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Pharmacokinetic–Pharmacodynamic Modeling of Rocuronium in Case of a Decreased Number of Acetylcholine Receptors

A Study in Myasthenic Pigs

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Background: **In myasthenic patients, the sensitivity for nondepolarizing relaxants is increased and the time course of effect is prolonged due to a reduced number of functional acetylcholine receptors at the neuromuscular junction. The authors investigated both the performance of the link model proposed by Sheiner and a pharmacodynamic–pharmacokinetic model taking into account the number of unbound acetylcholine receptors in myasthenic pigs.**

Methods: **After obtaining the approval of the Animal Experiments Committee of their institution, the authors studied eight myasthenic pigs and eight control pigs. Myasthenia gravis was induced by injecting Torpedo acetylcholine receptors in weeks 1 and 4. On the day of the experiments, the pigs were anesthetized and intubated, and the appropriate muscles and nerves were prepared for the measurements. Rocuronium was administered by infusion to reach 90% twitch height block. Arterial blood was sampled during onset and offset of effect, and the plasma concentration of rocuronium was measured with high-performance liquid chromatography. Plasma concentration–time effect data were analyzed using two different pharmacokinetic–pharmacodynamic models, the link model according to Sheiner and a pharmacokinetic–pharmacodynamic model taking into account the unbound receptor concentration. Muscles were removed after the experiment for laboratory analysis of the acetylcholine receptor concentration.**

Results: **All eight pigs of the myasthenic group developed clinical signs of myasthenia gravis (muscle weakness) and showed increased sensitivity toward rocuronium. Pharmacokinetic modeling revealed no significant differences between myasthenic and control pigs. In pharmacokinetic–pharmacodynamic analysis, visual inspection as well as the Akaike Information Criterion (3,605 *vs.* 3,769) and the residual SD (3.2 *vs.* 3.6%) revealed a better fit for the unbound receptor model in myasthenic animals compared to the Sheiner model. Pharmacokinetic–pharmacodynamic analysis with the unbound receptor model demonstrated a decreased EC_{50} of 0.27 μM (ranging from 0.17 to 0.59 μM) compared to 2.71 μM (ranging from 2.42 to 4.43 μM) in control animals. The results of the Sheiner pharmacokinetic–pharmacodynamic analysis were in the same range. Both the laboratory analysis and pharmacokinetic–pharmacodynamic modeling showed a decrease in receptor concentration of more than 75%.**

Conclusion: **Both the Sheiner model and the unbound receptor model may be used to fit plasma concentration–effect data of rocuronium in pigs. The unbound receptor concentration model, however, can explain the observed differences in the time course of effect, based on receptor concentration.**

MYASTHENIA gravis is a chronic autoimmune disease characterized by a diminished number of (functional) acetylcholine receptors (AChRs) at the neuromuscular junction. In the majority of patients with generalized myasthenia gravis, antibodies against the AChR are detectable in the serum. These antibodies cause a decrease in functional AChR in three different ways: first, an increased turnover of AChR, induced by the antibodies; second, focal lysis of the postsynaptic membrane by complement activation; and third, pharmacological blockade of the AChR.[1,2] As a result, the sensitivity to nondepolarizing neuromuscular blocking agents (NMBAs) is increased; thus, lower doses of NMBAs are required to obtain a certain degree of neuromuscular block. Even after an equipotent dose, the time course of effect is prolonged.[3,4] There is currently no pharmacokinetic–pharmacodynamic model that will explain the typical combination of increased sensitivity and prolonged time course of NMBAs as found in myasthenic patients.

The most used pharmacokinetic–pharmacodynamic model is the Sheiner link model.[5] In most situations, it is an adequate model to describe the time course of effect of NMBAs. In simulations, the pharmacokinetic–pharmacodynamic model proposed by Sheiner may predict the higher sensitivity to and the longer recovery time of NMBAs in myasthenic patients based on a lower EC_{50} and γ. The Sheiner model, however, gives no insight as to why these parameters are changed in this group of patients. This model does not take into account the number of AChRs, and the decrease in the effective dose needed to obtain a 90% neuromuscular block (ED_{90}) in myasthenic patients can only be explained by a decreased effective concentration that corresponds with 50% neuromuscular block (EC_{50}), which does not alter the time course of effect of neuromuscular blocking drugs. Donati[6] proposed a model taking into account the AChR concentration at the neuromuscular junction. This model does not predict the alterations observed in myasthenic patients, either; it predicts a slightly decreased

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ED_{90} when the AChR concentration is decreased,[7] while the actual ED_{90} decreases by more than 50% in myasthenic patients.[8] The predicted interval 25–75% (time from 25% twitch depression to 75%) is decreased in sensitive patients, which is contrary to the increased interval 25–75% seen in myasthenic patients.

We developed a novel physiologically based pharmacokinetic–pharmacodynamic model assuming that the contractile force of a muscle after supramaximal stimulation is related to the concentration of free AChRs, *i.e.*, not blocked by NMBAs or antibodies against the AChR. This model predicts an increased potency and a prolonged duration of action of NMBAs in case of a reduced number of AChRs at the neuromuscular junction, in line with the changes observed in myasthenia gravis.

To study the Sheiner model in a myasthenic situation and to validate the proposed pharmacokinetic–pharmacodynamic model, we developed a pig myasthenic model and conducted a pharmacokinetic–pharmacodynamic modeling study.

Materials and Methods

Following approval of the Ethical Committee on Animal Experiments of the University of Groningen (Groningen, The Netherlands), 16 female pigs (Yorkshire F1 hybrid; body weight, 25–30 kg) were studied. They were divided in two groups: one control group and one myasthenic group.

Induction of Myasthenia Gravis

In the myasthenic group, pigs were injected intramuscularly on day 1 with 100 μg Torpedo AChR (tAChR), emulsified in Specol (id-dlo, Lelystad, The Netherlands). Three weeks after primary immunization, pigs were boosted with 100 μg tAChR, again emulsified in Specol. tAChR was prepared by purifying AChR from electric organs of Torpedo californica (Pacific Biomarine, Venice, CA) by affinity chromatography using Cobra toxin coupled to sepharose 4B (Pharmacia LKB, Woerden, The Netherlands).[9]

Clinical Assessment

The diagnosis of myasthenia gravis was made based on bending off of the growth curve, together with signs of muscular weakness. Pigs with myasthenia gravis lay down, or were only able to stand upright for a very short time. To objectify the degree of myasthenia gravis, a train-of-four (TOF) ratio was determined before the administration of rocuronium during the experiment under anesthesia (see Experimental Protocol). We diagnosed myasthenia gravis in the pigs without delay, by checking the pig's health and appetite every day after the second injection. If the pig had signs of myasthenia gravis and it was not possible to conduct the experiment immediately, the pig was temporarily treated with intramuscular pyridostigmine administered at 4-h intervals.

Experimental Protocol

After a fasting period of 16 h with free access to water, the pigs were anesthetized with 500 mg ketamine followed by 15 mg midazolam, given intramuscularly. Pigs were weighed, and an ear vein was cannulated to allow infusions of pentobarbital (2 $mg \cdot kg^{-1} \cdot h^{-1}$). Fentanyl bolus doses were given if needed (2–4 μg/kg). The trachea was intubated, and the lungs were artificially ventilated with an air–oxygen mixture, using a Cameco UV 705 respirator (Cameco, Sweden), with a frequency of 19 breaths/min, and 20 cm water pressure. The end-tidal carbon dioxide level was maintained between 4 and 5 kPa (Godart Capnograph Mark 11; E. Jaeger, Wuerzburg, Germany). Heart rate and blood pressure were measured continuously (Pressure Transducer, HP 78342A; Hewlett Packard, Boeblingen, Germany). Rectal temperature was measured continuously, and the temperature of the pig was maintained at 38°C with a heating blanket. The jugular vein was cannulated for infusion of fluids (2.5% glucose in 0.45% saline) and drugs (pentobarbital, fentanyl, and rocuronium). The left axillary artery was catheterized for continuous blood pressure measurements and for sampling of blood. The common peroneal nerve was exposed left and right and attached to two silver stimulation electrodes. To prevent repetitive backfiring, the nerve was ligated proximal to the electrodes. The overlying skin was closed, and the left nerve was stimulated supramaximally with square wave stimuli of 0.2 ms at a frequency of 0.1 Hz (Grass S88; Grass Instruments, Quincy, MA). The response of the left tibialis anterior muscle was registered mechanomyographically using a force transducer (LB 8000 25N; Maryland Instruments Corp., Baltimore, MD) connected to a muscle relaxation monitor MK11 and to a recorder (MT 9000; Astro-Med, West Warwick, RI). Preload was measured continuously and kept constant at approximately 75 g. The right nerve was stimulated every 15 s supramaximally with square wave TOF stimuli of 1 mA, with a duration of 0.2 ms at a frequency of 2 Hz. The muscle response was allowed to stabilize before starting an infusion with rocuronium. Before the rocuronium infusion, a blood sample was drawn for determination of circulating antibodies against the AChR (see Immunohistochemistry).

Each pig received an infusion with a muscle relaxant. After stabilization of the twitch, myasthenic pigs received an infusion of 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ rocuronium, until there was an 80% twitch depression. The infusion was then stopped. Control pigs received an infusion of rocuronium at a rate of 116.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ after stabilization of the twitch. The twitch height and TOF ratio (the ratio between the fourth and the first twitch response during TOF stimulation) were recorded continuously.

During onset of block and at maximal block, arterial samples of 2 ml were taken each minute. During recovery, samples were taken at 20, 40, 60, and 80% twitch height recovery Additional samples were taken regularly until an hour after the start of the rocuronium infusion. Blood was collected into lithium-heparin–coated tubes, stored at room temperature, and centrifuged for 10 min at 4,000*g* within an hour after taking the sample. Rocuronium has been shown to be stable in plasma at room temperature. The plasma was collected and stored at −20°C until analysis was done.

At the end of the experiments, the anterior tibial muscles were removed from all the pigs and stored at −80°C. Pigs were then terminated by an overdose of pentobarbital.

Determination of Rocuronium in Plasma

Determination of rocuronium in plasma was carried out by high-performance liquid chromatography using post column ion-pair extraction and fluorometric determination.[10] The method showed a linear relationship between the logarithm of the amount of rocuronium and the logarithm of the response ratio in a range of at least 10–1,000 ng. The mean precision, expressed in the coefficients of variation of the intraday variability and of the accuracy data, was 6%, whereby 200–1,000 μl plasma can be processed. The mean absolute deviation of the accuracy samples covering the concentration of the unknown samples was 5%. The lower limit of quantization, defined as the minimum concentration that could be detected with a precision and accuracy better than 15%, was 10 ng/ml. The lower limit of detection, the amount of rocuronium resulting in a signal-to-noise ratio of 5, was estimated to be 3 ng.

Pharmacokinetic Analysis

Plasma concentration–time data were analyzed by iterative Bayesian two-stage analysis[11] and using the program Multifit (Johannes H. Proost, Pharm.D., Ph.D., Department of Anesthesiology, University Hospital Groningen, Groningen, The Netherlands). Mean values and SDs of the pharmacokinetic population parameters of a one-compartment model (CL, V_1), a two-compartment model (CL_{10}, V_1, CL_{12}, V_2), and a three-compartment model (CL_{10}, V_1, CL_{12}, V_2, CL_{13}, V_3) and the residual variance were estimated by an iterative Bayesian procedure using the plasma concentration–time data of each examined pig. A log-normal distribution for both the pharmacokinetic population parameters and the plasma concentration measurement errors was assumed. The correctness of the latter assumption was tested by visual inspection of the graphs of the residuals plotted against time and against concentration. Goodness of fit was evaluated from visual inspection of the measured and calculated data points and of the residuals plotted against time and against concentration. The choice between a one-, two-, and three-compartment model was based on the Akaike Information Criterion (AIC).[12] The AIC was calculated as −2 times the sum of the log likelihood of all individual animals within the group and increased by a value of 2 for each variable parameter. The groups of myasthenic and control pigs were fitted separately.

Pharmacokinetic–Pharmacodynamic Analysis

Two pharmacokinetic–pharmacodynamic models were applied to the data. First, the Sheiner model, with an effect compartment linked to the plasma compartment, was applied to the data using the actual plasma concentration data.[13] Second, we applied a new link model, which takes into account the number of the free AChR concentration, the unbound receptor model (see Appendix). The twitch height–time data were analyzed by an iterative Bayesian two-stage analysis using the program PkPdFit (written by Johannes H. Proost, Pharm.D., Ph.D.). The goodness of the fit was evaluated by visual inspection of the measured and calculated data, by the degree of minimization of the hysteresis loop, by the residual SD, and by the AIC value. The groups of myasthenic and control pigs were fitted separately. The molecular weight of rocuronium is 609.69.

Determination of AChR

The concentration of AChR was determined in anterior tibial muscles of control pigs and myasthenic pigs as described previously,[14] with minor modifications. Briefly, frozen tissue was homogenized, and AChR was extracted with 2% Triton X-100 (Sigma, Brunschwig Chemie b.v., Amsterdam, The Netherlands). An aliquot of 500 μl of each extract was labeled with ^{125}I-α-bungarotoxin (74 TBq/mmol; Amersham Pharmacia Biotech UK Limited, Little Chalfont, United Kingdom), incubated overnight with excess rat anti-AChR IgG (MAb35) and precipitated by goat antirat antibodies. Radioactivity was measured in a γ counter.

Immunohistochemistry

Muscle biopsy cryosections (thickness, 8 μm) of anterior tibial muscle of myasthenic and control pigs were acetone-fixed for 10 min at −20°C and air-dried for 5 min. The sections were preincubated in PBS containing 2% bovine albumin serum and 2% normal rabbit serum and subsequently incubated with plasma from pigs who developed clinical signs of myasthenia gravis, together with rhodamine-labeled α-bungarotoxin (Molecular Probes, Eugene, OR) overnight at 4°C to colocalize AChR in the same section. After being washed three times with PBS, the sections were incubated for an hour with FITC-labeled rabbit antiswine IgG antibodies.

Statistical Analysis

Differences between myasthenic and control pigs were tested with the Mann–Whitney U test; statistical

Table 1. Train-of-four Ratio, Rocuronium Dose Requirement, and Interval in Control Pigs and Myasthenic Pigs

	Control Pigs	Myasthenic Pigs
TOF ratio, %	104 (100–118)	58 (27–75)
Rocuronium dose, mg/kg	705 (470–930)	96.5 (62–220)
Interval 25–75%, s	169 (110–314)	479 (353–685)

Values are shown as median with range in parentheses. TOF ratio is measured before rocuronium administration.

Interval 25–75% = time elapsed from 25 to 75% twitch height during offset of block; TOF= train-of-four.

Table 2. Results of Pharmacokinetic Analysis

	Control	Myasthenic	*P*
CL_{10}, ml · kg^{-1} · min^{-1}	26 (18–35)	28 (17–51)	NS
V_1, ml/kg	53 (39–67)	41 (34–60)	NS
CL_{12}, ml · kg^{-1} · min^{-1}	10.5 (6.0–14.5)	10.7 (9.1–12.2)	NS
V_2, ml/kg	90 (65–105)	60 (50–103)	NS

Values are shown as median with range in parentheses.

CL_{10} = clearance; CL_{12} = distribution clearance from central to peripheral compartment; V_1 = distribution volume of central compartment; V_2 = distribution volume of peripheral compartment.

significance was assumed if *P* was smaller than 0.05. The interval 25–75% was defined as the time elapsed between 25% twitch height and 75% twitch height. The AIC was used for comparison between two pharmacokinetic–pharmacodynamic models; a lower value of the AIC represents a better fit.[12]

Results

We induced myasthenia gravis in eight pigs. All pigs became myasthenic, six pigs became myasthenic between 7 and 9 days after the second injection with tAChR, one pig needed three injections with tAChR before it became myasthenic, and one pig became myasthenic 1 week after the first injection. Most pigs showed, apart from signs of muscular weakness, bending in their weight gain after the second injection with tAChR.

The baseline TOF ratio, which was measured before rocuronium administration, was less than 90% in all myasthenic pigs, and the dose of rocuronium needed to obtain a block between 80–90% twitch height depression was reduced by 83% compared to control pigs. There was a significant difference between intervals 25–75% ($P < 0.0005$) between myasthenic pigs and control pigs (table 1).

Pharmacokinetic Analysis

For each group, the plasma concentration–time data of all pigs were included in an iterative Bayesian two-stage analysis. According to the AIC, a three-compartment model did not fit significantly better than a two-compartment model. The AIC values were for myasthenic pigs 255, 97, and 128 and for control pigs 327, −153, and −148 for one-, two- and three-compartment models, respectively. The results of the analysis are summarized in table 2.

Pharmacokinetic–Pharmacodynamic Modeling

The time course of effect of the neuromuscular block is depicted in figure 1, both for control pigs (A) and myasthenic pigs (B).

The results of the fitting procedure with the unbound receptor model are presented in table 3. The AIC after the fitting procedure with the unbound receptor model was 3,605 *versus* 3,769 with the Sheiner model, the residual SD being 3.16 *versus* 3.58%, respectively. This better fit with the unbound receptor model arises from a better performance at profound block and late recovery than the Sheiner model for myasthenic pigs. An example of how this model improves fitting is shown by figure 2. Both models performed almost equally well for control pigs with an AIC of 2,334 *versus* 2,323 and a residual SD of 2.66 *versus* 2.62% for the unbound receptor model and Sheiner model, respectively. There was no significant difference between the EC_{50} derived with the Sheiner model or the unbound receptor model, both for control pigs and myasthenic pigs. In tables 3 and 4, the results of the fitting procedure with the unbound receptor model and the link model proposed by Sheiner, respectively, are summarized.

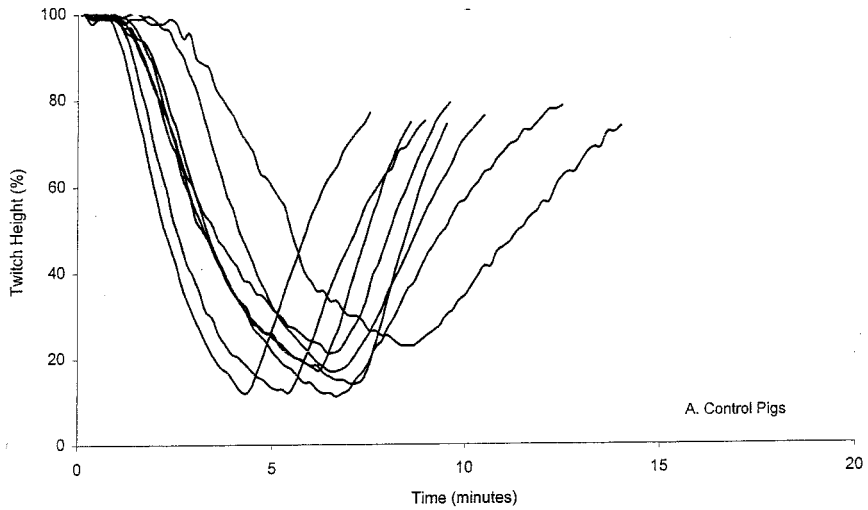
The unbound receptor model calculates an AChR concentration of 0.27 μM in myasthenic pigs and an AChR concentration of 1.47 μM in control pigs. This equals a decrease in AChR of 81% compared to control pigs. Using the *in vitro* radioactive immunoassay, we found a decrease in AChR concentration in myasthenic pigs of 76% compared to control pigs.

The serum of all myasthenic pigs contains antibodies directed against the neuromuscular junction, as shown by the indirect immunofluorescence assay (fig. 3).

Discussion

We developed an animal myasthenia gravis model that enabled us to study the time course of effect of NMBAs *in vivo*. We preferred to develop a pig model because it would allow us to draw a substantial number of arterial samples to determine the concentration of rocuronium in plasma over time. Furthermore, over time, a large number of pharmacokinetic–pharmacodynamic data about rocuronium in pigs is available[15,16] for comparison.[17] We also choose the pig as the animal species because it is an omnivore in which pharmacokinetic behavior of rocuronium may more closely resemble this behavior in humans.

For the first time, a reproducible myasthenia gravis model in pigs has been established since all immunized



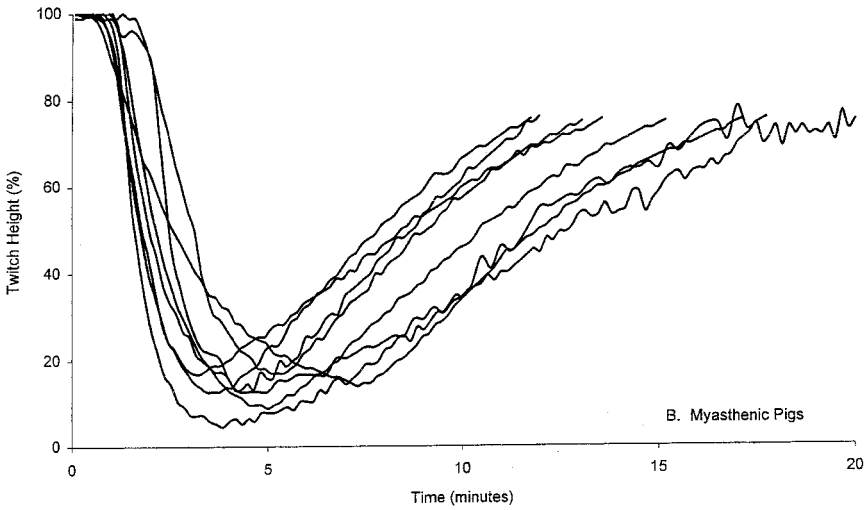


Fig. 1. Raw data on the time course of effect of the neuromuscular block in control pigs (*A*) and myasthenic pigs (*B*). The infusion rates were 116.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in control pigs and 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in myasthenic pigs.

pigs developed clinical signs of myasthenia gravis. The pigs showed a decreased need for rocuronium and the control TOF ratio was decreased to 58%, before the start of rocuronium administration. A decrement is also seen in humans with myasthenia gravis before the start of relaxant infusion,[18–20] although to some lesser extent. Our myasthenic pigs were more severely myasthenic than patients who undergo surgery after treatment of their myasthenic condition by plasmapheresis or immunosuppressant drugs. There seems to be some heterogeneity in the disease state of these myasthenic pigs as observed clinically and as measured by AChR loss; some are more affected by the disease than others. Probably, the immune response to the tAChR injection is quantitatively different among different pigs. This heterogeneity has been described earlier for humans[21] and rats.[22]

Table 3. Pharmacokinetic–Pharmacodynamic Fitting Results for the Unbound Receptor Model

	Control URM	Myasthenic URM	*P*
k_{e0}, min	0.93 (0.40–1.29)	0.41 (0.16–0.71)	0.010
β	2.84 (2.58–3.31)	2.63 (1.75–9.02)	NS
R_{tot}, μM	1.47 (1.33–2.29)	0.27 (0.08–0.42)	<0.001
EC_{50}, μM	2.71 (2.42–4.43)	0.27 (0.17–0.59)	<0.001

EC_{50} is a calculated value and not a model parameter; molecular-weight rocuronium = 609.69. Values are shown as median with range in parentheses.

β = exponential coefficient of twitch height–unbound receptor concentration relation; EC_{50} = rocuronium concentration at 50% effect; k_{e0} = rate constant between plasma and effect compartment; R_{tot} = total receptor concentration; URM = unbound receptor model.

The intervals 25–75% (table 1) in myasthenic pigs are significantly longer than those of control pigs, which can be understood from the myasthenic disease which leads to less AChR at the neuromuscular junction. This means that the safety margin is decreased. In our opinion, the unbound receptor model may predict this prolonged

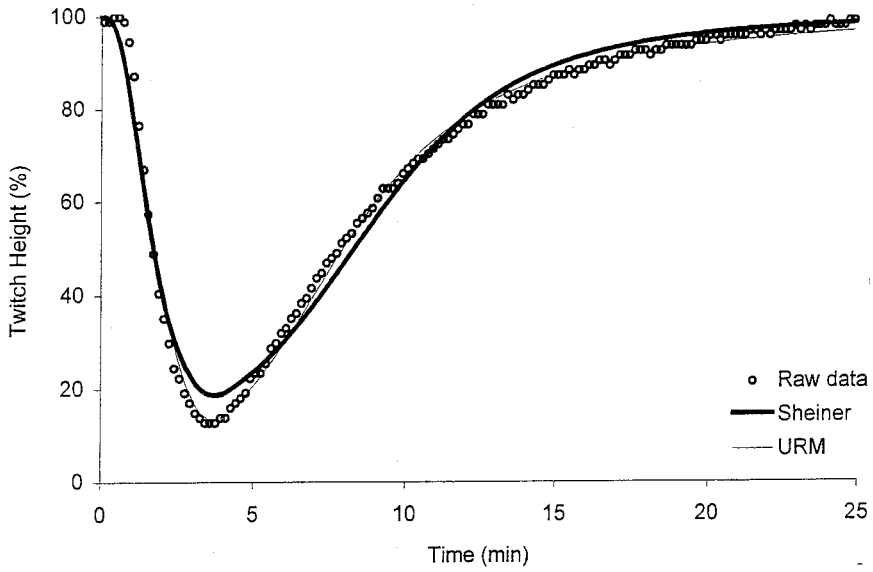


Fig. 2. Twitch height *versus* time in a typical myasthenic pig. The thin solid line represents the results of the fitting procedure with the unbound receptor model (URM), the thick solid line represents the fitting procedure with the Sheiner model, and the unfilled circles are the actually measured points.

recovery more adequately, based on receptor concentration, than the Sheiner model, based on a different γ.

The pharmacokinetic modeling revealed that the pharmacokinetic parameters did not differ widely between both groups, which could be expected because myasthenia gravis only affects the neuromuscular junction. Although the circulating volume may be decreased in the myasthenic pigs since the myasthenic state prevents the animal to maintain its normal food and water intake, nevertheless, from the kinetic analysis, it is clear that this does not influence the kinetic parameters to a large extent because we started the experimental procedure shortly after the occurrence of myasthenic weakness in the pigs.

The use of a pharmacokinetic–pharmacodynamic model that takes the number of unbound (free) receptors into account improves pharmacokinetic–pharmacodynamic modeling of the concentration–effect relationship of rocuronium in myasthenic pigs when compared to the link model proposed by Sheiner.[5] The most relevant advance of the unbound receptor model over the Sheiner model is that the unbound receptor model is designed to give a more physiologic explanation of the observed changes in the time course of effect. We chose to model the unbound receptor concentration because it is well known that different effects cause the diminishing of functional AChR at the neuromuscular junction of myasthenic animals. Antibodies against AChR interfere with the neuromuscular junction in three ways.[23] First, the antibodies against the AChR accelerate the rate of degradation of the receptor, resulting in a decrease of AChR at the neuromuscular junction. A second mechanism is activation of the complement that causes destruction of the postsynaptic membrane by a lytic membrane attack complex (this also decreases the AChR concentration). The third mechanism is the direct blockade of the AChR by the antibodies that bind to the AChR, which causes allosteric inhibition of the receptor function, the antibody being many times as large as an ACh molecule or an NMBA.[2]

Table 4. Pharmacokinetic–Pharmacodynamic Fitting Results for the Sheiner Model

	Control Sheiner	Myasthenic Sheiner	*P*
k_{e0}, min	0.76 (0.31–1.08)	0.35 (0.11–0.57)	0.021
γ	2.87 (2.67–3.34)	1.70 (1.11–3.04)	0.010
EC_{50}, μM	2.83 (2.50–4.61)	0.27 (0.17–0.60)	<0.001

Molecular-weight rocuronium = 609.69. Values are shown as median with range in parentheses.

γ = steepness of concentration–effect relation; EC_{50} = rocuronium concentration at 50% effect, molecular weight rocuronium = 609.69; k_{e0} = rate constant between plasma and effect compartment.

The Sheiner model shows a decreased EC_{50} in myasthenic pigs (0.27 *vs.* 2.8 μM in control pigs) and a decreased γ in myasthenic animals (1.70 *vs.* 2.87 in control animals). Both the EC_{50} and γ differ significantly between myasthenic animals and the control group. The lower EC_{50} reflects the increased sensitivity to rocuronium in myasthenic pigs (and patients), while the lower γ reflects the prolonged duration of neuromuscular block. The Sheiner model describes the observed differences in the time course of myasthenic pigs by a lower EC_{50} and γ, but it does not provide an explanation to why these values would be lower. The unbound receptor model calculates a significantly decreased receptor concentration in myasthenic pigs (R_{tot}, 0.27 *vs.* 1.47 μM in control animals). This decreased receptor concentration leads to the increased sensitivity to NMBAs and thus the lower EC_{50}. The decrease in receptor concentration, calculated by the unbound receptor model, is in agreement with the decrease we measured during the radioimmunoassay. This observation supports the explanatory value of the unbound receptor model. There is no

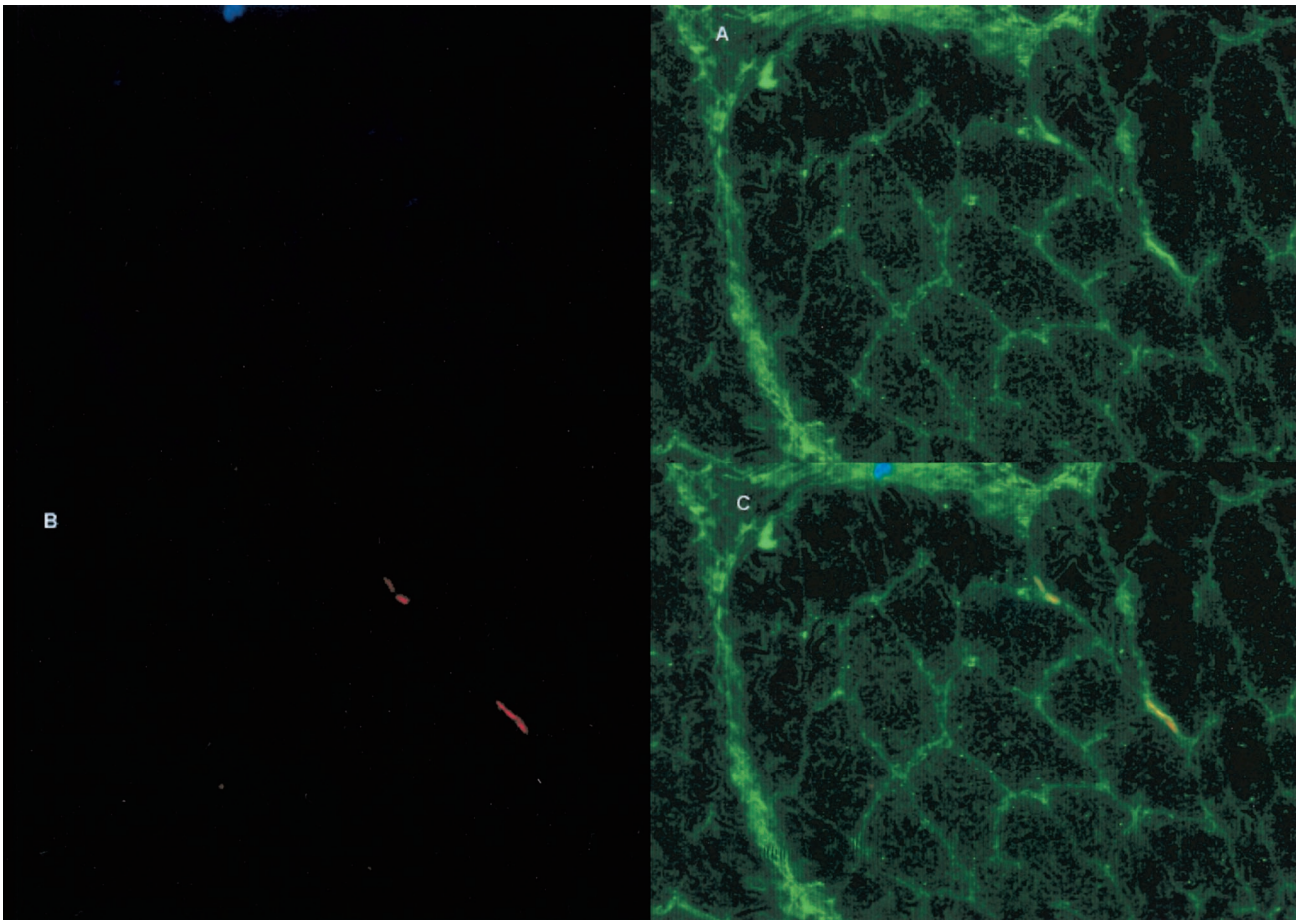


Fig. 3. The plasma of myasthenic pigs contains circulating antibodies, directed against the neuromuscular junction. (*B*) Rhodamine staining of AchR, indicating the place of the neuromuscular junction in the anterior tibial muscle. (*A*) The same muscle section incubated with EAMG serum and subsequently with FITC-conjugated rabbit antiswine IgG antibodies. The same structures as in panel B can be seen, stained green. (*C*) Colocalization of rhodamine α-BTX– and FITC-conjugated rabbit antiswine IgG antibodies (orange).

significant difference in β between myasthenic and control pigs. This is because the unbound receptor model explains the observed differences in potency and time course of effect of NMBAs from the decrease in receptor concentration in myasthenic pigs, without the need for a change in the steepness of the concentration-*versus*-effect relationship (β).

The Sheiner model contains three parameters, which can be assessed reliably. The unbound receptor model contains five parameters. These five parameters could not be uniquely identified, indicating that the model is overparameterized in relation to the available data. Therefore, we estimated two parameters in a different way and used these estimates as fixed parameters. K_d was estimated from the observed mean EC_{50} in control pigs and the assumption that in control pigs, receptor occupancy is 87.5% at 50% neuromuscular block. The parameter K_d in the unbound receptor model is the dissociation constant of the rocuronium-receptor complex. Since it is likely that receptors are the same in all normal pigs, it follows that the value of K_d is the same in all normal pigs. In myasthenic pigs, part of the AChRs is blocked by antibodies or internalized. There is no *a priori* reason why the remaining functional receptors would be different, for the very reason that they are functional. In the unbound receptor model, R_{tot} reflects these functional receptors only. Similarly, we assumed that R_{free50} was the same in all pigs.

To investigate the implications of the assumption of 87.5% receptor occupancy at 50% in control pigs on the presented results, we repeated the analysis with other values for this assumption. Assuming that 50% block is reached at 75% receptor occupancy in control pigs, K_d is increased by a factor 7/3 to 0.94 μM. An R_{free50} value of 0.63 μM was obtained by minimizing of the AIC during fitting of the animals of both groups simultaneously. After fitting of the groups separately, the values of k_{e0} were only slightly affected, and median β was increased from 2.63 to 4.22 in myasthenic pigs and from 2.84 to 3.29 in control pigs, but median R_{tot} markedly increased from 0.27 to 0.72 μM in myasthenic pigs, and from 1.47 to 2.42 μM in control pigs. The ratio between the median

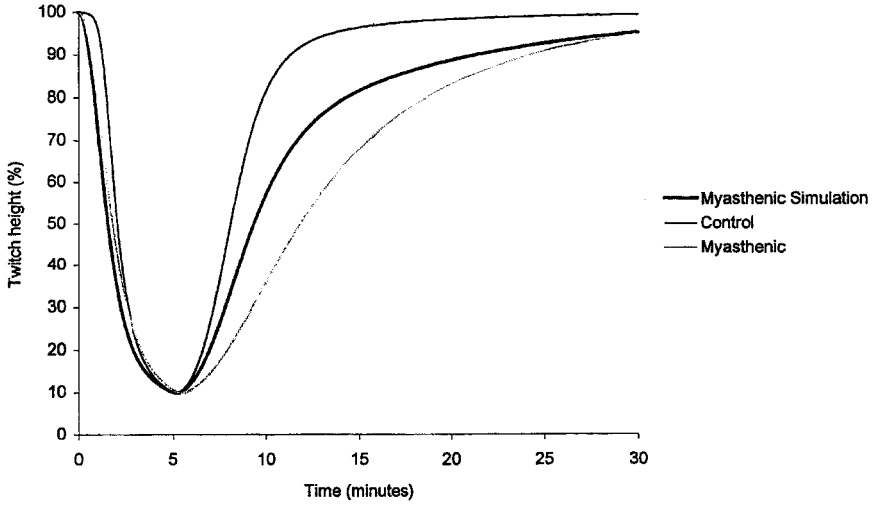


Fig. 4. Simulations of the time course of effect in control and myasthenic pigs. This simulation is carried out with the median pharmacokinetic and pharmacodynamic data for control (Control) and myasthenic pigs (Myasthenic) (tables 2 and 3) and an infusion time of 5 min. For control pigs, the simulated ED_{90} for a 5-min infusion was 790 μg/kg rocuronium and 160 μg/kg in myasthenic pigs. We added a simulation based on data for control pigs but with a value of R_{tot} equal to that in myasthenic pigs (Myasthenic Simulation). The simulated ED_{90} for this curve was 125 μg/kg. The predicted interval 25–75% is 2.5 min in control pigs *versus* 5.3 min in the myasthenic simulation, a prolongation that is due to the difference in receptor concentration only (1.47 μM in control pigs and 0.27 μM in myasthenic pigs).

values of both groups was moderately affected, from 0.19 in the original analysis to 0.29 for a K_d value of 0.94 μM. Both values agree reasonably well with the decrease of 76% obtained from the radioimmunoassay. From this analysis, it can be concluded that the absolute values of R_{tot} are indeed dependent on the values for K_d and R_{free50}, but the conclusions of the study are not markedly affected by the assumptions with respect to receptor occupancy at 50% block.

We did a pharmacokinetic–pharmacodynamic simulation of the time course of effect of an ED_{90} infusion of rocuronium with the data obtained in this study (fig. 4). We used the median pharmacokinetic and pharmacodynamic values of control pigs (control) and myasthenic pigs (myasthenic) (tables 2 and 3). We also used the results of the control pigs and only reduced the receptor concentration from 1.47 μM to 0.27 μM to show the effect of receptor concentration (myasthenic simulation).

The figure shows that the receptor concentration has little influence on the interval 75–25% during onset (1.3 min in control pigs *vs.* 1.5 min in the myasthenic simulation *vs.* 1.8 min in myasthenic pigs), but the interval 25–75% during recovery is increased in the myasthenic simulation (5.3 min) and in myasthenic pigs (8.3 min) compared to controls (2.5 min). The myasthenic simulation predicts not exactly the measured interval 25–75% we measured in myasthenic pigs, but in the myasthenic pigs, the k_{e0} was different from the k_{e0} of control pigs, which may explain the difference between the simulation and the results in the *in vivo* pig. This may probably be related to the existence of a relatively hypovolemic state in these myasthenic animals, resulting in a diminution of the cardiac output and consequently in a smaller value of the rate of equilibration k_{e0}. This assumption is supported by the observation that the initial volume of distribution is lower in six out of eight myasthenic pigs compared to control pigs.

As far as we know, this is the first study on pharmacokinetic–pharmacodynamic modeling of NMBAs in a myasthenic situation. There have been dose–response studies[19,24,25]; case reports about the use of NMBAs in myasthenic patients,[8,26–30] which have in common that the effective dose of a nondepolarizing NMBA is decreased; and reviews with advice for anesthetic management in myasthenic patients.[31,32]

We conclude that the unbound receptor pharmacokinetic–pharmacodynamic model explains the observed changes in the time course of effect of rocuronium in the myasthenic pig on a more physiologic base than the Sheiner model. Further studies to validate the model in myasthenic patients will need to be conducted.

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Appendix: Unbound Receptor Model

The existing pharmacokinetic–pharmacodynamic models assume that the neuromuscular blocking effect is related to the (unbound) concentration of the drug in the effect compartment, *i.e.*, the neuromuscular junction.[5,6] However, this assumption makes no sense from a mechanistic point of view because the effect is the result of binding of the NMBA to the receptor, and not just of the presence of free drug in the biophase. Alternatively, one might relate the effect to the concentration of bound NMBA. This hardly affects the model in a qualitative way since the unbound concentration and the bound concentration are strongly correlated.

NMBAs are antagonists of acetylcholine, which is in turn responsible for neuromuscular transmission. Therefore, we postulated that the contractile force of a muscle after supramaximal stimulation, measured as twitch height (TH), is related to the free AChR concentration according to the sigmoid E_{max} model (Hill equation):

$$TH = TH_{max} \cdot \frac{R_{free}^{\beta}}{R_{free}^{\beta} + R_{free50}^{\beta}} \quad (1)$$

where TH_{max} = maximum twitch height, *i.e.*, TH in case the number of free receptors is infinitely high; R_{free} = concentration of free recep-

tors; R_{free50} = concentration of free receptors at which TH is 50% of TH_{max}; and β = exponential coefficient.

In the absence of NMBA, the concentration of free receptors equals the total receptor concentration; on substitution in equation 1, it follows:

$$TH_c = TH_{max} \cdot \frac{R_{tot}^{\beta}}{R_{tot}^{\beta} + R_{free50}^{\beta}} \quad (2)$$

where TH_c = twitch height in the absence of NMBA, and R_{tot} = total receptor concentration.

The neuromuscular blocking effect of an NMBA is defined as:

$$E = 1 - \frac{TH}{TH_c} \quad (3)$$

Substituting equations 1 and 2 in equation 3 yields:

$$E = \frac{1 - \left[\frac{R_{free}}{R_{tot}}\right]^{\beta}}{1 + \left[\frac{R_{free}}{R_{free50}}\right]^{\beta}} \quad (4)$$

Equation 4 describes the neuromuscular blocking effect as a function of the free AChR concentration. R_{tot}, R_{free50}, and β are model parameters.

Binding of the NMBA to the AChR binding sites is characterized by the equilibrium dissociation constant of the drug-receptor complex (K_d):

$$Kd = \frac{Cu_e \cdot R_{free}}{R_{bound}} \quad (5)$$

where Cu_e = unbound concentration of NMBA in the effect compartment, R_{free} = concentration of free binding sites of AChR, and R_{bound} = concentration of AChR receptor sites to which an NMBA molecule is bound.

Defining R_{tot} as the total concentration of AChR binding sites, it follows upon rearrangement:

$$R_{free} = \frac{R_{tot} \cdot K_d}{Cu_e + K_d} \quad (6)$$

The time course of the unbound concentration in the effect compartment can be evaluated as described earlier.[7] Equation 16 of that article was simplified, assuming Cu = C (fu = 1), $fu_e = 1$, $ku_e = k_{e0}$, and $Pu_e = 1$, resulting in:

$$\frac{dCu_e}{dt} = \frac{k_{e0}}{1 + \frac{R_{tot} \cdot K_d}{(Cu_e + K_d)^2}} \cdot (C - Cu_e) \quad (7)$$

where Cu is the unbound relaxant concentration in plasma compartment, C is the concentration in plasma compartment, fu is the fraction unbound relaxant in plasma compartment, fu_e is the fraction unbound relaxant in effect compartment, ku_e is the transport rate constant, and Pu_e is the ratio between unbound concentration of relaxant in plasma compartment and effect compartment during steady state.

Fitting of each of the model parameters (k_{e0}, K_d, R_{tot}, R_{free50}, and β) did not result in reliable parameter estimates due to the strong correlation between the parameters, in particular between K_d, R_{tot}, and R_{free50}. Since the aim of the study was to investigate the influence of the AChR concentration (R_{tot}) on the potency and time course of effect, this parameter was assumed to be variable between the animals. In contrast, the values for K_d and R_{free50} were assumed to be the same for each animal. Estimates for these values were obtained as follows: Assuming that a neuromuscular block of 50% is reached at a receptor occupancy of 87.5% in case of a normal AChR density (*i.e.*, in controls), the value of R_{free} at 50% block is 12.5% of the normal total receptor concentration.[33] Using the Sheiner model, the median EC_{50} in control animals was 2.83 μM. On substitution of this value of EC_{50} for Cu_e in equation 6, and $R_{free}/R_{tot} = 0.125$, it follows that $K_d = 0.40$ μM. This value was used for each animal of both groups. A value of R_{free50} of 0.19 μM was estimated by minimization of the AIC during fitting of the animals of both groups simultaneously.

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