BINDING OF THEOPHYLLINE AND ITS METABOLITES TO HUMAN PLASMA PROTEINS

The binding of theophylline to human serum and plasma proteins has been the subject of numerous investigations. The value for protein binding in normal subjects is reported to differ between 37 and 79% depending on the procedure and conditions used (see Shaw *et al.*, 1982).

It has recently been shown that the renal clearance of theophylline metabolites is higher than the glomerular filtration rate (Tang-Liu *et al.*, 1982; Gundert-Remy *et al.*, 1983) and in order to calculate the relative contribution of glomerular filtration to the overall renal clearance, the degree of protein binding of the theophylline metabolites has to be known. Since there is an evident lack of information in this regard, the aim of the present investigations was to study the binding of theophylline, 1-methyluric acid (1-MU), 1,3-dimethyluric acid (1,3-DMU) and 3-methylxanthine (3-MX) to human plasma proteins.

Plasma samples were prepared from blood collected in heparinized tubes (0.5%, NH₄-heparinate) from two female healthy adults (24 and 38 years) receiving no drugs, who had abstained from coffee, caffeine containing beverages, e.g. coca cola, tea, chocolate, foods and beverages containing cocoa, e.g. biscuits for at least 3 days. Protein binding was estimated by equilibrium dialysis. Theophylline and metabolites were added separately to fresh plasma samples to give

initial concentrations of either 20 mg l or 2.5 mg l. In one separate experiment, all four compounds (theophylline, 1-MU (purchased from Sigma, Munich, FRG), 1,3-DMU and 3-MX (purchased from Fluka, Neu-Ulm, FRG) were added to the plasma to give initial concentrations of 2.5 mg l.

In all experiments, the compounds (in aqueous solution) were added in a volume of 0.1 ml to 9.9 ml of plasma.

Plasma samples (1 ml) containing the compound(s) was placed in dialysis chambers (Dianorm, Bachhofer, Reutlingen, FRG) separated by dialysis membranes, with a molecular cut-off of 5000 daltons (Visking, Serva, Heidelberg, FRG) and dialysed against 1 ml phosphate buffered saline (0.15 M NaCl, 0.01 M K₂HPO₄, 0.05 M NaH₂PO₄) at pH 7.4. Dialysis was carried out at 37°C. Under these conditions equilibrium was attained within 4 h. At the end of dialysis, the pH of the plasma samples was measured. Since it was found to vary only between 7.35 and 7.45, the results could not have been influenced by differences in pH as has been demonstrated by other authors (Shaw *et al.*, 1982; Vallner *et al.*, 1979).

The concentration of theophylline and its metabolites was measured by h.p.l.c. (Tang-Lui *et al.*, 1982). The percentage of compound bound to plasma proteins (%B) was calculated as follows: %B = (con-

Table 1 Binding of theophylline and its metabolites to human plasma proteins. Values represent the mean $\pm$ s.d. The number of experiments are in parenthesis.

	A	B	C
Initial concentration	(20 mg l)	(2.5 mg l)	Mixture (2.5 mg l)
Theophylline	34.8 $\pm$ 4.8 (7)	42.7 $\pm$ 3.1 ^a (8)	30.4 $\pm$ 2.6 ^b (8)
1,3-DMU	70.9 $\pm$ 2.4 (8)	73.7 $\pm$ 0.6 ^c (8)	66.3 $\pm$ 1.8 ^d (8)
1-MU	0 (7)	0 (8)	0 (8)
3-MX	7.9 $\pm$ 4.7 (13)	11.9 $\pm$ 4.7 ^e (6)	11.1 $\pm$ 4.5 ^f (8)

^a $P < 0.01$ (A vs B)

^c $P < 0.01$ (A vs B)

^e $P > 0.1$ (A vs B)

^b $P < 0.01$ (C vs B)

^d $P < 0.01$ (C vs B)

^f $P > 0.1$ (C vs B)

centration in plasma – concentration in buffer) concentration in plasma.

No binding of the compounds to dialysis membranes was observed.

The statistical analysis of data was performed using two tailed *t*-test.

As shown in Table 1, there was a significant difference ($P < 0.01$) between the plasma protein binding of theophylline at an initial concentration of 20 mg l (about 35% bound) and that at a concentration of 2.5 mg l (about 44% bound). These results showing binding to be concentration-dependent, confirm those of Shaw *et al.* (1982), who, under well-controlled conditions found a similar low degree of binding compared to the findings of other authors. The binding of theophylline was even lower in the presence of metabolites (see Table 1), presumably due to displacement from binding sites.

The binding of 1,3-DMU was also dependent on concentration (Table 1) with 71% bound at 20 mg l and 74% at 2.5 mg l ($P < 0.01$). This greater degree of binding of 1,3-DMU to protein compared to that of the parent compound was similar to the results of Eichman *et al.* (1962) who showed that 1,3,7-trimethyluric acid was 83% bound, whereas the degree of theophylline binding in their experiment was only 20%. As with theophylline, the binding of 1,3-DMU was significantly lower ($P < 0.01$) in the presence of all four compounds (Table 1).

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1-MU displayed no binding to plasma proteins, which accords with the low protein binding of uric acid reported by others (Eichman *et al.*, 1962; Klinenberg & Kippen, 1980; Alvsaker & Seegmiller, 1972; Boldrini & Tirone, 1982).

The protein binding of 3-MX was low, showed no concentration-dependency and was not influenced by the presence of the parent compound and other metabolites (Table 1).

The relatively minor effects of changing concentrations on the binding of theophylline and 1,3-DMU suggests that the contribution of concentration-dependent binding to the observed non-linearity of theophylline kinetics (Tang-Liu *et al.*, 1982; Gundert-Remy *et al.*, 1983) is small. On the other hand, displacement of theophylline from its binding sites by metabolites may contribute to the time-dependent renal clearance of theophylline as demonstrated by different authors (Tang-Liu *et al.*, 1982; Gundert-Remy *et al.*, 1983).

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OPHTHALMOLOGICAL MONITORING OF PATIENTS RECEIVING LONG-TERM ACEBUTOLOL

Because of the eye damage which occurred with practolol, and the possibility that other β -adrenoceptor blockers might have a similar effect, patients on long-term acebutolol were monitored for any changes that might occur in the cornea or conjunctiva.

Patients under treatment for hypertension or angina

with acebutolol whose physicians intended them to continue with the medication were selected for study. On entering the study each patient was subjected to an ophthalmological assessment, including the following tests of lacrimal gland function and corneal integrity: