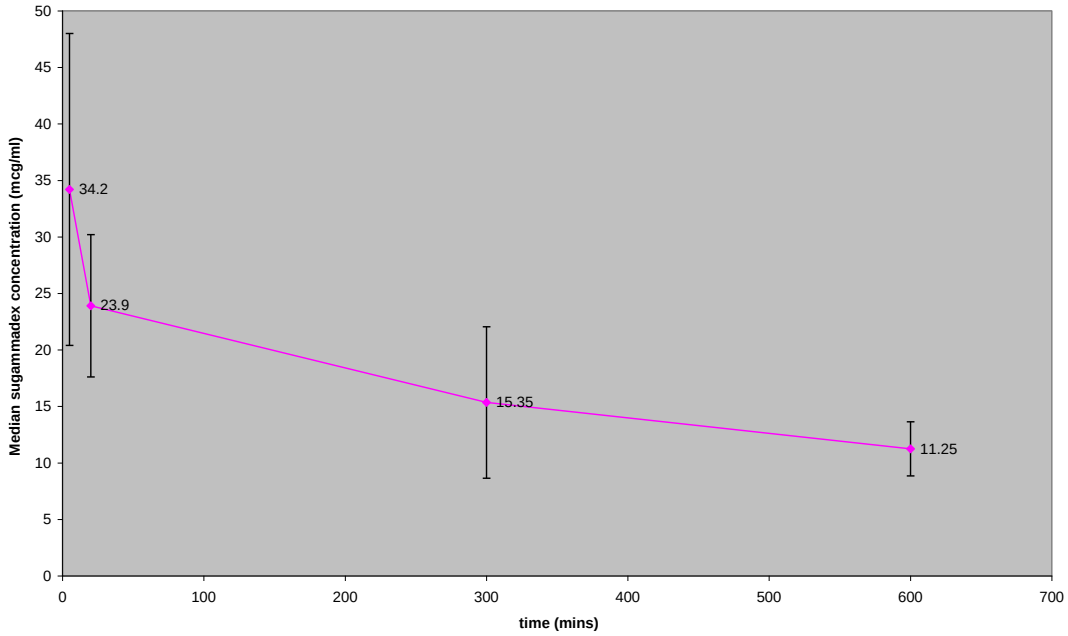


Figure 4.13: Median concentration:time plot ± 1 SD for the renal group n=8

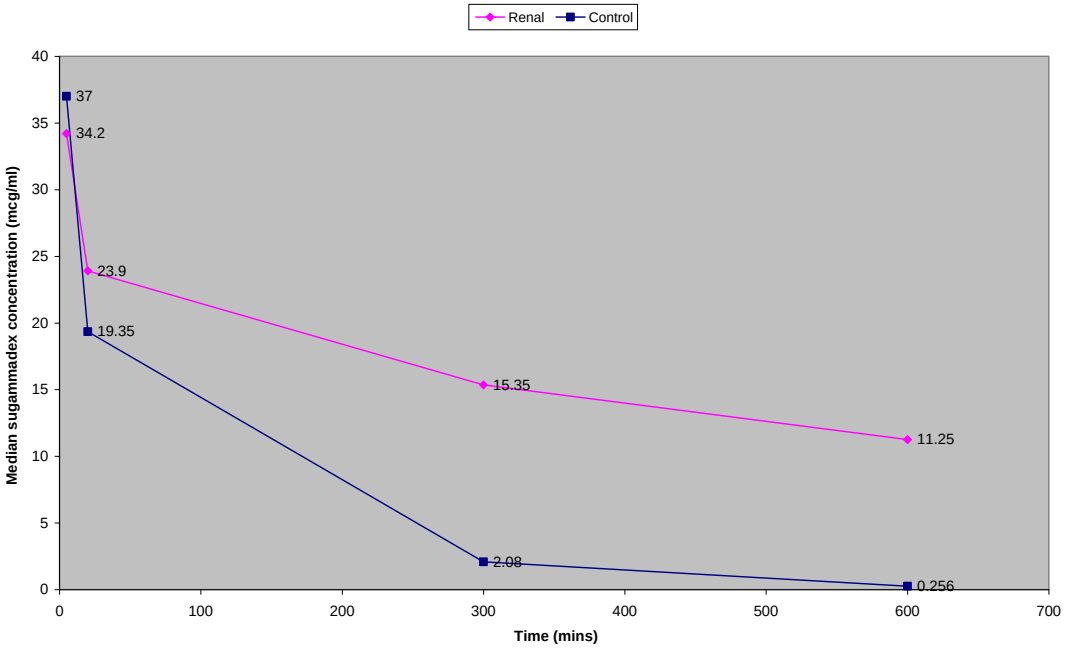


Figure 4.14: Median concentration:time plot comparing renal and control groups

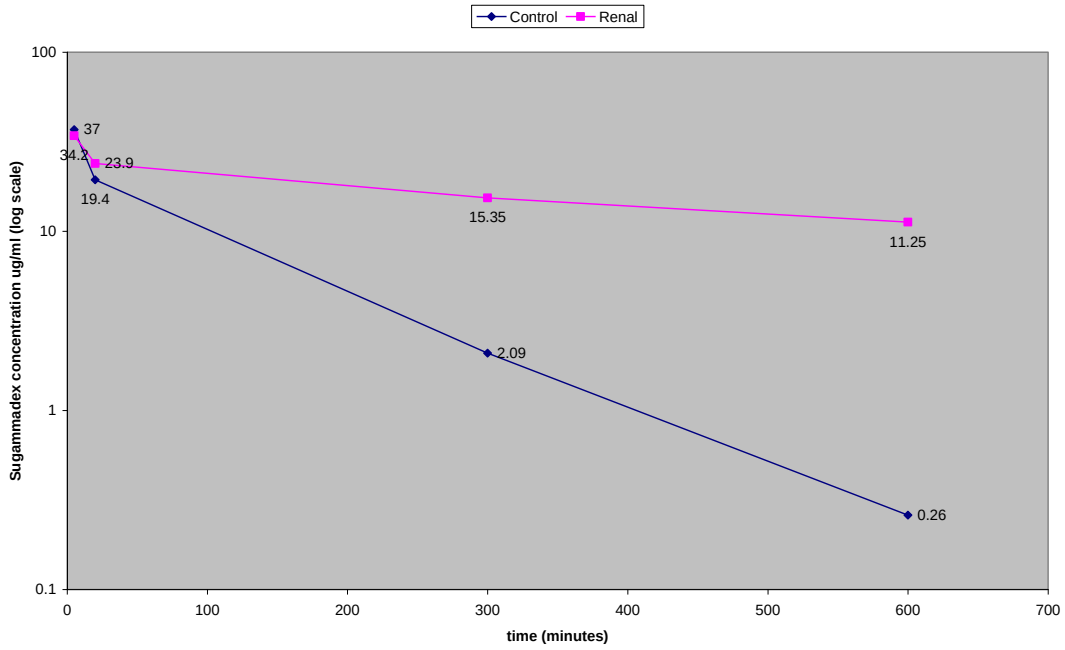


Figure 4.15: Median concentration:time plot (on logarithmic scale) comparing renal and control groups

4.8.3 Group pharmacokinetic parameters

When comparing the two study groups, statistically significant differences were found between the PK parameters relating to sugammadex listed in table 4.12 below using the Student t-test on $\log_e$ transformed values.

To summarise, the renal group had a much lower clearance of sugammadex, leading to a longer $t_{1/2}$ and MRT and in turn a larger AUC 0-∞. The control group had a smaller V_{ss} than the renal group. Weight normalised Cl and V_{ss} showed similar degrees of differences between the groups. In addition, there was found to be greater variability as described by percentage coefficient of variation in the renal group for all the parameters except V_{ss}.

Table 4.16: Pharmacokinetic parameter comparisons between study groups

Pharmacokinetic parameter	Control n=6	Renal n=8
AUC 0-∞ *µg.min/ml* range	3985* (20.7) 2832-5323	28569 (27.8) 16529-44099
Cl *ml/min* range	74.7* (10.4) 62.3-83.2	8.2 (31.0) 5.66-11.6
Wn-Cl *ml/min/kg* Range	1.00* (22.2) 0.75-1.41	0.14 (32.1) 0.09-0.24
V_{ss} *litre* range	5.49* (24.1) 4.2-7.7	11.8(14.7) 10.2-14.7
Wn-V_{ss} *litre/kg* range	0.074* (25.4) 0.048-0.120	0.201 (37.0) 0.114-0.337
$t_{1/2}$ eff *min* range	50.9* (23.3) 35.4-67.5	995 (45.1) 659-1782
MRT *min* range	73.5* (23.3) 51.1-97.4	1435 (45.1) 951-2572
Data shown as geometric mean (coefficient of variation %) *=statistically significant (Student t-test p<0.001)		
AUC=area under curve, Cl= plasma clearance, Wn-Cl=weight normalised clearance, V_{ss}= volume of distribution at steady state, Wn-V_{ss}=weight normalised V_{ss}, $t_{1/2}$ eff= effective half life, MRT= mean residence time		

Table 4.17 Per subject pharmacokinetic data for sugammadex for the ASPE group

Subject	Group	MRT	Vss	Cl	AUC	$t_{1/2}$ eff	wnVss	WNCL
003	Renal	2571.58	14545	5.66	30586.36	1782.49	336.69	0.13
004	Renal	1189.72	11732	9.86	16529.14	824.65	288.26	0.24
008	Renal	951.30	10159	10.68	23972.77	659.39	158.73	0.17
009	Renal	1671.56	10743	6.43	29251.37	1158.63	228.58	0.14
012	Renal	964.05	11000	11.41	26994.49	668.23	142.85	0.15
014	Renal	1666.23	11162	6.70	30901.12	1154.94	215.89	0.13
015	Renal	2382.12	14693	6.17	44099.19	1651.16	216.07	0.09
019	Renal	970.08	11231	11.58	34030.61	672.41	114.14	0.12
002	CONTROL	85.27	6502	76.26	2832.58	59.10	120.41	1.41
005	CONTROL	51.06	4249	83.22	4277.74	35.39	47.75	0.94
006	CONTROL	63.19	4397	69.59	3621.18	43.80	69.80	1.10
010	CONTROL	97.38	7745	79.54	4274.79	67.50	91.12	0.94
017	CONTROL	68.64	5472	79.72	4014.17	47.58	68.40	1.00
018	CONTROL	85.59	5338	62.36	5323.60	59.33	64.31	0.75

AUC=area under curve, Cl= plasma clearance, Wn-Cl=weight normalised clearance, V_{ss}= volume of distribution at steady state, Wn-V_{ss}=weight normalised V_{ss}, $t_{1/2}$ eff= effective half life, MRT= mean residence time

4.8.4 Dialysis data

4 patients in the renal group underwent low flux haemodialysis within the first 48 hours after administration of sugammadex. The reduction ratio and corrected half time ($t_{1/2}$corr) were calculated as described in section 3.10 and are presented in table 4.18 below and discussed in section 5.4.

Table 4.18: Summary of dialysis data for sugammadex

Subject	Dialysis machine/ Dialysis membrane	Time on dialysis (*h*)	Pre dialysis sug. conc (*mcg/ml*)	Post dialysis sug. conc (*mcg/ml*)	Reduction ratio %	$t_{1/2}$ corrected *min*
003	Fresenius 5008 / *Helixone FX8*	3.5	6.8	2.7	60.1	173.7
004	Fresenius 4008 / *Helixone FX8*	4	5.7	4.3	25.0	1834.5
014	Fresenius 4008 / *Helixone FX8*	4	12.6	6.5	48.8	314.7
015	Gambro 200 / *Helixone FX8*	3	9.6	6.7	29.8	443.6

4.9 Body Mass Index(BMI), Age and Duration of anaesthesia comparisons

To investigate any link between BMI, age or duration of anaesthesia and the time to recovery of the TOF ratio ≥0.9 the data from the PP group were plotted as scatter charts and linear regression was carried out using Microsoft Excel 2003. The results of this analysis appear in table 4.19 below.

These data do not demonstrate a link between the comparators and the time to recovery of TOF ratio ≥0.9 with the exception of duration of anaesthesia in the Control Group. However, due to the small sample size, a link cannot be claimed.

Table 4.19: Linear regression data for recovery times to comparators; BMI, Age and Duration of Anaesthesia (Renal group n=7, Control group n=5)

Study Group	Comparator	R^2 value
Renal	BMI	0.206
Control	BMI	0.065
Renal	Age	0.016
Control	Age	0.246
Renal	Duration of anaesthesia	0.100
Control	Duration of anaesthesia	0.712

Section 5: Discussion

5.1 Efficacy results

The primary objective of this study was to investigate whether sugammadex 4.0mk/kg when given at 1-2 PTC has equivalent efficacy, as measured by time to TOF ratio≥ 0.9, in subjects with normal or severely impaired renal function. As discussed in section 4.6, there were statistically significant differences in times to recovery of the TOF ratio ≥0.9 between the groups, (post hoc analysis using Wilcoxon rank sum p=0.004). In addition, the originally planned analysis of efficacy using the confidence interval approach of Hodges, Lehman and Moses delineated the magnitude of the difference between the groups and demonstrated that equivalence between the groups could not be claimed. The geometric mean recovery time in the renal group was 176 sec (95% confidence interval (CI):112-278) compared to 50 sec (95% CI: 30-83) for the control group with an estimated treatment difference in median recovery time to be 126 sec which is not within the pre-defined range of -60 sec to +60 sec. The results of the efficacy analysis using the confidence interval approach for the entire study population were similar.

Results for recovery of the TOF ratio≥0.7 and ≥0.8 were also reported in table 4.6 and 4.7 which similarly suggest a statistically significant difference between the groups. However, there is no clinical application to this finding as TOF ratios <0.9 are associated with signs and symptoms of residual neuromuscular blockade [100].

It is of interest to compare our results with the study by Jones et al. [147] of reversal of rocuronium induced neuromuscular blockade by sugammadex 4.0mk/kg or neostigmine 70μg/kg with gylcopyrrolate 14μg/kg given at 1-2 PTC. Geometric mean recovery to TOF ratio ≥0.9 was 174 sec for the sugammadex group compared to 3024 sec (50.4 minutes) for the neostigmine group. Comparisons with our study are limited by the fact that the Jones et al. study used sevoflurane and opioid rather than propofol and opioid to maintain anaesthesia and as shown by Reid et al.[182] sevoflurane can delay the reversal of rocuronium induced blockade by neostigmine.

One cannot state that the use of sugammadex 4.0mk/kg for reversal of deep neuromuscular block (1-2 PTC) is equivalent in patients with and without severe renal impairment. However, in this study sugammadex has been shown to reverse neuromuscular blockade efficaciously i.e. no evidence of post operative recurarization, and in a clinically useful timeframe when compared to neostigmine.

Potential reasons for the intergroup differences in recovery times are discussed below:

Study design

Section 5.6 will discuss the factors involved in study design.

Statistical error

The data I have presented is from one study centre which treated 16 out of a total of 68 patients treated in this clinical trial, therefore it is inappropriate to judge equivalence on this group alone. In addition, the Wilcoxon rank sum analysis was a non-powered test carried out post hoc and therefore any findings must be qualified by this fact. The originally planned efficacy analysis using the non parametric methods of Hodges, Lehmann and Moses for comparing the groups are valid tests and are appropriate to be used and when examining the results of the entire study group the results were similar to my own.

The sample size calculations for the entire study group appear robust and therefore when analysing the entire study group if a difference was found, it is reasonable to suggest that the result is applicable.

Differences in study procedures

Only subjects in the Per Protocol (PP) group were included in the efficacy evaluations as the major protocol violations relating to timing of administration of sugammadex with reference to PTC could have affected the results (see section 3.6). Four subjects in the AST group were removed from the efficacy evaluations due to major protocol violations (see section 4.5) thereby removing their influence on the efficacy results.

It is unlikely that differences in the efficacy values could be ascribed to differences in study procedures between the groups as all subjects in the PP Group (renal and control) received the same dose of sugammadex per kg within 10 seconds through a fast running drip and sugammadex was only given when the PTC= 1-2. In addition, all subjects in the PP group received total intravenous anaesthesia (inhalational anaesthetics can interfere with neuromuscular function [183]) and no subject received any medication throughout the study period which was thought could potentially interact with the study medication i.e flucloxacillin, toremifene, fusidic acid.

The mean duration of anaesthesia was longer in the control group in our study centre however, when analysing relationships between time to TOF ratio ≥0.9 and duration of anaesthesia there were no strong associations found (see table 4.19.) and I do not feel that this difference influenced the results.

It is of interest to note that as PTC of 1-2 was maintained throughout surgery with boluses of rocuronium 0.1-0.2mg/kg this usually resulted in a higher total dose of rocuronium being given if the duration of anaesthesia was longer. However as the PTC was 1-2 at the time of sugammadex administration, reflecting a comparable efficacy of rocuronium at the NMJ [161, 184], the increased total dose of rocuronium in the control group should not be a factor in the time to recovery of TOF ratio ≥0.9. As discussed in section 4.8.1, there was a statistically significant difference in the mean rocuronium concentration between the groups in our study centre at the time that sugammadex was given (the pre-sugammadex sampling timepoint) although I do not feel that this is a clinically significant result. The mean plasma rocuronium concentration of the control group was actually lower than that of the renal group but as can be seen there was overlap of the 95% confidence intervals; control mean (95%CI) = 2270 ng/ml(1801-2739), renal mean (95%CI) = 2894 ng/ml (2565-3221), Wilcoxon rank sum: p=0.05. I suggest that the higher rocuronium concentration in the renal group at the pre-sugammadex sampling timepoint could be a consequence of decreased clearance of rocuronium in patients with renal failure [129] although, as the results for the entire study group showed no large difference in median concentrations, the result from my study group may be an anomaly.

Furthermore, when analysing any relationship between rocuronium concentrations at the time that sugammadex was given and time to TOF ratio ≥0.9, no strong association was found (see section 4.8.1) adding credence to the suggestion that this was not a clinically significant result. I must state once more that this is post hoc analysis of subgroups within a larger study and this limits the ability to extrapolate these results to a wider population.

Differences in patient characteristics

The renal group had a higher mean age and a lower mean height and weight. The control group had an equal gender distribution; 4:4 f:m, the renal group was more unequal; 6:2 f:m. The renal group, by definition, had renal failure which is a significant disease with systemic effects and as such the renal group are all classified in ASA group III whereas the controls were all without significant systemic disease (ASA I-II). The renal group also had more pre-existing medical conditions compared to the controls (table 4.5).

In section 4.9, when analysing the relationships between BMI or age and the efficacy variable, no links were demonstrable. However this was a very small sample studied and I do not feel that reliable conclusions can be made on these parameters.

Fuchs-Buder et al. state that age, gender, weight and systemic disease can affect the action of neuromuscular blocking agents [105]. Kuipers et al. have demonstrated that cardiac output has been shown to influence the pharmacokinetics of rocuronium [185] and Henthorn et al. state that early drug distribution, which is a determinate of the pharmacokinetics of rapidly acting intravenous drugs, is affected by cardiac output, age, gender and body habitus [186].

With regards to sugammadex, the summary of product characteristics states that no gender differences have been demonstrated [165] and I could find no further evidence to claim any difference. McDonagh et al. found an increase in mean recovery times with increases in age group: <65 yrs = 2.3min, 65-74 yrs = 2.6min, >75 yrs = 3.6min [155]. Monk et al showed no difference in recovery times between patients with BMI> 30mg/kg^2 or BMI< 30mg/kg^2 [156].

When considering the patient characteristics I feel that it is very difficult to state that these factors; age, height, gender, weight, pre-existing medical conditions and systemic disease did not influence the efficacy results. As discussed in section 2.4, chronic renal failure in its own right can have significant effects on pharmacokinetics. In addition, all of these factors could combine to produce comparative differences in the cardiovascular status i.e. cardiac output, of patients in the renal group, which may have led to the longer time to TOF ratio≥0.9 found in this study.

To be able to investigate the effect of age, height, weight, pre-existing medical conditions and systemic disease and in turn make more valid comparisons the control group should be selected from a more comparable group, i.e. this should have been a randomised controlled trial using sugammadex and neostigmine for reversal with both study groups having severe renal impairment and being more closely matched for age, height and weight. One key issue with this suggestion would be giving rocuronium and then neostigmine as a reversal agent (mean time from PTC 1-2 to TOF ratio ≥0.9 =50.4 minutes in patients with *normal* renal function [147]) to a group of patients with severe renal impairment who will already have a prolonged recovery time [129].

Pharmacokinetic differences

Analysis of the plasma concentrations of sugammadex at 5 minutes and 20 minutes did not show a statistically significant difference between the groups hence we can propose that the concentrations were comparable between the groups, (table 4.14). As the time to TOF ratio ≥0.9 occurred entirely within 20 minutes, this may suggest that although there was a difference in pharmacodynamics (efficacy) between the groups, this was not due to differences in pharmacokinetics. However, the plasma concentrations reflected the concentrations at the effect site i.e. the NMJ and were not directly measured there. It may be possible that the measured differences in V_{ss} (significantly larger V_{ss} in renal group) may have influenced the results although this would be difficult to quantify without a matched control group with renal failure as discussed above. As will be discussed in section 5.3, the V_{ss} of sugammadex in the control group was underestimated and therefore the difference in V_{ss} between the groups may not be as large. In addition, Staals et al. found no significant differences

between renal and control groups in V_{ss} of sugammadex 2.0mg/kg given at return of the second twitch of the TOF [162].

Depth of blockade

Staals et al. reported comparable recovery times between groups when sugammadex 2.0mg/kg was given to patients with renal failure and to controls after rocuronium induced neuromuscular blockade at the return of the second twitch of the TOF, i.e. moderate block [54]. In both the Stalls study and our own the renal group had longer geometric mean times to recovery of the TOF ratio≥0.9;

Staals 2008 : renal = 120s (SD 43.2), Control = 109s (SD 37.8)

Our results : renal = 176s(SD 95.6), Control = 50s (SD 20.4)

One could hypothesise that the increased depth of blockade at which sugammadex is given may affect and augment any differences in recovery times between the groups. However, the cause for this effect and whether it is a true effect is unknown and potentially a subject of further study.

Another point to consider is that if this hypothesis is true, there could be a more prolonged recovery time in patients with renal failure if sugammadex 16mg/kg is to be used after high dose rocuronium (1-1.2mg/kg) as in a modified rapid sequence induction.

5.2 Safety

In clinical trials and since European licensing in 2008 the safety record of sugammadex has been acceptable and there have not been any common significant side effects reported [150]. There have been a small number of reports of hypersensitivity reactions [165] and the product characteristics have been updated to include a potential effect of sugammadex on haemostasis parameters [149].

One of the secondary objectives of this trial was to collect more safety data on the use of sugammadex in patients with renal failure and in controls. The study as a whole was not designed to be able to make any statements about the safety of sugammadex in this population. In our study centre there were no incidences of recurarization, no AEs or

SAEs considered to be related to sugammadex and no cases reported of vital signs or laboratory parameters relating to sugammadex.

Post hoc, non-powered statistical analyses of differences between the groups for all the vital sign parameters at all of the assessment timepoints were carried out using Student pooled t test, (see table 4.9) At 5 and 10 minutes post administration of sugammadex, the control group had statistically significant higher mean SBP when compared to the renal group; control 5 minute mean (95% CI) SBP = 134 (115-154), renal 5 minute mean (95% CI) SBP = 105 (87-124) p=0.049, control 10 minute mean (95% CI) SBP = 137 (125-150), renal 10 minute mean (95% CI) SBP = 107 (90-124) p=0.015. The mean DBP at the 28 day assessment was lower in the control group; control 28 day mean (95% CI) DBP= 74 (71-77), renal 28 day mean(95% CI) = 83 (75-91), p=0.046. It is noted that there is overlap between the groups in the 95% confidence intervals with regards the 5 minute mean SBP and the 28 day DBP which may reduce the significance of any difference found between the groups at these timepoints. There were no other statistically significant differences in mean SBP, DBP and HR between the groups.

This study was not powered to make conclusions about the safety of sugammadex, and the analysis of vital signs was non-powered and post hoc. Therefore I do not feel able to make extrapolations to the wider population with regards to the safety of sugammadex.

It is possible that the inclusion of a neostigmine control group with renal failure as mentioned above may have allowed comparisons on safety to be made but the number of subjects to be included would probably have to have been much larger.

5.3 Pharmacokinetic results

5.3.1 Rocuronium

The plasma rocuronium concentrations have been discussed in the discussion of efficacy, section 5.1. There was found to be a statistically significant difference in the plasma rocuronium concentrations at the pre-sugammadex sampling timepoint although for the reasons given above, this was not thought to be a clinically significant result. A confounding factor in the comparison of the plasma rocuronium

concentrations was the dosing schedule during the study i.e. intubating dose 0.6mg/kg, followed by doses of 0.1-0.2 mg/kg to maintain the PTC 1-2. As the control group tended to have longer surgery, this would suggest a larger total dose of rocuronium being given to the control group. Table 4.13 and figure 4.9 demonstrate that at the sampling timepoints after sugammadex was given the renal group had higher median plasma rocuronium concentrations. As discussed in section 2.1 rocuronium is primarily eliminated via hepatic uptake and excretion of the unchanged drug in the bile and ultimately the faeces, approximately a quarter of excretion is via the renal route [167]. However, once encapsulated by sugammadex, the complexed drug is primarily eliminated via renal pathways[165] which will be greatly reduced in the renal group and hence I suggest that this is the reason for the trend.

Group pharmacokinetic parameters relating to rocuronium were not calculated in this study for the reasons discussed in section 3.10.

5.3.2 Sugammadex

The plasma sugammadex concentrations at 5 and 20 minutes were not statistically significantly different but the 5 hour and 10 hours samples showed statistically significant differences between the groups, the renal group having consistently higher sugammadex concentrations. This was post hoc subgroup analysis and as such any statistical conclusions drawn have to reflect this fact. At the 24 hour sampling point, the control group all had sugammadex concentrations below 0.1mcg/ml. However, as can be seen in table 4.15. the sugammadex complex was present for considerably longer in the renal group, i.e. up to 7 days in 4/7 samples taken.

Sugammadex and the sugammadex-rocuronium complex are excreted via renal pathways, therefore it was expected that there would be a decreased sugammadex clearance and increased effective half life in the renal group. The results bore this out (table 4.16and table 4.17) and an increased exposure to sugammadex was experienced by the renal group as shown by a large difference in values for geometric mean AUC 0-∞ between the groups (an approximate seven fold increase from control to renal groups.)

It could be suggested that the prolonged exposure may submit the renal group to an increased risk of the sugammadex-rocuronium complex disassociating although the association constant: K_A =1.8 x 10_7 M^{-1} suggests a very strong host-guest affinity that makes disassociation unlikely [133, 142]. Prolonged exposure to sugammadex may increase the risk of patients experiencing potential side effects and it also raises the question of which NMBA to use if further neuromuscular blockade is required within a 28 day period. Advice on this may have to be included in the summary of product characteristics (SmPC) if the drug is to be recommended in this group of patients.

The sampling scheme in this study was chosen to allow comparisons between the groups over a longer period, and in particular to collect PK samples in the renal group up to the 28 day sampling time point which resulted in using a sparse sampling scheme (table 3.1). This can be seen if the sampling scheme is compared to the Staals sugammadex PK study in renal patients and controls when samples were taken at 2, 3, 5, 10, 15, 30, 60 minutes and 2, 4, 6, 8, 12, 18, 24, 36, 48 hours after sugammadex 2.0mg/kg was given at return of the second twitch of the TOF [162]. Therefore any PK comparisons must be qualified by this fact.

Analysis of the PK data generated (table 4.16and 4.17) has shown significant differences between the groups in the way in which sugammadex is handled. This may be due to the differences in the patient characteristics having an effect on cardiac output and early drug distribution as discussed above [185, 186], or the differences in protein binding associated with renal failure [173]. This may be expected given what is known about the pharmacokinetics of patients with renal failure (section 2.2.) It is possible that in renal failure the non-renal excretion pathways (faecal and respiratory) of sugammadex play a greater role or it may be the case that the renal route is still the primary, although much slower route.

In addition, the PK data has shown a larger degree of variability (as shown by coefficient of variation) in the renal group. This was also found by Staals et al. although their results showed an even larger variability (table 2.2)[162]. Information about interpatient variability may also have to be included in the SmPC.

As noted above, due to the calculation methods used and the sparse sampling schedule the V_{ss} for the control group was underestimated. This occurred as the sparse sampling schedule resulted in an overestimate of the AUC for the control group. The renal group was only slightly affected as PK sampling continued to the 28 day sampling point giving more sampling points and a more accurate result. Sugammadex clearance was also underestimated in the Control group which explains why the results for V_{ss} and Cl are both lower than those reported by Staals et al. (table 2.2) [162].

5.4 Dialysis

Previous *in vivo* studies [188] had predicted that low flux dialysis (performed in patients at our study centre) would be less effective than high flux dialysis (performed in patients at other study centres.) This was generally found to be the case; however there was a high degree of variability in reduction ratio and thus corrected $t_{1/2}$ in this small sample to the extent that subjects 003 and 014 had reduction ratios (60.1% and 48.8% respectively) more in keeping with 5 patients in other study centres that underwent high flux haemodialysis (range 37.2% to 74.1%).

The variability in results was discussed with Dr Sandeep Mitra, a Renal Physician responsible for the dialysis unit at our study centre. He suggested that variations in the factors affecting the diffusive clearance (blood flow rate, dialysate flow rate, individual volume of distribution, time on dialysis) and the convective clearance (amount of fluid removed) may have affected the results. In addition, depending on the sampling methods used at the end of the dialysis cycle there may have been a large rebound of solute which will not be picked up by the sample and will lead to an overestimation of the reduction ratio. Any future studies will need to accommodate for or standardise these factors to avoid potentially misleading results.

The fact that the dialysis parameters, such as blood flow rate, were not standardised and that the timing and method blood sampling was not standardised, this significantly limits any conclusions that can be drawn from the data collected in this study with regards to the dialysability of sugammadex.

However, more data has been collected on the dialysability of sugammadex and this information will be added to the current data set. The current understanding that high flux haemodialysis should be used to remove sugammadex still holds true although future dialysis studies are planned which can investigate this further.

5.5 Summary

In this clinical trial, differences were shown in the efficacy and pharmacokinetics of sugammadex when used in patients with severe renal impairment compared to patients with normal renal function. In my post hoc subgroup analysis, the time to reversal of neuromuscular blockade was statistically significantly longer in the renal group; geometric mean time from start of administration of sugammadex to recovery of the TOF ratio≥ 0.9 was 50 sec (SD 20.4, 95% CI: 30-83) for the control group and 176 sec (SD 95.6, 95% confidence interval (CI):112-278) for the renal group (p=0.004). In addition, the exposure to sugammadex in the renal group was statistically significantly higher than that of the control group; geometric mean AUC 0-∞ *μg.min/ml* (coefficient of variation*)* control group 3985 (20.7), renal group 28569 (27.8) p<0.001.

There were no incidences of recurrence of neuromuscular blockade in either group and there appeared to be no difference in the safety profile between the groups.

The fact that recovery from deep blockade is likely to be slower and that exposure to the sugammadex-rocuronium complex will be longer in patients with renal failure will need to be taken into consideration if sugammadex is used in this group of patients. However, the ability to provide deep neuromuscular block throughout a surgical procedure which can then be reversed in a clinically useful timeframe, in addition to the possibility of avoiding the need to use suxamethonium are two key potential benefits that the use of sugammadex will confer to patients with severe renal impairment which may outweigh the aforementioned issues.

Future work

The secondary objectives in this study related to collection of more safety, pharmacokinetic and dialysis data. The safety data will be added to the safety database to inform future drug development. The PK data may be combined with data from other sugammadex trials to aid a greater understanding of the PK profile of sugammadex in renal failure. This will also happen with the dialysis data, although due to the flaws in the dialysis data a new dialysis study may be of more benefit.

A future study which may be of great interest would be one in which the control group has renal impairment and are given neostigmine for reversal. This may be difficult to design but it should enable more valid pharmacokinetic and safety comparisons to be made. In addition, a study using high dose rocuronium, 1-1.2mg/kg, followed by high dose sugammadex, 16mg/kg, in patients with renal failure and matched controls should be carried out to see if there is a clinically significant difference in recovery times which may affect the use of rocuronium and sugammadex as a potential replacement for suxamethonium in rapid sequence induction in patients with renal failure.

5.6 Critical appraisal of study design

The study was designed to evaluate the use of sugammadex in patients with severe renal impairment with a primary objective of comparing efficacy with a control group with normal renal function. Secondary objectives were to collect more data on safety, pharmacokinetics and dialysability of sugammadex. Sugammadex is not currently recommended in patients with severe renal impairment however, as discussed in section 1.9 this group of patients that may benefit greatly from the safe application of the drug. With this in mind I feel that the research questions were valid and had the potential to add new information about the use of the drug in this special population.

The primary research question related to the efficacy of sugammadex and the study was appropriately designed to answer this. The guidelines set out by of Fuchs-Buder et al. in ‘Good clinical research practice in pharmacodynamic studies of

neuromuscular blocking agents II: the Stockholm revision' [105] were adhered to with regards to protocol design, sample size calculations, neuromuscular calibration and monitoring and reporting of results. One aspect which could be improved in further studies would be the inclusion of core temperature in addition to surface temperature measurement. This would allow more confidence to state that temperature did not affect the measurement of neuromuscular function.

The use of healthy controls rather than controls with severe renal impairment has limited the ability to make comparisons relating to the safety of sugammadex in this trial. In addition, the use of a control group with severe renal impairment may have allowed more in depth analysis of any influence that renal impairment, age, height, weight and pre-existing medical conditions may have had on the efficacy results. However, as discussed in section 5.1, I feel that designing a randomised controlled trial using an alternative reversal agent i.e. neostigmine, that could take a significantly longer time to reverse deep neuromuscular blockade, has significant ethical considerations that may not be acceptable.

When the study was designed it was decided to stop pharmacokinetic assessments of plasma rocuronium and sugammadex concentrations in the control group at the 24 hour sampling timepoint. I understand this was due to the fact that both of these drugs had been studied in healthy patients in previous trials in addition to time and resource constraints. I feel that this was an omission on the part of the study designers as it limited the ability to make comparisons between the groups beyond this timepoint. Furthermore, the pharmacokinetic parameters relating to rocuronium were not calculated. As discussed in section 3.10 this was due in part to time and resource constraints and to the fact that the dosing schedule of rocuronium (intubating dose plus maintenance) may have lead to spurious results. Once more I feel that this was an omission in the study as the ability to compare rocuronium parameters, both between the groups and with other pharmacokinetic studies, may have allowed more validation of the results from this study. Furthermore, if the results were very different, it would have cast doubt as to the validity of the study.

Although this was not a randomised or blinded trial, certain steps were taken to minimise the possibility of bias affecting the results:

Selection bias

- The protocol stated that no more than two patients from each group, control or renal, could be consecutively recruited. This was an attempt to reduce the risk of selection bias by introducing a degree of random sampling, albeit a small one.
    - There is a possibility of *Volunteer Bias* occurring; i.e. only subjects motivated either personally or altruistically will be included. However, the use of a control group should have minimised any potential influence on outcome.

Measurement bias

- The primary efficacy outcome measure was the time to recovery of the TOF ratio ≥ 0.9 as measured by the TOF-Watch®SX. This is objective data and should not be open to interpretation although the data must be robust in the first instance.
    - The use of validated and checked equipment was intended to minimise any *Instrument Bias*.
    - The calibration procedure detailed in section 3.8 was intended to decrease any variability between patients.
    - To ensure consistency of trace quality, training was undertaken in the use of the TOF-Watch®SX and test traces had to be approved by the Trace Team before a centre was allowed to recruit patients.
    - The TOF traces were reviewed by the investigators at site, the Trace team and ultimately by a Central Independent Adjudication Committee (CIAC) which should add to the objectivity of any interpretation of the data. However, none of these teams were blinded to the groups into which the subject belonged. This had a possibility of introducing *Expectation Bias* and in future studies I would suggest that the Trace Team and the CIAC be blinded to the subject group. The investigators in theatre could not be blinded due to the nature of the surgery as this would not have been possible to reliably enforce i.e. renal patients were often undergoing surgery relating to renal dialysis access.
    - The safety data collected had the possibility to be open to *Expectation Bias*, however, there was strict adherence to ICHGCP guidelines with regards to S/AE reporting. In addition to the study doctors being involved in the

reporting procedure, clinical research assistants employed by the sponsors regularly reviewed all patient notes for S/AEs.

- The laboratory safety and pharmacokinetic data had the possibility to be open to *Instrument* or *Insensitive Measurement Bias*. The use of a central laboratory for all analysis, using validated and quality controlled techniques should have minimised any bias, especially as the samples from both groups were analysed in the same laboratory.

The study was mainly carried out in line with the original protocol, with regards to study procedures, recruitment numbers, statistical analysis etc. There was an amendment to the exclusion criteria submitted and accepted in May 2009 which shortened the list of concomitant medication which would result in patient exclusion. This has a potential to reduce the relevance of any findings, however, it was thought that the original list contained a prohibitive and slightly speculative list of medications that may interfere with neuromuscular blockade. After the amendment, the drugs which remained (section 3.4) were known to potentially interfere with the action of sugammadex and were therefore more relevant to act as excluding criteria.

Conflict of interests

From November 2008 to November 2009 a portion of my salary was paid out of funds received by CMFT NHS Trust from MSD for patient participation in the clinical trial.

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Appendix

Methods of sugammadex assay

Details of the methods of sugammadex assay have been provided by the bioanalytical team at MSD. They have given me an advanced copy of an article accepted for publication in the Journal of Chromatography B entitled "Determination of sugammadex in human plasma, urine, and dialysate using a high-performance liquid chromatography/tandem mass spectrometry assay "and therefore there is no formal reference.

Liquid chromatography in tandem with mass spectrometry has been developed and validated in the quantification of sugammadex and rocuronium samples in guinea pigs [144]. The assay method for determination of sugammadex in human plasma has been developed in accordance with the Food and Drug Administration guidelines on bioanalytical method validation. Details of the assay method appear below;

- Samples from plasma were analysed on a Polaris® C18-A PEEK (polyaryletheretherketone) analytical column (50 mm x 4.6 mm internal diameter, 5 µm) with a linear mobile phase gradient of 0.1% v/v formic acid in water:methanol from 70:30 to 20:80.
- The flow rate was 1 mL/min with a total run time for each injection of 6 min.
- Tandem mass spectrometric detection was conducted using multiple reaction monitoring under negative ion mode with a turbo ion-spray interface to quantify the concentration of sugammadex.

The accuracy of the assay method was determined using inter- and intra-assay precision and accuracy measurements which were within pre-defined acceptance limits (inter-assay coefficient of variation from 4.9-7.3%, intra-assay coefficient of variation from 3.4-7.8%). The lower limit of quantification was 0.1 mcg/ml and the upper limit 40 mcg/ml. There was no interference to the assay method by the presence of rocuronium in the plasma. It was found during the studies on guinea pigs that the processing of the samples led to some disruption of the sugammadex-rocuronium complex [144] and as such the assay method yields a total sugammadex

concentration independent of the drug being bound or unbound. Sugammadex was found to be stable in plasma in the short-term at room temperature and at long-term at –20 °C (this was how the samples were stored in our study until transportation under ‘dry-ice’ to the central laboratory.)

In addition to our clinical study, this method for determination of sugammadex in the plasma has been used in numerous other clinical trials, is a validated bioanalytical method developed under FDA guidelines and carried out in full compliance with Good Laboratory Practice regulation.

Table A1: Details of surgery carried out in AST group

Surgery data : All Subjects Treated Group			
Subject	Group	Indication for surgery	Surgical procedure
003	Renal	Dialysis dependant renal failure	Dialysis access surgery (thigh loop graft)
004	Renal	Infected abdominal wound	Change of vacuum dressing with peritoneal washout
008	Renal	Dialysis dependant renal failure	Formation of brachial AV fistula
009	Renal	Inguinal hernia	Repair of inguinal hernia
012	Renal	Dialysis dependant renal failure	Insertion of Tenchkoff peritoneal dialysis catheter
014	Renal	Dialysis dependant renal failure	Insertion of Tenchkoff peritoneal dialysis catheter
015	Renal	Adult polycystic kidney disease	Native nephrectomy
019	Renal	Dialysis dependant renal failure	Insertion of Tenchkoff peritoneal dialysis catheter
002	Control	Cosmesis	Maxillary advancement osteotomy with genioplasty
005	Control	Cosmesis	Bimaxillary osteotomy
006	Control	Restorative dental surgery	Removal and replacement of dental implants
010	Control	Previous facial trauma	Plastic surgery to right cheek
011	Control	Previous bowel surgery	Reversal of loop ileostomy
016	Control	Cosmesis	Lefort I impactosteotomy
017	Control	Cosmesis	Lefort I impact osteotomy
018	Control	Absent dentition upper jaw	Right iliac crest bone harvest to pre-maxilla

Table A2: Details of the medical conditions reported in the AST group

		Subject Group	
System organ class Medra V12.1	Pre-existing medical conditions	CLcr <30 mL/min	CLcr ≥80 mLmin
Infections and infestations	Hepatitis C	1	0
	Pneumocystis Pneumonii	1	0
	Upper respiratory tract infection	0	1
Neoplasms benign, malignant and unspecified	Benign breast neoplasm	1	0
	Metastatic neoplasm	0	1
	Uterine leiomyoma	1	0
Blood and lymphatic system disorders	Anaemia	3	0
Endocrine disorders	Hyperparathyroidism	1	0
	Hyperthyroidism	1	1
Metabolism and nutrition disorders	Gout	1	0
	Hypercholesterolaemia	2	1
	Hyperlipidaemia	2	0
	Obesity	1	1
	Type 2 diabetes mellitus	1	0
Psychiatric disorders	Anxiety	1	0
	Depression	1	1
Ear and labyrinth disorders	Ototoxicity	1	0
Vascular disorders	Arteriovenous fistula	1	0
	Hypertension	6	1
	Intermittent claudication	1	0
	Secondary hypertension	1	0
Respiratory, thoracic and mediastinal disorders	Asthma	1	1
Gastrointestinal disorders	Constipation	5	0
	Gastrooesophageal reflux disease	3	0
	Nausea	1	0
	Peritonitis sclerosing	1	0
Skin and subcutaneous tissue disorders	Actinic keratosis	1	0
	Pruritus	1	0
	Rosacea	0	1
Musculoskeletal and connective tissue disorders	Osteoarthritis	1	0
	Osteoporosis	1	0
	Spinal osteoarthritis	0	1
	Systemic lupus erythematosus	1	0
Renal and urinary disorders	Renal failure	1	0
	Renal failure chronic	7	0
Reproductive system and breast disorders	Benign prostatic hyperplasia	0	1
	Menopausal symptoms	0	1
	Postmenopausal haemorrhage	1	0
Investigations	Hysteroscopy	1	0
Injury, poisoning and procedural complications	Transplant failure	1	0

Surgical and medical procedures	Abdominal cavity drainage	1	0
	Appendicectomy	1	0
	Colectomy	0	1
	Hepatectomy	0	1
	Hip arthroplasty	1	0
	Hysterectomy	1	0
	Hysterosalpingo−oophorectomy	0	1
	Jaw operation	0	1
	Malignant tumour excision	0	1
	Mammoplasty	0	1
	Peritonectomy	1	0
	Rhinoplasty	0	1
	Sterilisation	1	0
	Transgender operation	0	1
	Wound treatment	1	0
Social circumstances	Drug abuser	1	0
	Trans−sexualism	0	1
	The medical conditions were classified using MedDRA version 12.1 used to classify adverse event information associated with the use of biopharmaceuticals and other medical products [189].		