Title: QUANTITATION OF ELECTROPHILIC METABOLITES FORMED FROM ATRACURIUM IN VITRO

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Introduction. Acrylates, a product of Hofmann elimination of atracurium, avidly bind to nucleophiles in a Michael type addition reaction (Figure 1). We searched for experimental evidence that spontaneous degradation of atracurium leads to formation of these electrophilic metabolites. This was accomplished by adding a known amount of *l*-cysteine to solutions containing atracurium or its degradation products. Repeated determinations of the amount of mercapto groups consumed during the incubation provided an estimate of the rate and the amount of electrophilic metabolites formed from atracurium.

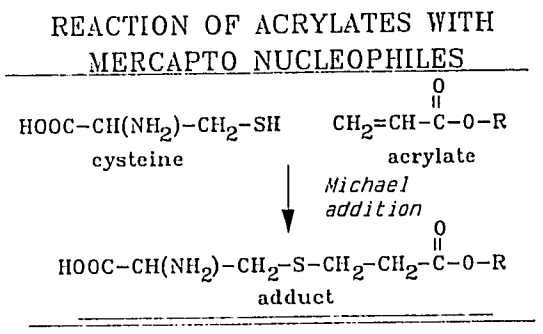

Figure 1

Methods. Variable amounts of atracurium (4, 8 or 12 μmol) were incubated for 120 min in saline (10 ml) at pH 8.0 ± 0.1 and 37°C (under nitrogen atmosphere). Thirty μmol of cysteine was then added and the incubation continued at pH 7.4 ± 0.1 and 37°C. Samples of the incubation solutions were collected frequently and analyzed for sulfhydryl groups by the method of Ellman. Variations of the experimental design included the addition of carboxylesterase during or after the initial incubation period (but prior to the addition of cysteine) or the addition of cysteine immediately at the start of incubation of atracurium (pH 7.4 and 37°C).

Results. Mercapto groups of cysteine were not consumed when cysteine was added to the plain saline. In solutions containing degradation products of atracurium, two SH-groups were maximally consumed for each molecule of atracurium (Figure 2). Of these, one group was consumed very rapidly. The rate of consumption of the second SH-group was slower ($t_{\frac{1}{2}}$ 3 to 4 min) and was independent of the initial amount of atracurium. Addition of cysteine directly to solutions containing atracurium also led to consumption of two mercapto groups per molecule of atracurium but at a much slower rate ($t_{\frac{1}{2}}$ 70 to 80 min). The presence of carboxylesterase, either during the incubation of atracurium or inclusion of the enzyme at the end of incubation but prior to the addition of cysteine, markedly reduced the consumption of mercapto groups.

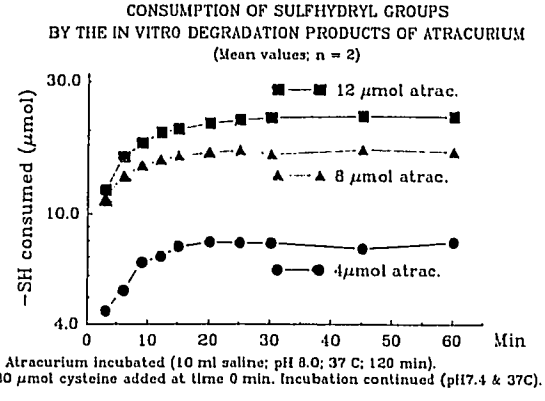

Figure 2

Discussion. We conclude that spontaneous degradation of atracurium, presumably by Hofmann elimination, leads to formation of two equivalents of electrophilic metabolites. The slower consumption of SH groups when cysteine was added directly to atracurium indicates that the addition reaction was secondary to, and dependent on, an antecedent slower reaction, presumably the spontaneous degradation via Hofmann elimination. Diminution of the consumption of SH groups by carboxylesterase indicates that the electrophilic species are esters, most likely acrylates. The cytotoxic effects of atracurium, previously observed in isolated hepatocytes, may be due to Michael addition of electrophilic metabolites of atracurium to structurally or functionally important endogenous nucleophiles.

References.

Ellman GL: Tissue sulfhydryl groups. Arch Biochem Biophys 82:70-77, 1959.