Title: THE BINDING OF NEUROMUSCULAR BLOCKING AGENTS TO PLASMA PROTEINS

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Introduction: Binding of drugs to plasma proteins is important from the point of view of their speed of onset, intensity and duration of action. The dialysis, ultrafiltration or centrifugation methods hitherto employed for the determination of protein binding of drugs are either unreliable or complex. The protein binding of compounds that inhibit plasma butyrylcholinesterase (BuChE; EC 3.1.1.8), i.e. neuromuscular (NM) blocking agents (muscle relaxants; MR) can be assessed reliably and simply by determining their I50 in two assay systems containing different concentrations of plasma. The method is based on two assumptions: a) that the percentage of a compound adsorbed to plasma proteins is proportional to the concentration of plasma in the assay system; and b) that the concentration of the free (unbound) drug causing the same degree of inhibition of BuChE is constant. These two principles were utilized for the determination of the binding of 13 MR to human plasma proteins.

Methods. The I50 of MR (see table) were determined in assay systems containing 5 and 50 or 5 and 90% pooled heparinized human plasma obtained from 20 healthy, young adult, volunteers of both sexes. BuChE activity was determined with a null-point potentiometric titration procedure, with a pH-stat (Radiometer, Copenhagen) at pH 7.4 and 37°C with 0.1 or 0.5N NaOH titrant. The concentration of the acetylcholine (ACh) substrate was $2\times10^{-2}$M. The MR were incubated with the plasma dilution used for 10 min before the addition of ACh. Heat inactivated enzyme blanks were used to correct for non-enzymatic hydrolysis. The I50 values of the compounds were calculated from log dose - inhibition regression lines obtained with at least 5 inhibitor concentrations. Determinations were done in triplicate. The calculation of protein binding was carried out as follows: If in undiluted plasma the total concentration of a drug is T and of this B is bound to proteins and the free drug concentration is F then

$$T = B + F \quad \text{----------1}$$

When the plasma concentration is x or y% then

$$T_x = x\cdot B + F \quad \text{--------2 and}$$

$$T_y = y\cdot B + F \quad \text{--------3}$$

Subtracting equation 3 from equation 3 and solving for B

$$B = \frac{T_x - T_y}{x-y} \quad \text{----------4}$$

Substituting experimentally determined I50 (or other inhibitory values) for $T_x$ and $T_y$

$$B = \frac{I50_x - I50_y}{x-y} \quad \text{---------5}$$

Rearranging equation 2 and substituting I50 for $T_x$ and B from equation 5

$$F = I50_x - x\cdot\frac{I50_x - I50_y}{x - y} \quad \text{---------6}$$

and substituting the calculated values of B (equation 5) and F (equation 6) into equation 1

$$T = I50_x + (1-x)\cdot\frac{I50_x - I50_y}{x - y} \quad \text{-----7}$$

and the percentage protein binding

$$P = \frac{B}{T}\cdot 100 \quad \text{----------8}$$

Results. Of the MR investigated pancuronium and vecuronium are bound to plasma proteins the most and metocurine and decamethonium the least (see table).

Discussion. Calculating the protein binding from I30, I50 or I50 values determined in assays in the presence of 5, 50 or 90% plasma showed that the margin of error of the method is less than 5%. Theoretically the described method should be suitable for the determination of protein binding of any compound that inhibits any enzymatic (or other) reaction in plasma. The applicability of the method is limited by the relationship between the inhibitory potency of and the available binding sites for the compound to be tested. If the inhibitory potency is low (high I50 value) and the number of binding sites is relatively small the percentage of protein binding cannot be reliably calculated with the described method.

| | I50 (M) With Plasma Concentration (%) of 5 | 90 | Protein Binding (%) |
|---|---|---|---|
| d-Tubocurarine | $2.2\times10^{-4}$ | $5.6\times10^{-4}$ | 66.1 |
| Metocurine | $1.8\times10^{-4}$* | $2.6\times10^{-4}$* | 37.1 |
| Toxiferine | $1.3\times10^{-5}$ | $5.0\times10^{-5}$ | 80.1 |
| Alcuronium | $5.4\times10^{-5}$ | $1.9\times10^{-4}$ | 77.1 |
| Pancuronium | $6.1\times10^{-8}$ | $3.3\times10^{-7}$ | 87.1 |
| Vecuronium | $6.9\times10^{-7}$ | $4.5\times10^{-6}$ | 90.6 |
| Duador | $6.0\times10^{-7}$ | $1.3\times10^{-6}$† | 79.8 |
| Arduan | $2.2\times10^{-6}$ | $4.8\times10^{-6}$† | 75.8 |
| Atracurium | $1.2\times10^{-4}$ | $3.3\times10^{-4}$† | 81.9 |
| Gallamine | $4.5\times10^{-5}$ | $1.4\times10^{-4}$ | 73.9 |
| Decamethonium | $1.4\times10^{-5}$ | $3.0\times10^{-5}$ | 59.0 |
| Succinylcholine | $6.2\times10^{-4}$ | $2.3\times10^{-3}$ | 79.1 |
| Suxethonium | $6.4\times10^{-4}$ | $2.7\times10^{-3}$ | 82.4 |

*I30 values used for calculation of protein binding with metocurine.

†I50 in 50% instead of 90% plasma.