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Effect of age and sex on lorazepam protein binding

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Lorazepam is a 3-hydroxy-1,4-benzodiazepine derivative biotransformed in the liver by glucuronide conjugation (Greenblatt 1981). Previous studies of lorazepam kinetics have demonstrated no important alterations in the elderly (Greenblatt et al 1979; Kraus et al 1978). Since only the free fraction of a drug present in plasma is available for diffusion out of the vascular system to sites of pharmacological activity and metabolic biotransformation, kinetic variables based upon total (free plus bound) concentrations of extensively protein-bound drugs can be influenced by differences in unbound fraction between individuals (Koch-Weser & Sellers 1976; Greenblatt et al 1980). This study evaluated the effect of age and sex on lorazepam protein binding and the influence of such differences on the outcome of a pharmacokinetic study.

Methods

Fifteen young and 15 elderly volunteers aged 19 to 84 years participated in a kinetic study of intravenous lorazepam described previously (Greenblatt et al 1979). Volume of distribution, elimination half-life, and clearance of total (free plus bound) were determined based on lorazepam plasma concentrations determined by gas chromatography (Greenblatt et al 1978) at multiple points in time for up to 72 h after the dose (see Table 1).

The free fraction (percent unbound) of lorazepam was determined by a modified equilibrium dialysis technique (Woo & Greenblatt 1979) using a single sample drawn during the study in the nonfasting state. Duplicate 1 ml plasma samples were spiked to contain 2 nCi ml^{-1} of [^{14}C]lorazepam (spec. act. 101 μCi = 3·3 mg) and placed in dialysis membranes suspended in 7 ml of an isotonic phosphate buffer, pH 7·4. The tubes were capped and shaken horizontally at 37 °C for 18 h. A control derived from a pooled sample was included in each run.

Aliquots of both plasma and buffer were mixed with 15 ml of Aquasol (New England Nuclear) and the radioactivity was determined in a Beckman liquid scintillation counter. The free fraction of lorazepam was calculated as the ratio of counts min^{-1} in the buffer phase to that in the plasma remaining in the interior of the dialysis bag. The mean coefficient of variation between duplicate samples was less than 3·5%. The mean coefficient of variation between runs of the control sample was 2·7%.

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Results

The free fraction of lorazepam ranged from 9·07 to 12·80% with a mean of 11·08% for the 30 subjects (Fig. 1, Table 1). Sex did not influence the free fraction, but it was positively correlated with age ($r = 0·45$; $P < 0·01$), and was higher in the elderly than in young subjects of either sex (11·46 vs 10·71%; $P < 0·025$).

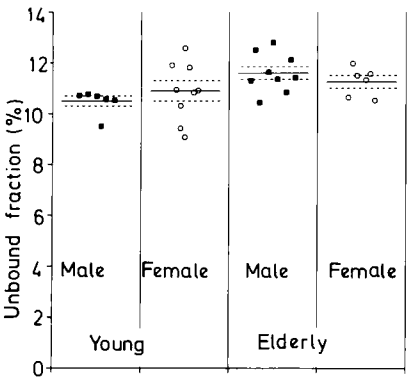

FIG. 1. Protein binding of lorazepam in relation to age and sex. Individual and mean (±s.e.) values for each group are shown.

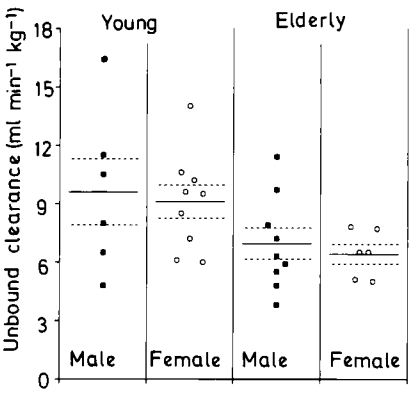

FIG. 2. Clearance of unbound lorazepam in relation to age and sex. Individual and mean (±s.e.) values for each group are shown.

Table 1. Subject characteristics and pharmacokinetics of lorazepam.

	Mean values (with range) for:			
Subject characteristics	Young male (n = 6)	Young female (n = 9)	Elderly male (n = 9)	Elderly female (n = 6)
Age (y)	27·8[a] (22–38)	27·0[a] (19–32)	69·1[a] (64–76)	70·8[a] (60–84)
Weight (kg)	76·0 (69·5–86·4)	57·1 (48·6–70·0)	82·0 (65·5–90·9)	59·9 (45·5–72·7)
Albumin (g/100 ml)	4·68[a] (4·20–5·10)	4·41 (3·70–5·0)	4·04[a] (3·60–4·70)	4·20 (3·70–4·50)
Kinetics of total (free plus bound lorazepam				
Volume of Distribution (litre kg^{-1})	1·07 (0·91–1·13)	1·14[a] (0·93–1·30)	1·02 (0·83–1·21)	0·95[a] (0·89–1·0)
Clearance (ml min^{-1} kg^{-1})	1·0 (0·52–1·56)	0·98[a] (0·71–1·52)	0·80 (0·49–1·30)	0·72[a] (0·58–0·89)
Kinetics of unbound lorazepam				
Free fraction (%)	10·47[a] (9·51–10·77)	10·87 (9·07–12·58)	11·60[a] (10·44–12·80)	11·25 (10·52–11·97)
Unbound Vd (litre kg^{-1})	10·25[b] (8·61–11·88)	10·52[a] (7·79–11·73)	8·81[b] (7·13–11·16)	8·44[a] (8·19–8·84)
Unbound clearance (ml min^{-1} kg^{-1})	9·63 (4·82–16·40)	9·08[b] (6·01–14·04)	6·95 (3·83–11·38)	6·42[b] (5·01–7·75)

Value of Student's independent t for young vs elderly of the same sex: a: $P < 0·01$; b: $P < 0·05$.

Vd of unbound or free lorazepam was also nearly identical between males and females. It was 18% lower in elderly than in young subjects, and was significantly negatively correlated with age in females ($r = -0·78$; $P < 0·001$). To a lesser extent, it was also negatively correlated with age in males ($r = -0·51$, $P < 0·05$). There was no difference in the clearance of unbound lorazepam in males and females (Fig. 2). It was 28% lower in the elderly than the young due to a significant reduction in elderly compared with young women ($P < 0·05$).

Discussion

Since only the free fraction of a drug diffuses across biological membranes to the site of pharmacological activity and metabolic biotransformation, kinetic variables for unbound rather than total drug may reflect characteristics of distribution and clearance more accurately (Koch-Weser & Sellers 1976). This study evaluated the extent of lorazepam binding to plasma protein, and the influence of binding changes on the interpretation of pharmacokinetic results based on total plasma concentrations.

The mean free fraction for lorazepam was 11·1%, and was significantly but only slightly increased in elderly as opposed to young subjects. Vd before correction for individual differences in binding was 11% less in elderly than in young individuals (Table 1). However, after correction for individual binding differences, the difference between age groups in unbound Vd increased to 18%. Clearance of total lorazepam was 22% less in the elderly than in the young group (Table 1). After correction for individual differences in binding, this difference increased to 28%. The age effect was more apparent in females than in males.

With lorazepam, our conclusions regarding the influence of age and sex on drug disposition are not greatly altered when kinetic evaluations are based on unbound as opposed to total drug concentrations. However, age-related increases in free fraction (reduction of the extent of protein binding) for extensively bound drugs may obscure important differences in actual volume of distribution on clearance of pharmacologically active unbound drug when kinetic studies are based on total drug concentrations. Assessment of the extent of drug binding to plasma protein is an important aspect of pharmacokinetic studies in the geriatric population.

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