Model of Neuromuscular Block Reversal in the Anaesthetised Rat

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1 Introduction

Reversal of neuromuscular block (NMB) is of strategic relevance to prevent risks of developing postoperative respiratory complications in clinical situations.

For this purpose, we have developed a rat model for assessing blocking of neuromuscular function by two drugs, and reversal of NMB with a prototypical agent, while monitoring main safety endpoints.

In a first step, ED_{90} of rocuronium and atracurium, as representative NMB agents, was determined. In a second step, reversal effect of neostigmine on NMB induced by both agents was studied at a dose of 3xED90 for each NMB agent.

2 Material and Methods

Test system: six male Sprague-Dawley rats (400-500g).

Animal preparation and monitoring: after induction of anaesthesia (pentobarbital 60 mg/kg IP + 10 mg/kg/h IV), rats were tracheotomised and artificially ventilated (50 rpm, 2.3 mL volume, 600 ms inspiratory time). A jugular vein was catheterised to allow intravenous administrations of the anaesthetic agent and test substances. ECG, heart rate and body temperature were monitored over the whole experiment duration.

NMB model (adapted from Pickett et al. 2008): the rat was placed on a custom-build unit where the hindlimb was secured between blocks, such that only the tibio-tarsal joint was mobile. An isometric force transducer was secured to the hind foot such that the tibio-tarsal angle was 90°. A pair of hypodermic monopolar needle electrodes (28G) was placed near the sciatic nerve to deliver electrical pulses in order to promote contraction of the *triceps surae* muscle group and extension of the foot, whose displacement was converted to force using a strain gage transducer.

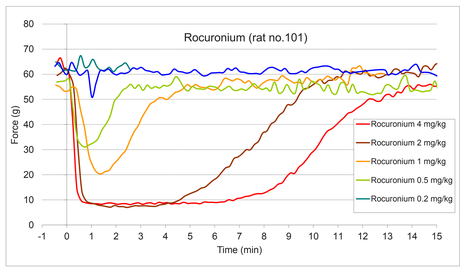
Signal acquisition and data analyses: after a 10 to 15-minute stabilisation period, square pulses (40 V, 50 µs) were delivered at a frequency of 0.1Hz (kept constant over the whole experiment). The muscle force signal was captured on Notocord-hem® (Notocord Systems, France) data acquisition software.

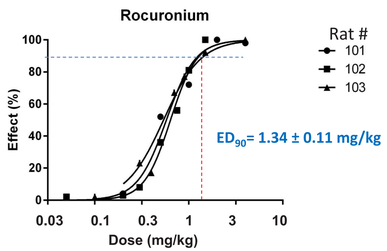
The maximum twitch amplitude was calculated for each stimulation. After dosing, and twitch amplitude was expressed as % from basal (pre-NMB) value. Non-linear sigmoid curve fitting was performed using Graphpad Prism®.

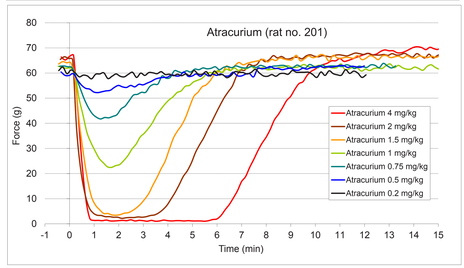
Treatment design: rocuronium bromide, atracurium besylate and neostigmine (Sigma) solutions were prepared by dilution of fresh stock solutions in water for injection (pH 4.0 ± 0.3) and administered as an IV bolus. Neostigmine was administered as an IV bolus, 2.5 minutes after each NMB agent administration.

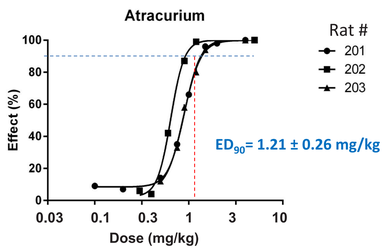
3 Results

Determination of rocuronium and atracurium ED_{90}



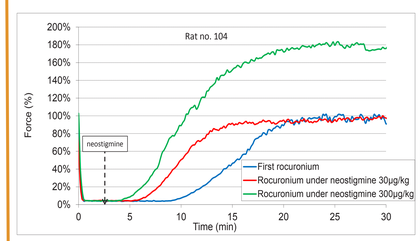


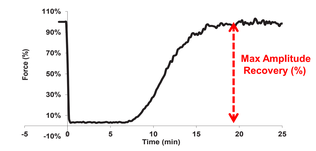


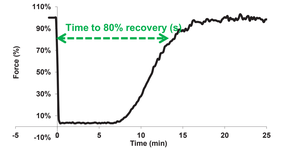


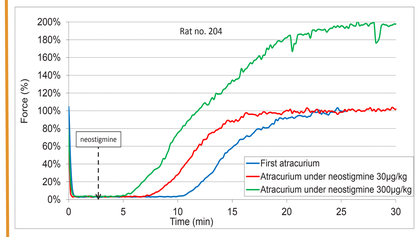
Time course of changes in twitch amplitude after NMB with rocuronium or atracurium (n=3 for each). ED_{90} of either blocker was determined to be 1.34 ± 0.11 mg/kg and 1.21 ± 0.26 mg/kg, respectively.

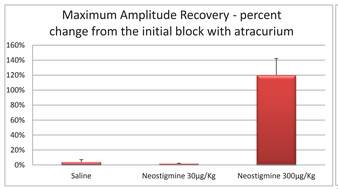
Neuromuscular block reversal by neostigmine

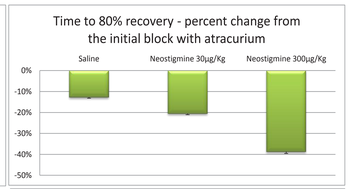


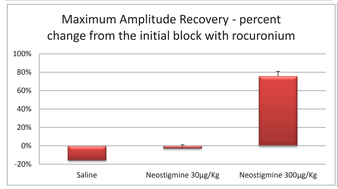


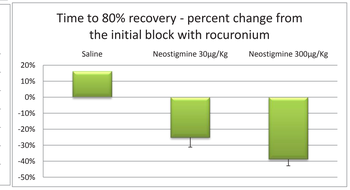












Time course of changes in twitch amplitude after NMB with rocuronium and atracurium at 3xED_{90}. The duration of NMB induced by either agent was shortened by neostigmine at 30 and 300 µg/kg. In addition, at 300 µg/kg, neostigmine over-reversed the neuromuscular block, as shown by the increase in twitch amplitude beyond basal level.

4 Conclusion

We have successfully developed a rat model of neuromuscular blockade considered to be reliable and robust in order to allow evaluation of efficacy of neuromuscular blockers and/or reversal agents, while monitoring main safety endpoints.

Since these experiments were conducted, this model has also been shown to be as suitable under isoflurane anaesthesia, while blood pressure could be monitored in addition to other vital endpoints.

5 References

Pickett *et al.* (2008). The in vivo rat muscle force model is a reliable and clinically relevant test of consistency among botulinum toxin preparations. Toxicon, 52, 455-464.