

Resistance to Atracurium in Thermally Injured Rats

The Roles of Time, Activity, and Pharmacodynamics

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Thermal injury induces resistance to nondepolarizing muscle relaxants in patients. Because the mechanism of the resistance is unknown, the authors have sought to establish thermally injured rats as a suitable model for subsequent detailed studies of mechanisms. Two hundred twenty-five- to 250-g rats sustained a 30% total body surface area thermal injury while anesthetized with pentobarbital. Another group had sham injury. Animal activity was monitored both by periods of direct observation and by use of activity cages. At 10, 20, 30, 40, 60, and 90 days after injury, rats were anesthetized and ventilated and the strength of contraction of their gastrocnemius produced by supramaximal stimulation of the sciatic nerve was measured before and after a bolus of atracurium (2.0 mg/kg) was administered. The plasma concentration required to diminish contraction to 50% of the preceding value (Cp_{50}) was determined by atracurium infusion. Animals displayed the greatest resistance to atracurium at 40 days. The Cp_{50} value was also greatest at this time. The protein binding of atracurium was identical for both sham and injured groups. Activity for thermally injured resistant rats and for sham animals was not different. It appears that pharmacodynamic mechanisms are involved, and inactivity and disuse atrophy are not necessary in rats for development of resistance to nondepolarizing muscle relaxants after thermal injury. (Key words: Burns. Neuromuscular relaxants, nondepolarizing; atracurium. Pharmacokinetics; protein binding. Pharmacodynamics.)

NONDEPOLARIZING MUSCLE RELAXANTS are used in patients with thermal injury in preference to depolarizing relaxants, such as succinylcholine, which may produce hyperkalemia and cardiac arrest.¹ However, thermal injury produces resistance to a variety of nondepolarizing muscle relaxants (NDMRs), such as *d*-tubocurarine,² metocurine,³ and atracurium.⁴ The mechanism for the increased requirements for NDMRs in burned patients is not clear. Martyn *et al.*⁵ have demonstrated no significant changes in pharmacokinetics of *d*-tubocurarine after thermal injury, but six of the eight patients included were studied less than 8 days after their injury—a time when one would not expect resistance to have developed.⁴ Disuse atrophy has been suggested to account for resistance, but the hypothesis has not been critically evaluated.

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In this study, we describe observations of the response to atracurium in thermally injured rats. The purposes of the study were as follows: 1) to determine if the burned rat was a suitable model for the study of resistance to NDMRs in thermally injured humans; 2) to establish the time course of the development of resistance to atracurium; 3) to determine whether pharmacodynamic mechanisms were involved in resistance to NDMRs; and 4) to assess the possible contribution of skeletal muscle disuse. Resistance to the effects of neuromuscular relaxants of muscle not directly under the burned area was studied to preclude an effect of direct injury. By using rats, we were able to study the effect of a uniform injury in the absence of various drugs, therapies, bed rest, and infections.

Methods

The studies were approved by the Animal Experimentation Committee of the University of Washington.

Sprague-Dawley female rats weighing 225–250 g were anesthetized with 43 mg/kg of sodium pentobarbital. The back of each was shaved, and the animal was placed in a template that exposed an area of the back equal to 30% of the total body surface area. The sham animals were removed from the template and allowed to recover. The experimental animals were exposed to boiling water for 10 s. This exposure has been shown by microscopic studies to invariably produce a full-thickness third degree burn.⁶ None of the skin over the rear extremities was injured. The animals received 10 ml of saline ip to replace fluid loss for the first 24 hours after injury and were allowed to recover. The 2-day mortality was 7% in both groups. One day after injury all animals were eating, drinking, and moving around their cages normally. Another study examining eight pain-related behaviors has shown little or no difference in these behaviors between thermally injured and sham-injured rats over a period of 4 weeks after injury.**

Starting at 10 days after injury, rats were anesthetized with urethane (1,250 mg/kg), and arterial and venous cannulae were inserted and a tracheostomy performed. The animal's lungs were mechanically ventilated with a Harvard® small animal ventilator, and rectal temperature

** Butler SH, Mackie A, Pavlin E, Haschke R, Slattery J: Behavioural changes in rats post 30% burn: Is nociception present? Pain 22 (suppl 4):S14, 1987.

was maintained from 36.5° C to 37.5° C with a warming pad and recorded by a rectal thermistor. The gastrocnemius muscle and sciatic nerve were exposed and cleaned and the femur and tibia fixed to a board. The Achilles tendon was severed and attached to a Grass® force transducer, the output of which went to a strip chart recorder. The sciatic nerve was then stimulated at a supramaximal voltage with a 1-ms square wave at a rate of 0.1 Hz. Atracurium, 2 mg/kg, was administered as an iv bolus, and the degree and time course of paralysis were measured. After return of twitch height to approximately 80% of the initial value, an additional bolus of atracurium was administered and an infusion of atracurium was begun, the infusion rate varied as necessary to maintain the twitch height at 50% of maximum (*i.e.*, 50% of preatracurium value). After 20 min, during which twitch response to stimulation was stable (at 50% depression), an arterial blood sample was drawn for atracurium assay performed as described below.

In another group of five animals, twitch depression was carried out exactly as above with maintenance of 50% twitch depression. Blood samples were drawn at 10 and 20 min after a stable twitch depression of 50% was reached and compared, to assess whether a stable blood level had been achieved.

Thermally injured animals were studied at 10, 20, 30, 40, 60, and 90 days after injury with six to eight rats at each time point. Sham-injured animals were studied at 10, 40, and 90 days. After the bolus dose, data obtained were the percentage maximal twitch depression and the time to return to 50% of control (preatracurium) twitch. When a study 50% twitch depression had been present for 20 min, the arterial blood sample was obtained for analysis of the atracurium concentration. The blood samples (5 ml) were drawn into heparinized plastic syringes and quickly transferred to polypropylene tubes containing 100 μ l 3 N HCl (to prevent degradation of atracurium in plasma). The plasma was immediately separated in a cold centrifuge and stored in a plastic tube at -70° C before analysis.

MEASUREMENT OF ATRACURIUM IN PLASMA

The procedure of Stiller *et al.*⁷ was modified for atracurium measurement in plasma. Alcuronium (Hoffman-LaRoche) was added to 0.1 N HCl to achieve a concentration of 1 mg/ml and allowed to stand at room temperature for 8 h to allow conversion to a product (not identified) stable in HCl. This solution was stored at 4° C and diluted 1:1 in 1 mM HCl for analysis. The plasma sample was thawed and 0.5 ml was combined with 10 μ l of internal standard solution and applied to a C-18 Sep-Pak® column (Waters). The column was preconditioned with methanol and 0.1 M phosphate buffer (pH 5.4). After

the sample was applied, the column was then washed with 2 ml of the phosphate buffer followed by 1 ml of methanol to water (60:40). The drug and internal standard were eluted with 1 ml acetonitrile to 0.1 N HCl (75:25).

Separation was achieved by high-performance liquid chromatography (HPLC) on a 5- μ m C-18 radially compressed column (Waters) with a mobile phase consisting of 30 mM tetramethyl ammonium hydroxide in 30 mM KH₂ PO₄ (pH 3.0) and acetonitrile (64:36) delivered at a rate of 1 ml/min. The system was maintained at ambient temperature, and peaks were quantified by fluorescence (235 nm excitation, 310 nm emission) (Kratos Analytical®). Peak height was determined manually from a strip-chart recorder. The assay was linear over a concentration range of 1-10 μ g/ml, and recovery after extraction was about 90%.

ATRACURIUM PROTEIN BINDING

The binding of atracurium to plasma proteins was determined in seven rats at 40 days after injury and in seven sham-injured animals. Plasma samples obtained for Cp₅₀ measurement were thawed, and a 150- μ l aliquot was quickly removed and frozen again for determining total atracurium concentration. The pH of the rest of the plasma was adjusted to 6.0-6.2. This range was chosen to be acidic enough to prevent rapid degradation of the atracurium (no detectable change in concentration by HPLC over 30 min under these conditions) and to minimally affect plasma protein binding. The pH-adjusted plasma (800 μ l) was transferred to an Amicon® micro-partition system with a YMT membrane that retains 99.9% of the serum proteins and demonstrates very low nonspecific binding of small molecules. Plasma was centrifuged in this device for 15 minutes at room temperature at 1,200 \times g. Protein-free filtrate (150 μ l) was combined with 10 μ l of the internal standard and extracted with the use of a Sep-Pak® as described before.

ANIMAL MOVEMENT

Animal activity was assessed to determine if skeletal muscle disuse and, hence, atrophy could contribute to altered response to NDMRs. Two methods were used.

1) Rats were observed singly in an isolated 30" \times 30" \times 30" observation chamber in a dark room. The chamber was made of plywood, except for a single wall of one-way glass, and was illuminated by a 15-watt bulb in the roof. Rats were introduced to the chamber before the injury for accommodation and baseline measurements. Thirty-minute observations of individual thermally and sham-injured rats were performed at 1, 2, 3, and 4 weeks after injury. Activity (movement of at least one limb) was recorded as seconds per 30-min observation. These observations were performed by a trained observer (Dr. S. H.

Butler) using techniques validated in rats in previous studies.⁸

2) Twelve-hour night-time activity was quantified for the first 4 weeks by placing rats in individual cages mounted on activity detectors. The preinjury activity was quantified for the 12-h night-time period (6 P.M. to 6 A.M.) of the night before injury. (Rats are typically most active at night; therefore, any variation between burned and unburned animals would be most evident.) After injury (or sham injury) movement was recorded over the 12-h night-time period on nine to 13 occasions during the 4 weeks after the injury. The activity ratio reported was obtained by dividing postinjury scores by the preinjury score.

STATISTICS

The experimental parameters in groups of animals at different times were compared by one-way analysis of variance (ANOVA) with Dunnett's *t* test.

Results

The results of the bolus iv injection of 2 mg/kg of atracurium are seen in figure 1 and table 1. This dose caused almost a 100% depression of preatracurium twitch in unburned animals. Because this response did not vary with time after sham injury (ANOVA), the three groups of sham-injured animals were combined for comparison with the burn group and are represented by time 0 in figure 1. The thermally injured animals demonstrated no change in sensitivity to atracurium at 10 days, a slight resistance at 20 days, significant resistance at 30 days, peak resistance at 40 days (47% of preatracurium twitch depression), a decreased but significant resistance at 60 days, and a return to normal response at 90 days after injury (fig. 1). The twitch depression at 30, 40, and 60 days was significantly less than that of the sham animals. Similarly, the time from administration of the drug to the return of 50% of control (preatracurium) twitch was significantly shorter at 30, 40, and 60 days than in the unburned sham animals (table 1).

The relationship between days after injury and the atracurium concentration required to depress twitch to 50% of the preatracurium value (Cp_{50}) is shown in figure 2. The Cp_{50} increased in a pattern similar to that of resistance to atracurium (fig. 1). The highest Cp_{50} was obtained at 40 days after injury and was significantly different from sham controls. There was no statistical difference between Cp_{50} obtained in the sham animals at the various times.

The attainment of stable atracurium concentration in plasma was verified in five control rats in which a bolus of atracurium was administered followed by infusion to maintain twitch depression at 50% of control (method

identical to that used for fig. 2 and table 2). Samples obtained at 10 and 20 min of infusion showed a difference of 8.6% for the five rats.

ATRACURIUM BINDING TO PLASMA PROTEINS

Atracurium plasma-free fraction was determined at 40 days after injury and 40 days after sham injury (table 2). The fraction of unbound drug was the same in both groups at 57%. The free Cp_{50} was elevated by 75% in the thermally injured rats.

ANIMAL ACTIVITY

Animal activity was quantified for both sham and injured groups (table 3). Morning activity (assessed by 30-min visual observation) was not different between the two groups at any time before or up to 4 weeks after injury (table 3). Animal movement as monitored over 12-h night-time periods with activity cages is shown in table 4. For individual animals, the ratio of 12-h activity postburn to 12-h activity preburn was determined for each day, measured during the first 4 weeks after injury, then averaged for the number of days of observation for each animal. There was no decrease in activity in the burned compared with sham-injured animals over the first 4 weeks after injury.

Discussion

The principal findings of this study are as follows: 1) thermally injured rats develop resistance to NDMRs with a time course similar to that of humans⁴; 2) changes in protein binding do not explain this resistance; 3) although pharmacokinetic contributions are not ruled out, pharmacodynamic mechanisms are involved; and 4) disuse of skeletal muscle does not account for the development of resistance to NDMRs.

The propensity for hyperkalemia and cardiac arrest in burned patients^{9,10} has led to the use of NDMRs. Although burned patients exhibit a resistance to *d*-tubocurarine,² metocurine,³ and atracurium,⁴ the mechanism has not been elucidated.

Martyn *et al.*⁵ examined the pharmacokinetics of *d*-tubocurarine in a burned population and found no changes from normal patients that would account for the resistance to NDMRs observed.^{3,4} However, six of the eight patients were studied less than 7 days after burn, a time when another study⁴ showed that burned patients had not yet developed resistance.

Any examination of mechanisms underlying the resistance to NDMRs must take into account the pattern of development of resistance. Dwersteg *et al.*⁴ showed a relationship between the total body surface area (TBSA) of burn and the decrease of muscle twitch after a fixed dose

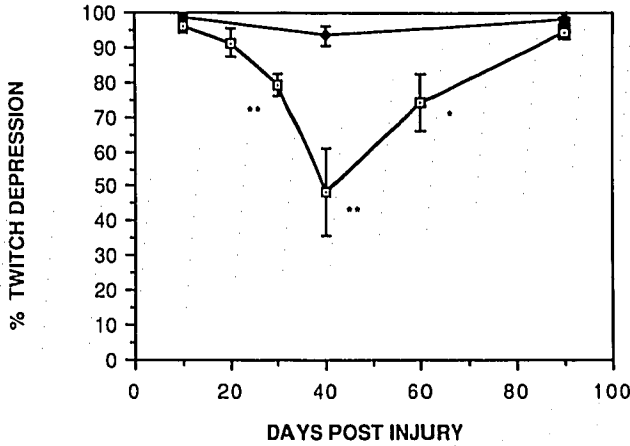


FIG. 1. Maximum percentage of twitch depression in rat gastrocnemius muscle after iv bolus of 2 mg/kg of atracurium. Peak resistance develops at 40 days and decreases thereafter. * $P < 0.05$ compared with grouped sham-injured controls. ** $P < 0.01$ compared with grouped sham-injured controls. □ = Thermally injured rats; ■ = sham-injured rats.

of atracurium. Few patients with a TBSA of less than 30% showed resistance, whereas patients with larger burns showed an increased dose requirement for the same response proportional to the fraction of TBSA involved. Furthermore, twitch depression and time to return to 50% of control twitch were normal until 7 days after injury, then decreased with peak resistance between 30 and 60 days. Thereafter, the response returned toward control.

Burned patients present difficulties in examining mechanisms, which might account for resistance. Although they may have surgery several times, they do so at irregular times, depending on their state of health, healing, and availability of autologous donor skin. Furthermore, the site and size of burns vary. Analyses of results are complicated by the presence of sepsis and infections; a wide variety of other drugs, such as antibiotics; varying degree of activity; and other factors. Thus, we examined rats in which these variables could be controlled and from which tissues could be obtained for further *in vitro* study. We found that the rat developed resistance in a manner very similar to humans. The pattern of resistance with respect to time was remarkably similar to the study of Dwersteg *et al.* in humans.⁴ At 10 days after injury the degree of twitch depression and time to return to 50% of control were normal (fig. 1). Peak resistance had occurred by 40 days and tended to decrease after that time. A 30% TBSA burn was sufficient in these animals to demonstrate resistance to the effects of atracurium. No animal studied showed signs of systemic infection. No animals received any type of medication that might attenuate or increase effects of NDMRs.

An infusion study was carried out to determine the Cp_{50} , *i.e.*, the stable concentration of atracurium in plasma

TABLE 1. Effect of Thermal Injury on Response to Atracurium

Day Postinjury	n	Percentage Twitch Depression*	Time to Return to 50%† (min)
0	19	96.6 ± 1.2	15.6 ± 0.8
10	7	96.1 ± 2.0	14.4 ± 1.6
20	6	91.5 ± 3.8	11.5 ± 2.4
30	24	79.3 ± 3.4‡	5.8 ± 0.61§
40	8	48.5 ± 12.4‡	6.8 ± 1.8§¶
60	7	74.3 ± 8.2§	8.7 ± 1.7†§
90	8	94.4 ± 1.8	12.5 ± 1.2

Mean ± SE. Animals observed 0 days after injury are sham-injured controls.

* Maximum depression of twitch elicited by supramaximal nerve stimulation following atracurium bolus.

† Time after bolus of atracurium for twitch response to return to 50% of the preatracurium value.

‡ $P < 0.01$ compared with group studied at 0 days after injury (sham-injured controls).

§ $P < 0.05$ compared with group studied at 0 days after injury (sham-injured controls).

¶ $n = 4$, in other animals twitch was not depressed by 50%.

required to cause a 50% depression in gastrocnemius contraction. The peak Cp_{50} correlated well with times of greatest resistance and decreased as responses to atracurium returned toward normal (fig. 2). This study does not rule out alterations in pharmacokinetic mechanisms as contributing to a changed response to a bolus injection, but it strongly suggests pharmacodynamic mechanisms are involved.

Leibel *et al.*¹¹ showed an increased plasma protein binding of *d*-tubocurarine in patients burned to varying degrees and studied at various times after injury. In our animals, protein binding of atracurium at 40 days after

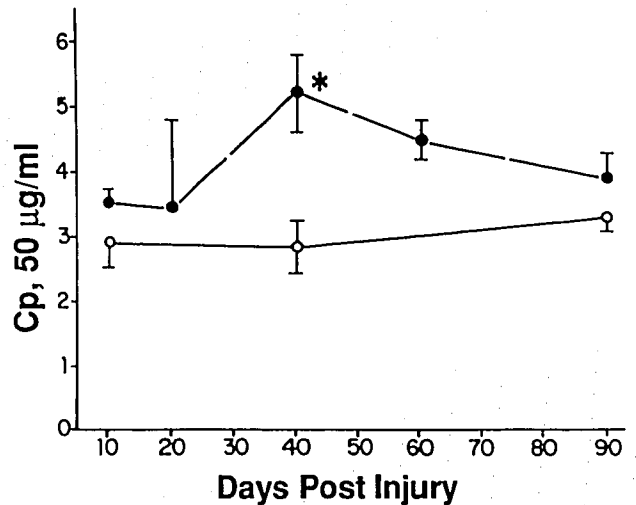


FIG. 2. Steady-state plasma concentration of atracurium required to produce 50% depression of twitch. Highest concentration is at 40 days. * $P < 0.05$ compared with grouped sham controls. ● = Thermally injured rats; ○ = sham-injured rats.

TABLE 2. Effect of Thermal Injury on Protein Binding of Atracurium in Rat Plasma

	Sham-injured Control	40 Days After Injury
n	7	7
Total Cp ₅₀ * (μg/ml)	3.16 ± 0.35	5.21 ± 0.61†
Fraction of unbound drug‡	0.57 ± 0.06	0.57 ± 0.03
Unbound Cp ₅₀ § (μg/ml)	1.75 ± 0.19	3.08 ± 0.52†

Mean ± SE.

* Stable total concentration (free + bound) of atracurium in plasma at which twitch is depressed to 50% of the preatracurium value.

† P < 0.05 compared with sham group.

‡ Fraction of atracurium in plasma unbound to protein (determined at pH 6.2)

§ Unbound steady-state concentration of atracurium in plasma at which twitch is depressed to 50% of the preatracurium value.

TABLE 3. Effect of Thermal Injury on Movement Determined by Observation

Weeks Postinjury	Sham (Seconds of Movement/30 min)	Injured (Seconds of Movement/30 min)
0	1,645 ± 21	1,599 ± 67
1	1,451 ± 45	1,197 ± 66
2	1,342 ± 80	1,192 ± 81
3	1,258 ± 87	1,224 ± 78
4	1,291 ± 116	1,089 ± 83

Mean ± SE. n = 12, sham-injured group; n = 11, thermally injured group. The total time (in seconds) at least one extremity was in movement was recorded for a 30-min observation period. Observation period was always at the same time of morning for each rat. Differences between groups were not statistically significant.

injury (peak resistance to atracurium) was no different from the sham-injured group. This would preclude a decreased free fraction of atracurium as an explanation for the decreased relaxation and more rapid return for normal twitch seen in thermally injured animals (table 1). Binding studies were carried out at an acidic pH (6.0–

6.2) to decrease the rate of atracurium degradation. The degree of binding at physiologic pH of 7.4 might be different. However, because the free fraction of atracurium was identical in both groups, it is not likely that protein binding differs *in vivo*.

We considered the possibility that the burn area may result in direct muscle damage beneath injured skin, which may alter response to NDMRs. We excluded the hind extremity from thermal injury to preclude any direct involvement. The resistance displayed by the gastrocnemius shows a systemic response to injury as opposed to a direct injury. Whether the mechanisms responsible for the alterations in muscle function are neuronal or blood borne is not clear.

Gronert¹² and Gronert and Theye¹³ have observed that of the variety of patients who have abnormal hyperkalemic responses to succinylcholine (upper motor neurone lesion, lower motor neurone lesion, muscle disease, head injury, thermal injury, inactivity) or resistance to nondepolarizing neuromuscular relaxants (upper motor neurone lesion, thermal injury, limb immobilization), inactivity and disuse atrophy seem to be a common characteristic. Waud *et al.*¹⁴ reported resistance to *d*-tubocurarine in the foreleg of the guinea pig after the limb had been immobilized in plaster for 2 weeks. It has been suggested that disuse atrophy may account for the resistance to NDMRs after thermal injury. Using two different methods of assessing activity, we found no difference in activity between burn- and sham-injured rats. Our data show that disuse atrophy or inactivity, *per se*, do not seem to be factors contributing to the phenomenon in burned animals.

Evidence for a pharmacodynamic causation for resistance to NDMRs is offered by Kim *et al.*¹⁵ In rats with up to 55% TBSA thermal injury, these authors demonstrated a 60% increase in diaphragmatic acetylcholine receptors (AChRs) 14 days postburn after which the number of receptors declined. The issue of whether this increase is

TABLE 4. Night Activity of Sham- and Thermally Injured Rats Determined by Activity Cage

Sham Injured			Thermally Injured		
Animal	Number of Nights Recorded After Injury*	Activity Ratio†	Animal	Number of Nights Recorded After Injury*	Activity Ratio†
1	9	1.7 ± 0.03	1	11	1.1 ± 0.12
2	11	1.4 ± 0.18	2	11	1.1 ± 0.09
3	7	1.3 ± 0.15	3	10	1.2 ± 0.19
4	9	1.5 ± 0.03	4	9	1.8 ± 0.27
5	10	0.6 ± 0.13	5	10	0.4 ± 0.06
6	10	1.1 ± 0.09	6	13	2.0 ± 0.22
			7	13	2.6 ± 0.28
			8	13	3.1 ± 0.31

Mean ± SE.

* The number of nights that data were actually recorded during the first 4 weeks after injury.

† Activity ratio was determined by dividing the motion score de-

termined over a 12-h period (6 P.M. to 6 A.M.) after injury by that before injury. Motion score was determined in activity cages as described in Methods. Differences across time and groups are not statistically significant.

junctional or nonjunctional is important. Elevation of junctional AChRs might cause an increase in NDMRs required for effective competitive blockade of acetylcholine. If the AChR increase is extrajunctional, the mechanism for increased NDMR requirements would be to decrease neuromuscular junction (NMJ) availability of NDMR because of increased binding of antagonist at extrajunctional sites. In the latter case, steady-state infusion experiments such as those in this study would not yield a Cp_{50} different from that of normal controls. The study does demonstrate structural changes in a skeletal muscle that is distant from the site of burn injury and that is not immobilized. We do not have information that the diaphragms in the animals in which AChR changes occurred were resistant to NDMRs.

In summary, we have demonstrated that rats with a 30% TBSA thermal injury demonstrate a resistance to atracurium with a time course similar to that observed in humans. Infusion experiments show that a pharmacodynamic mechanism must at least partially account for the resistance. Furthermore, we have shown that neither direct thermal injury to muscle nor inactivity and disuse atrophy are required for the development of the pharmacodynamically altered responses to NDMRs.

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