

Enzymatic Hydrolysis of Atracurium In Vivo

Vladimir Nigrovic, M.D.,* Melissa Auen, B.S.,† Aron Wajskol, M.D.‡

Inactivation of atracurium *in vivo* has been postulated to proceed along two pathways: Hofmann elimination and ester hydrolysis. Since an end product of Hofmann elimination (acrylate) may be potentially toxic, the authors conducted a study to determine the extent of degradation via enzyme-catalyzed hydrolysis relative to that via Hofmann elimination. The enzyme carboxylesterase was inhibited by the pretreatment of rats with an organophosphorous compound, triorthotolyl phosphate (TOTP). Skeletal muscle relaxation produced either by *d*-tubocurarine or succinylcholine was not influenced by the pretreatment. This indicates that TOTP does not alter directly the paralyzing properties of either depolarizing or nondepolarizing muscle relaxants. Relaxation produced by atracurium, however, was prolonged markedly and the rate of recovery from relaxation was decreased. The authors conclude: 1) enzyme-catalyzed hydrolysis is probably responsible for the short duration of action of atracurium; 2) Hofmann elimination, at least in rats, probably is not the principal degradation pathway; and 3) the opportunity for *in vivo* generation of large amounts of potentially toxic end products is less than previously implied. (Key words: Biotransformation (drug): Hofmann elimination. Enzymes: carboxylesterase. Neuromuscular relaxants: atracurium. Toxicity: metabolites, acrylate.)

A SHORTER DURATION of action of atracurium in comparison with other competitive muscle relaxants was demonstrated in animal experiments¹ and documented clinically in humans.²⁻⁵ The brevity of relaxation produced by atracurium is attributed to the unique biodegradation that involves the cleavage of the bond between the quaternary nitrogen and the vicinal carbon in the aliphatic side chain.^{6,7} Since the aliphatic chain connects the two quaternary nitrogens and since the bis-quaternary structure presumably is required for the competitive blockade of the nicotinic receptors on the end plate, the rupture of the aliphatic chain is believed responsible for the rapid termination of relaxation.^{6,7} For chemical reasons, the cleavage of the N⁺-C bond—termed Hofmann elimination—is enhanced by an increase in the alkalinity of the milieu as well as by an increase in temperature. However, the fact that the *in vitro* degradation of atracurium proceeds faster in plasma than in a buffer solution at the same pH and temperature

necessitated a postulate of an additional degradation pathway involving ester hydrolysis, either spontaneous or enzymatically catalyzed.⁸ A role for plasma pseudocholinesterase was ruled out.⁸ Curiously, the relative contributions of each of the two postulated degradation pathways—Hofmann elimination and ester hydrolysis—have not been studied.

We previously have expressed our concern⁹ that a product of Hofmann elimination, *i.e.*, acrylate, might not be innocuous *in vivo*. We based our concern on the high reactivity of acrylate toward nucleophiles, *e.g.*, sulfhydryl groups or amines. Such a reaction might result in alkylation of these groups *in vivo* with unpredictable consequences. Since the amount of acrylate generated would be dependent on the extent of the *in vivo* degradation via Hofmann elimination relative to that via ester hydrolysis, we considered it important to evaluate the relative contribution of each pathway. Based on the uniformly expressed opinion¹⁻⁵ that the Hofmann elimination represents the major route of atracurium degradation, we hypothesized that the inhibition of carboxylesterase, the enzyme that presumably catalyzes the ester hydrolysis, would not influence greatly the duration of action of atracurium. The hypothesis was tested in rats by comparing the effect of an inhibitor of carboxylesterase, triorthotolyl phosphate (TOTP), on the duration of skeletal muscle relaxation produced by atracurium, *d*-tubocurarine, and succinylcholine.

Materials and Methods

Male Wistar rats, 250–350 g body weight, were pretreated by intraperitoneal injection of either TOTP (125 mg · kg⁻¹) in corn oil or of an equal volume of the vehicle alone (1 ml · kg⁻¹). Eighteen to 20 hours later, anesthesia was induced by the intraperitoneal injection of pentobarbital 50 mg · kg⁻¹, and if signs of light anesthesia appeared, *e.g.*, increased blood pressure, increased heart rate, or movements, the injection was repeated at one-tenth of the initial dose. Tracheotomy and tracheal cannulation were performed, and the animals were ventilated mechanically with air. Ventilatory settings were obtained from a nomogram for small rodents. The right femoral artery and vein were exposed and cannulated. The arterial catheter was linked to a pressure transducer, and its output was recorded. The venous cannula was utilized for the infusion of 0.9% NaCl (at 0.1 ml · kg⁻¹ · min⁻¹) and the injection of drugs. The left gastrocnemius muscle was exposed; its tendon was cut close to its osseous insertion and connected via

* Assistant Professor, Anesthesiology, Associate Professor, Pharmacology.

† Research Assistant, Anesthesiology.

‡ Associate Professor, Anesthesiology.

Received from the Departments of Anesthesiology and Pharmacology, Medical College of Ohio, Toledo, Ohio. Accepted for publication December 6, 1984. An abstract, based on the present work, has been accepted for oral presentation at the meeting of IARS, Spring 1985.

Address reprint requests to Dr. V. Nigrovic: Department of Anesthesiology, Medical College of Ohio, C.S. #10008, Toledo, Ohio 43699.

TABLE 1. Characteristics of Skeletal Muscle Relaxation Produced by Atracurium, *d*-Tubocurarine, and Succinylcholine in Rats Pretreated with TOTP and in Control Animals (n = 4)

Pretreatment with TOTP (125 mg·kg ⁻¹ ip)	Muscle Relaxant (mg·kg ⁻¹ iv)	Time to Maximal Relaxation (s)	Duration of Complete Relaxation (s)		Rate of Recovery of T ₁ (Per cent of T ₁ Recovered per Minute)	
			Mean	95% Confidence Interval	Mean	95% Confidence Interval
-	Atracurium	45.0	210	118-377	34.5	20.1-59.3
+	(1.2)	46.5	506*	283-906	16.1*	9.4-27.7
-	<i>d</i> -Tubocurarine	38.0	470	262-841	9.8	5.7-16.9
+	(0.15)	67.8	388	217-695	12.6	7.3-21.6
-	Succinylcholine	60.3	170	95-304	29.2	17.0-50.2
+	(0.5)	56.5	207	116-371	30.2	17.6-51.9
	Pooled SEM	±13.8				

* P < 0.01 for the comparison with the corresponding control group.

a thread to a force transducer (Grass FT.03®). The distal end of the left femur was immobilized by a pin inserted perpendicularly to the long axis of the bone, and the pin was held firmly in place by a set of clamps. The force transducer was mounted via a rack and pinion attachment to a steel rod. This arrangement permitted adjustment of the preload to optimize the isometric contractions. The output of the transducer was recorded continuously on a polygraph at the paper speed of 60 mm·min⁻¹. The ipsilateral sciatic nerve was severed, and its distal stump was attached to a bipolar electrode and supramaximally stimulated at 2 Hz for 2 s with a 10-s interval between the trains (Grass S-88® nerve stimulator). A heating lamp was used to maintain the animal's body temperature. Exposed incisions were covered with paraffin oil to avoid desiccation. A stabilization period of 30-60 min was allowed prior to drug administration. At the termination of experiments, arterial blood samples were collected for blood gas analysis.

In preliminary experiments, we determined that the smallest dose of each relaxant required to produce a greater than 95% inhibition of the first twitch response was for atracurium 1.2 mg·kg⁻¹, for *d*-tubocurarine 0.15 mg·kg⁻¹, and for succinylcholine 0.5 mg·kg⁻¹. Immediately before administration, the desired dose was diluted with 5% dextrose in water so that the injected volume was 1 ml·kg⁻¹. Intravenous injection of the dextrose solution alone had no influence on muscular contraction.

The following variables were derived from the recordings of the muscle contractions: 1) Onset of the maximal relaxation: this was defined as the time interval, in seconds, between the injection of the relaxant and the time of the maximal reduction or disappearance of the first twitch in response to nerve stimulation. 2) Duration of the complete muscle relaxation: this variable was expressed as the interval, in seconds, between the

injection of the drug and the recovery of the first twitch to 10% of its preinjection value. 3) Rate of recovery: We observed that during the initial recovery (2-5 min) the peaks of the first twitch in each train fell on a straight line. We determined the slope of this line by calculating the ratio between the change in the height of the first twitch (expressed in per cent of the preinjection value) and the corresponding change in time (in minutes). Thus, the slope was representative of a series of observational points and, we believe, gave the best quantitative estimate of the rate of recovery under our experimental conditions. We deliberately omitted any evaluation of the train-of-four ratio, since it did not provide any additional information.

Each muscle relaxant was administered to a group of four rats pretreated with oil (control) and to another similar group pretreated with TOTP. The assignment was randomized. Analysis of variance was performed for each of the three measured variables. Due to the lack of homogeneity of variances for two of the parameters, duration of relaxation and rate of recovery, the analysis was repeated after logarithmic transformation of the data to approximate normal distribution. As expected,¹⁰ this transformation established the homogeneity but it necessitated the utilization of the confidence intervals for the mean values of the two variables rather than the customary statistical parameters.

Results

As illustrated in table 1, skeletal muscle relaxation produced either by *d*-tubocurarine or succinylcholine was not altered by pretreating the rats with TOTP. In contrast, the results obtained with atracurium indicate a pronounced effect of TOTP: the duration of relaxation produced by atracurium was prolonged and the rate of recovery from muscle paralysis was markedly slowed in

rats pretreated with TOTP in comparison with control animals.

Blood gas analysis revealed no effect of TOTP; blood pH was within normal limits in all groups (data not presented).

Discussion

The choice of the experimental animal for testing the working hypothesis was governed by the availability of data on the time course and the extent of inhibition of carboxylesterase by the organophosphorus compound TOTP. We based our TOTP treatment schedule on the results of Silver and Murphy,¹¹ who found that 18 h after the administration of TOTP 125 mg · kg⁻¹ body weight, a marked but incomplete inhibition of carboxylesterase could be produced in the plasma and in various tissues of rats. We desired to contrast the relaxation produced by an individual muscle relaxant in animals with normal *versus* those with lowered levels of carboxylesterase. Comparison among the three muscle relaxants was not intended.

Carboxylesterase (carboxyl ester hydrolase) is distributed widely in mammalian tissues and plasma.¹² The principal biochemical characteristic of the enzyme, *i.e.*, hydrolysis of the carboxyl esters, is shared by a number of enzymes that differ in their substrate specificity and in their susceptibility to inhibition by various compounds.¹³ Considering the possibility that both acetylcholinesterase and pseudocholinesterase also might be inhibited by TOTP, it was necessary to design the experiments to separately examine the possibility of any nonspecific interference by TOTP on the impulse transmission across the neuromuscular junction. *d*-Tubocurarine appeared suitable for this purpose, since its structure does not contain an ester linkage and, therefore, its inactivation should be independent of carboxylesterase. Were TOTP to produce an inhibition of acetylcholinesterase, an antagonism of the relaxant action of *d*-tubocurarine would be expected. Succinylcholine, on the other hand, was included in our study to rule out the possibility that TOTP inhibits pseudocholinesterase.

The results showed that the administration of TOTP did not influence any of the measured parameters of muscle relaxation produced either by *d*-tubocurarine or succinylcholine. We conclude that a pharmacologically relevant inhibition of acetylcholinesterase or of pseudocholinesterase by TOTP is highly unlikely. Therefore, any alteration of the pharmacologic effect of atracurium in the TOTP pretreated rats could be attributed to the inhibition of carboxylesterase.

In our study, inhibition of carboxylesterase more than doubled the duration of muscle relaxation produced by

atracurium and halved the rate of recovery from muscle paralysis. This finding documents the importance of ester hydrolysis in determining the duration of action of atracurium. The null hypothesis that the enzymatically catalyzed hydrolysis is only of minor importance in the biodegradation now can be rejected, at least in the rat. A reexamination of the basic premise of our study, *viz.*, the Hofmann elimination as the principal route of degradation of atracurium, appears indicated.

The evidence provided to support the dominant role of Hofmann elimination was based on the following findings: 1) The guiding concept in the synthesis of atracurium was the desire to incorporate in its molecule structural elements conducive for Hofmann elimination.^{6,7} 2) The *in vitro* rate of degradation of atracurium in solutions buffered to different pH values (6.9–8.0) was consonant with the base-catalyzed character of Hofmann elimination.⁸ In parallel experiments, incubation of atracurium in human plasma (tested at only two pH values), resulted in an even faster degradation: the half-life in plasma was 44 min as opposed to 76 min in a buffered solution (pH 7.4 and 37° C). The enhanced rate of degradation in plasma was attributed to ester hydrolysis, either spontaneous or enzyme mediated. Hofmann elimination nevertheless was deemed the major degradation pathway.⁸ 3) Two of the metabolites detected in the urine and bile of cats treated with radiocarbon labeled atracurium were considered to be products of Hofmann elimination.¹⁴

To our knowledge, there is no further documentation available in the original literature supporting the quantitative preeminence of Hofmann elimination. In clinical trials, Hofmann elimination appeared likely, since atracurium produced a rather short-lasting relaxation even in patients with hepatic¹⁵ or renal¹⁶ failure; tacitly this was taken as evidence supporting the dominance of Hofmann elimination. The clinical desirability of Hofmann elimination as the dominant biodegradation pathway was expounded.^{2-5,17}

Our concern that acrylate—an obligatory end product of Hofmann elimination—might be toxic,⁹ made us examine in more detail the evidence for the quantitative importance of Hofmann elimination. Because of the following points, the experimental evidence for the prevalence of the Hofmann degradation pathway was not convincing: 1) Base-catalyzed degradation⁸ is consistent not only with Hofmann elimination but also with ester hydrolysis. 2) Carboxylesterase has a pH-optimum in the alkaline range.¹² It is, therefore, conceivable that when atracurium was incubated in human plasma⁸ the increased rate of degradation of atracurium was due to enzyme-catalyzed hydrolysis enhanced by the shift of the pH in the incubating solution (plasma) toward the

alkaline values. 3) The two metabolites of Hofmann elimination measured in the urine and bile after intravenous injection (two cats) add up to less than 14% of the injected atracurium.¹⁴ In spite of the methodologic problems described in the article,¹⁴ one still would expect a higher yield of metabolites from a "major" degradation pathway. 4) Pharmacokinetic determinations in humans¹⁸ showed the plasma half-life to be much shorter (approximately 20 min) than that obtained in *in vitro* experiments (45 min).⁸ No explanations of this discrepancy were offered. Our first suspicion was that the facilitated removal (alkylation) of acrylates by the abundant supply of nucleophiles *in vivo* even further enhanced Hofmann elimination. Alternatively, we considered that plasma and tissue carboxylesterase could have enhanced enzymatic hydrolysis.

Since the end products of the two degradation pathways have markedly different toxicologic consequences—with acrylates being theoretically undesirable—we concluded that a direct evaluation of the role of enzymatic hydrolysis of atracurium *versus* that of Hofmann degradation was indicated. If Hofmann elimination is the minor metabolic pathway, the potential toxicity of atracurium due to acrylates generated *in vivo* via Hofmann elimination would be lessened although not totally eliminated.

The rat generally is not considered to be the most suitable animal for the study of muscle relaxants. Whether the data obtained in rats could be extrapolated to humans frequently is questioned. Our goal, however, was not to study skeletal muscle relaxation, *per se*, but to use the rat as a means for the bioassay of atracurium. Although Hofmann elimination evidently is not species dependent, the activity and substrate specificity of carboxylesterase might differ among species. Similarities and dissimilarities between rat and human carboxylesterase regarding the substrates and inhibitors are known¹⁹ and are such that only a direct determination of the hydrolytic degradation of atracurium by human carboxylesterase can give a definitive answer. Presently, we are conducting experiments to verify in humans the possibility that enzymatic hydrolysis and not Hofmann elimination may play the dominant role in the inactivation of atracurium.

The authors thank Howard Rosenberg, M.D., Ph.D., Department of Pharmacology, for providing laboratory space and equipment, and John T. Martin, M.D., Department of Anesthesiology, for reviewing the manuscript.

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