

Title: ATRACURIUM: INHIBITION OF PLASMA CHOLINESTERASE AND DEGRADATION *IN VITRO*

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Introduction. Though atracurium (ATCR) may be degraded either by Hoffman elimination or by ester hydrolysis (1) there is no agreement on the relative importance of these pathways *in vivo* or on whether the esterolysis is enzymatic. Since plasma cholinesterase (PCE) might cleave ATCR and since substrates of PCE should behave as inhibitors of this enzyme (2), we studied the degradation of ATCR and its inhibition of PCE *in vitro*.

Methods. Pure ATCR was the gift of Burroughs Wellcome. All chemicals were of reagent quality. Reactions were performed in M/15 MOPS buffer, pH 7.14. Human plasma was the source of PCE; assays used 50 μ M benzoylcholine (BzCh) as substrate (3). Quadruplicate assays were done without drug and with each concentration of ATCR or laudanosine. *In vitro* degradation studies at 37° C began with 80 μ M ATCR in buffer containing 1/50 strength plasma, heat-treated plasma, plasma and 20 μ M NaF, or no plasma. Timed aliquots were removed, cooled to -15° C, and assayed in quadruplicate by high performance liquid chromatography (0.4 x 25 cm Whatman SCX; solvent 40% A: 0.1 M KH₂PO₄, 5 mM H₂SO₄, and 60% B: CH₃CN, at 2 mL/m). Peaks were detected spectrophotometrically. After atracurium (80, 40, and 20 μ M) was incubated at 37° C for 2 h, BzCh and plasma were added to initiate the PCE assays.

Results. ATCR inhibits PCE in a simple competitive manner, obeying the equation:

$$V_{\max}/V = (K_m/[S])(1/K_i)[1] + (1 + K_m/[S])$$

where V_{\max} is the rate without ATCR, V is the rate, K_m the Michaelis constant, S the substrate, K_i the inhibitor constant, and I the inhibitor. Figure 1 shows the inhibition graphically; K_i is $\sim 3 \mu$ M, assuming the K_m for BzCh to be 4 μ M. The degradation of ATCR was invariably a simple first-order process, described by the equation:

$$[S] = [S_0] \exp(-kt)$$

where $[S]_0$ is the initial concentration, t is time, and k is the rate constant. Figure 2 shows the course of a typical reaction. The values of k were 0.021, 0.023, 0.022, and 0.021 m^{-1} , corresponding to half-times ($t_{1/2}$) of 33, 30, 31, and 33 m for reactions in the presence of native plasma, heat-treated plasma, plasma and NaF, and no plasma, respectively. Though fragments of ATCR inhibited the PCE reaction to the same extent as intact ATCR, laudanosine had no effect.

Discussion. Though ATCR at clinical concentrations inhibits PCE, it is not degraded by it. The values of k and $t_{1/2}$ ($\sim 1.5 - 15 \mu$ M) agree with those reported *in vivo* and in plasma and blood *in vitro* (4,5). Since ATCR is degraded as readily in buffer as *in vivo*, spontaneous degradation thus seems to be sufficient to account for its elimination and termination of action.

Neither PCE nor any organ-based enzyme seems to be required. A fragment of ATCR, probably the quaternary monoacrylate, also inhibits PCE. Though the inhibition of PCE by ATCR and its fragments may, at clinical concentrations, cause measurable prolongation of the action of succinylcholine (~ 10 min @ .2 mg/kg), the effect should not be large enough to hinder anesthetic management.

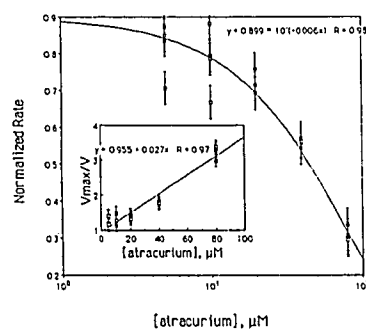


Fig. 1. Inhibition of PCE by ATCR. Normalized rate vs. log [ATCR]. Inset: V_{\max}/V vs. [ATCR]. Bars show regression SE of data.

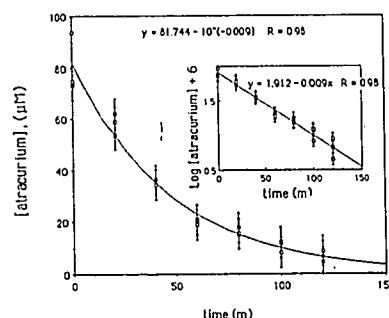


Fig. 2. Degradation of ATCR *in vitro*. [ATCR] vs. time. Inset: Log [ATCR] + 6 vs. time. Bars show regression SE of data.

References.

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