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Concentration-independent plasma protein binding of benzodiazepines

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Benzodiazepine derivatives are extensively bound to plasma protein (Johnson et al 1979; Abel et al 1979; Klotz et al 1980; Greenblatt 1981; Divoll & Greenblatt 1981, 1982). Quantitation of the extent of binding is of considerable importance, since binding can influence the interpretation of pharmacokinetic data based on total plasma concentrations (Faed 1981; Greenblatt et al 1982). These problems are complicated when binding is dependent on total plasma concentration. The present study evaluated the extent of plasma protein binding of 10 different benzodiazepines, and the influence of total plasma concentration on free fraction in plasma.

Methods

All studies were performed using a pool of human blood collected into heparinized tubes. Samples were centrifuged, and the plasma separated and stored frozen without preservatives. All samples were taken from the same large pool.

Samples of [^{14}C] or [^{3}H]benzodiazepines were obtained from their pharmaceutical manufacturers. Table 1 shows the benzodiazepine derivatives studied, the radioactive label, and the specific activity. For each day's run, ten 3-ml aliquots of the plasma pool were spiked to contain identical concentrations of a given radiolabelled benzodiazepine (Table 1). Samples were also spiked to contain ten ascending concentrations of non-radioactive drug, ranging from 10 to 10 000 ng ml^{-1} (Table 2). Duplicate equilibrium dialyses were then performed on each sample. An aliquot of plasma containing both radioactive and non-radioactive drug was placed in standard cellulose dialysis tubing which was sealed and suspended in 7·25 ml of pH 7·4 phosphate buffer (Woo & Greenblatt 1979). Samples were dialysed at 37 °C for 20 h. At the completion of dialysis, radioactivity in aliquots of plasma and dialysate were determined using a liquid scintillation counter.

Free fraction was calculated as the ratio of radioactivity in the dialysate divided by that remaining in the dialysed plasma. (Concentrations of radioactive and non-radioactive drug in dialysed plasma at the termination of the dialysis procedure were lower than the initial pre-dialysis added concentrations because of the egress of drug from plasma to dialysate during the procedure, and to the approximately 10% increase in volume of the plasma component because of osmotic forces).

The total number of dialysis runs was 13, and all analyses were performed over 28 days. Between-day variability was evaluated using a separate pool of 'control' plasma in which the free fraction of lorazepam was determined as described above along with each day's run.

The influence of concentration on benzodiazepine free fraction was evaluated using two-way analysis of variance.

Results

Free fraction varied widely among drugs, ranging from less than 2% for diazepam to more than 30% with alprazolam (Table 2). Variations among drugs in free fraction were highly significant. However, analysis of variance indicated that concentration did not significantly influence free fraction ($F = 1·54$, d.f. = 9,90; N.S.).

Between-day variability in the free fraction of lorazepam in the control sample was 4·3% (n = 13). Culture of dialysate and plasma components using standard media revealed no evidence of bacterial growth.

Discussion

Consistent with previous reports, benzodiazepines are extensively bound to plasma protein. The free fraction in normal human plasma varied considerably among the various drugs evaluated, but in no case did total plasma concentration significantly influence the extent of protein binding, despite pre-dialysis total concentrations as high as 10 000 ng ml^{-1}. For all the drugs evaluated, plasma levels encountered during usual therapeutic use do not approach this upper limit,

Table 1. Experimental conditions.

Drug	Label	Specific activity (μCi mg^{-1})	Initial (pre-dialysis) concentrations of labelled isotope (ng ml^{-1})
Diazepam	^{14}C	65·8	31
Desmethyldiazepam	^{14}C	288	11
Temazepam	^{3}H	62·5	32
Midazolam	^{14}C	41·2	48
Oxazepam	^{14}C	21·7	46
Lorazepam	^{14}C	30·6	33
Clobazam	^{14}C	109	32
Triazolam	^{14}C	108	28
Flunitrazepam	^{3}H	280	12
Alprazolam	^{14}C	170	16

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Table 2. Effect of representative concentrations on free fraction (% unbound) at initial (pre-dialysis) total plasma concentrations of unlabelled drug from 10–10 000 ng ml^{-1}. (n = 10).

Drug	10	100	600	1000	4000	10000	Mean (± s.e.)
Diazepam	1·58	1·53	1·64	1·60	1·63	1·62	1·58 (± 0·01)
Desmethyldiazepam	3·53	3·51	3·72	3·25	3·54	3·64	3·47 (± 0·05)
Temazepam	3·59	3·40	3·97	3·79	3·68	3·50	3·62 (± 0·07)
Midazolam	3·71	3·72	3·62	3·61	3·55	3·58	3·66 (± 0·03)
Oxazepam	5·55	5·22	4·94	4·73	4·78	5·05	5·12 (± 0·08)
Lorazepam	9·85	9·76	9·61	9·78	9·49	9·77	9·74 (± 0·04)
Clobazam	16·8	16·3	16·2	16·9	16·4	18·1	16·9 (± 0·2)
Tiazolam	22·6	23·4	21·5	21·6	22·1	21·1	22·1 (± 0·28)
Flunitrazepam	21·6	22·6	20·2	21·4	22·1	23·1	22·5 (± 0·4)
Alprazolam	34·0	32·6	28·4	31·9	28·9	32·0	31·6 (± 0·6)

although in some cases of massive overdosage levels in this range have been reported (Greenblatt et al 1978). The findings suggest that concentration-dependent plasma protein binding should not complicate the interpretation of pharmacokinetic studies of benzodiazepines following usual therapeutic doses.

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(+)-Oxaprotiline but not (−)-oxaprotiline given chronically potentiates the aggressive behaviour induced by clonidine

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Long-term, but not acute, treatment with antidepressants (tricyclics, maprotiline, nisoxetine, mianserin, iprindole, zimelidine, levomepromazine, thioridazine) potentiates aggression induced by clonidine in mice (Maj et al 1980, 1981, 1982). Clonidine aggression is realized by α_1-adrenergic stimulation (Morpurgo 1968; Ozawa et al 1975; Maj et al 1981), then its potentiation by antidepressants can be attributed to the noradrenergic mechanism. To support such a hypothesis we have examined (+)- and (−)-oxaprotiline, of which only the (+)-form inhibits noradrenaline uptake, evokes the typical antidepressant pharmacological effects, and, given chronically, induces β-adrenergic subsensitivity (Bittiger et al 1981; Mishra et al 1981).

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Method

The experiments were carried out on male Albino Swiss mice (20–30 g) housed in groups and having free access to food and water throughout the experiment. (+)- and (−)-Oxaprotiline (hydrochloride, 10 mg kg^{-1}) or 0·9% NaCl (saline) were injected i.p. twice a day for 14 days. Clonidine (hydrochloride, 20 mg kg^{-1} i.p. in saline) was given 2 h after the last dose of oxaprotiline. Immediately thereafter groups of four mice each were placed together in glass cylinders and the number of biting attacks was counted for 1 h (Ozawa et al 1975). The acute experiments (single dose of (+)- or (−)-oxaprotiline) were performed in a similar manner. The dose of clonidine was choosen on the basis of previous experiments (Maj et al 1980, 1981) and was administered 2 h after oxaprotiline. Both drugs were dissolved in saline. In each (acute or chronic) experimental group