IN VITRO DEGRADATION OF ATRACURIUM IN HUMAN PLASMA

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SUMMARY

Degradation of the neuromuscular blocking agent atracurium has been examined in vitro in both Tris buffer and human plasma using a biological assessment in the mouse to estimate active drug concentrations. These studies show that at normal physiological pH and temperature, the major route of inactivation is by "Hofmann elimination". Therefore, in clinical use atracurium will be inactivated independently of hepatic, renal or circulatory function or plasma esterase activity.

The clinical usefulness of a neuromuscular blocking agent is dependent on the predictability of response, the ease of reversal of its effect and on the degree of separation between the paralysing dose and that causing side-effects on the cardiovascular system. The use of existing myoneural blockers can be complicated in patients with impaired renal function, liver disease or low plasma pseudocholinesterase activity because of marked increases in the potency, and duration of the effect, of the drug (Lehmann and Liddell, 1964; Wingard and Cook, 1977). In an attempt to overcome these problems a novel neuromuscular blocking agent, atracurium (2,2'-[(3,11-dioxo-4,10-dioxatridecylene)-bis-[6,7-dimethoxy-1-(3, 4 - dimethoxybenzyl-2-methyl-1,2,3,4-tetrahydroisoquinolinium]] dibenzene sulphonate) has been developed (Stenlake, 1979; Hughes and Chappie, 1981). The chemical structure of atracurium was expected to undergo spontaneous deactivation in vivo. In anesthetized man atracurium is a potent competitive neuromuscular blocking agent with no associated cardiovascular side-effects at doses required to induce muscular relaxation (Payne and Hughes, 1981). The major advantage of this drug appears to lie in its susceptibility to degradation by the process of "Hofmann elimination" (fig. 3), a process first described by A. W. Hofmann in 1851 in which quaternary ammonium compounds can be degraded at physiological pH and temperature (Stenlake, 1979) and is independent of enzymatic activity, or hepatic and renal function.

We have designed an in vitro technique to study the overall degradation of atracurium in both physiological and non-physiological fluids. In addition, we have compared the in vitro rates of degradation of atracurium and suxamethonium in plasma samples obtained from normal healthy subjects with those from subjects with low concentrations of plasma pseudocholinesterase.

METHODS

Assessment of neuromuscular blocking activity using an ED_{50} test in mice

In order to establish biological responses for atracurium and suxamethonium, doses of the drugs were administered i.v. to groups of six male, albino mice within a 5-g weight range. Each animal was carefully observed for up to 5 min following injection and its response classified as "positive" or "negative". A positive response to atracurium was characterized by a loss of motor activity and a decrease in the frequency of breathing occurring within 2 min of injection, whilst for suxamethonium an immediate loss of motor activity and collapse signified a positive effect. No animal was allowed to suffer from the effects of neuromuscular blockade beyond the short time taken to confirm the response. The number of animals displaying a positive response at each dose was recorded and probit analysis of the results provided an ED_{50} value for each drug.

Measurement of the rate of degradation of atracurium and suxamethonium in Tris buffer and human plasma

Solutions of atracurium 1 mg ml^{-1} or suxamethonium 350 μg ml^{-1} freshly prepared in Tris 0.05 mol litre^{-1} or phosphate 0.1 mol litre^{-1} buffers were added to test incubates at 37°C in the ratio 1:4.
and thoroughly mixed by inversion. These prepara-
tions, which had pH values of 6.9–8.0 unit (Tris) and 7.4–7.8 unit (plasma), initially contained, in a
volume of 0.1 ml, about twice the amount of drug
required to cause neuromuscular blockade when
administered i.v. to a 20-g mouse.

The samples mixed with neuromuscular blocking
agent were incubated at 37 °C and, at timed inter-
vals, 0.1 ml was withdrawn using a 1-ml glass
syringe and injected i.v. into the warmed tail vein of
a male albino mouse. The animal was observed for
up to 5 min and its response classified as positive
when sufficient active drug remained in the injec-
tion to cause neuromuscular blockade or negative
when in vitro inactivation had occurred in the sam-
ples, such that insufficient drug remained to cause
a response. The time in minutes from the start of
incubation to the change in response in a series of
individual animals from “positive” to “negative”
was noted and at this “end-point” the total amount
of active drug administered was assumed to repres-
ent an amount of drug equivalent to that causing
neuromuscular blockade in 50% of mice treated
(ED₅₀) as determined above.

The procedure was repeated in further series of
mice and additional end-points were obtained fol-
lowing the administration of the incubated
drug–sample mixtures in 0.1-ml increments to a
maximum of 0.5 ml per mouse. For each end-point
an estimated residual drug concentration was calcu-
lated using the ED₅₀ value, the volume administered
and the mean weight of the animals used. The
residual drug concentration was then plotted against
the time taken to achieve the effect and from this
graph a half-life for drug degradation was obtained.

Using this procedure, the in vitro degradation of
atracurium was assessed in Tris buffer solutions
0.05 mol litre⁻¹ at pH 6.9–8.0 unit and in normal
human plasma samples pH 7.4–7.9 unit.

In addition, a comparison was made of the rates of
degradation of atracurium and suxamethonium in
plasma from normal human subjects and in plasma
from subjects with low pseudocholinesterase en-
zyme activity adjusted to pH 7.4 unit with phos-
phate buffer 0.1 mol litre⁻¹.

Estimation of pseudocholinesterase activity
in human plasma

Pseudocholinesterase activities of normal and
atypical genetically pseudocholinesterase-deficient
human plasma were determined using a colorimetric
method based on that of Ellman and co-workers
(1961) in which enzyme activity is expressed as the
rate at which a 1:1000 dilution of plasma hydrolyses
acetylthiocholine iodide at 25 °C. Each unit of en-
zyme activity represents the hydrolysis of one mole
of substrate per litre min⁻¹ x 10⁻⁸.

RESULTS

Degradation of atracurium in Tris buffer

In Tris buffer 0.05 mol litre⁻¹ incubated at 37 °C,
atracurium was inactivated more quickly as the pH
value was increased. Under these conditions the rate
of increase in drug degradation was linear and was
related directly to pH (fig. 1).

An increase in pH from 6.9 to 8.0 unit resulted in
an 8.6-fold increase in the rate of drug inactivation,
whilst within the range of pH encountered in clinical
practice (6.9–7.6 unit) an increase of almost four-
fold was recorded.
Degradation of atracurium in human plasma

In a series of human plasma samples received with a mean pH value of 7.65 unit and incubated at 37 °C, the degradation of atracurium was significantly faster ($P < 0.001$) than in a second group of human plasma samples in which the pH was corrected to 7.4 unit with phosphate buffer before incubation. As shown in figure 1, the rate of drug breakdown in human plasma at the different pH values was consistently faster by a factor of 1.7–1.8 times when compared with the corresponding Tris buffer preparations. The individual values in table I show that the half-lives for the degradation of atracurium in human plasma samples from different volunteers at pH 7.4 unit are remarkably consistent.

When incubated in a sample of human plasma at pH 7.9 unit, the rate of degradation of atracurium was shown to be related to the temperature of incubation. A half-life value of 18 min obtained for the degradation of atracurium at body temperature (37 °C) was extended to 49 min at 23 °C, 15.5 h at 5 °C and 6.5 days at −22 °C.

Comparative degradation of atracurium and suxamethonium in normal and atypical (low in pseudocholinesterase activity) human plasma

The pseudocholinesterase activity of normal human plasma obtained from six subjects varied between 163 and 207 units (mean value 192 units) whilst in six subjects with atypical plasma the pseudocholinesterase activity ranged from 6 to 33 units (mean value 19 units).

Suxamethonium was degraded very quickly in all of the normal samples (mean half-life 2.6 min), but in the samples low in pseudocholinesterase activity, the rate of inactivation was dramatically slower, with a mean half-life greater than 4 h (fig. 2).

### TABLE I. The inactivation of atracurium at 37°C in plasma donated by human volunteers. *Unbuffered samples not used in calculation of mean, SD or SEM. Samples 1 to 6 were supplied by individual healthy volunteers and samples 7 to 12 were donated by volunteer patients known to be genetically deficient in the enzyme pseudocholinesterase.

<table>
<thead>
<tr>
<th>Plasma sample</th>
<th>pH (unit)</th>
<th>Pseudocholinesterase contents (units of enzyme activity)</th>
<th>Half-life for inactivation of atracurium (min)</th>
<th>Mean half-life (min) at pH 7.4 unit</th>
<th>SD of samples (min)</th>
<th>SEM (min)</th>
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In contrast, there was no difference in the rates of degradation for atracurium when incubated in either the normal or the atypical plasma samples.

DISCUSSION
Under conditions likely to be encountered at physiological pH and temperature atracurium is continually undergoing inactivation. This has caused particular problems in the chemical assay of this drug. Our method, which provides a means for the immediate biological estimation of the concentration of the drug without the need for the storage of samples, or delay in assay, enabled us to obtain meaningful half-life values for incubation under physiological conditions, and by using these we have been able to follow closely the inactivation of the drug.

The mechanism of drug inactivation is of vital importance in the clinical use of any neuromuscular blocking agent, since this controls the duration of its action. Inactivation of all of the established competitive neuromuscular blocking agents is dependent on either the rate of metabolism or excretion, or both. Thus, impairment of liver function or renal insufficiency increases their potency and prolongs their action (Wingard and Cook, 1977). Furthermore, small concentrations of plasma pseudocholinesterase which occur in genetically-deficient homozygote patients can substantially increase the duration of effect of suxamethonium (Goodman and Gilman, 1980) as demonstrated in this study.

It appears from our studies that the clinical use of atracurium is not limited by the physiological status of the patients, as its major route of inactivation appears to be that of "Hofmann elimination" which is independent of metabolism, excretion or circulatory function (fig. 3); also, under conditions most likely to be encountered in vivo (37°C and

\[
\text{Hoffmann elimination}
\]

![Diagram of Hofmann elimination]

\[
+ \text{CH}_2 \cdot \text{CHCO.O.[CH}_2\text{]}_5 \cdot \text{CO.CH}_2 \cdot \text{CH}_2
\]

Laudanosine

Pentamethylene-1, 5-Diacrylate

Fig. 3. The inactivation of atracurium by "Hofmann elimination".
pH 7.4 unit) the half-life values for the degradation of atracurium in samples of human plasma from 10 different volunteers were remarkably consistent and independent of pseudocholinesterase enzyme activity (table I). Although the concentrations of the drugs used in our experiments were greater than those likely to be achieved in plasma following i.v. injection, we would expect that under the same conditions the kinetics of degradation for smaller concentrations would remain unchanged.

Experiments in various Tris buffer solutions have shown that atracurium can be inactivated without the need for plasma enzymes and identification by high performance liquid chromatography of the major breakdown product, laudanosine, in the incubated buffer has verified that this inactivation is by “Hofmann elimination”. The presence of this process should make a significant contribution to in vivo recovery from neuromuscular blockade through the decrease of drug concentration at neuromuscular junctions. “Hofmann elimination” is favoured by increasing alkaline conditions (fig. 1) and temperature, as these studies showed clearly, and its major product, laudanosine, has no neuromuscular blocking activity (Hughes and Chappie, 1978). Conversely, since the inactivation by “Hofmann elimination” can be slowed by cooling, this feature could be used to advantage to prolong the action of atracurium in cardiopulmonary bypass.

Theoretically, in addition to the process of “Hofmann elimination”, an alternative degradation pathway, that of ester hydrolysis (fig. 4), is also available for the inactivation of atracurium to yield monoquaternary breakdown products which have minimal neuromuscular blocking properties. Chemical inactivation of the drug by this route would normally ensue at a smaller pH value than could occur physiologically, but it is possible that this route

![Diagram of atracurium inactivation](image)

**FIG. 4.** The inactivation of atracurium by ester hydrolysis.
could be mediated enzymatically. In human plasma the rate of drug inactivation is about 1.8 times faster than that in Tris buffer alone. One enzyme present in plasma which is likely to effect the ester hydrolysis of atracurium is pseudocholinesterase. However, the studies in human plasma indicated that the importance of this enzyme can be discounted in man as there is no difference in the rate of drug degradation when incubated in normal or atypical (pseudocholinesterase deficient) plasma. In contrast, suxamethonium, which is known to be susceptible to the action of pseudocholinesterase, was rapidly degraded in the plasma from normal patients, but only poorly degraded in those plasma-deficient in the enzyme. These findings indicate that atracurium is not contraindicated in patients who might be deficient in pseudocholinesterase.

Although our present work has not conclusively established the reason for the increased degradation of atracurium obtained in human plasma, it is possible that non-specific enzymes such as the aliesterases may be involved.

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References
Hughes, R., and Chapple, D. J. (1978). Application for the issue of a clinical trial certificate to the Committee for the Safety of Medicines, Compound BW 33A.