The Association of Age and Frailty with Paracetamol Conjugation in Man

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Summary

The association of age, physical frailty and liver size upon hepatic conjugation reactions was studied using paracetamol as a model drug. Nineteen fit subjects (mean age 26 years), 20 fit subjects (mean age 73 years), and eight frail, hospitalized subjects (mean age 82 years) were recruited. Paracetamol clearance expressed in terms of body weight was significantly lower in the fit elderly than in the fit young subjects, and was lowest in the frail elderly subjects ($p < 0.01$). There was no difference in paracetamol clearance expressed per unit volume of liver between the fit young and fit elderly subjects but it was significantly reduced in the frail subjects. Although the partial metabolic clearance to paracetamol sulphate was preserved per unit volume of liver with ageing and frailty, the partial metabolic clearance to paracetamol glucuronide per unit volume of liver was markedly reduced in the frail elderly ($p < 0.01$) when compared with the fit subjects.

These results show that age-associated changes in paracetamol clearance are attributable to both changes in liver volume and in general health. The findings underline the important influences of the elderly person's physical state upon drug clearance.

Introduction

Studies on the effect of ageing upon drug elimination have largely concentrated upon phase I metabolism. Less information is available about age-associated effects upon phase II conjugation reactions. Thus, whilst some workers report a small decline in clearance of some conjugated drugs with age [1–4], others show rates to be unaffected by age [5–9]. Such studies as are available have often failed to control for factors such as the clinical state of the elderly volunteers, or oral contraceptive ingestion by young volunteers, although such influences can have marked effects upon drug conjugation [10–12].

Liver size, which is a determinant of the elimination of drugs undergoing capacity-limited hepatic metabolism, falls with age [13]. We set out to examine the influence of age, physical frailty and liver size upon important phase II reactions, using the clearance of paracetamol as a model of hepatic conjugation.

Methods

Nineteen healthy subjects (two men, aged 18–31 years (25 ± 1) (mean ± SEM) and 20 healthy subjects (three men) aged over 60 years (73 ± 1) were recruited from amongst colleagues and from local social organizations for the elderly. No subject was suffering from hepatic, renal, respiratory or cardiac disorders as assessed by clinical history and examination. All had normal blood count, serum bilirubin, alkaline phosphatase, aspartate aminotransferase, plasma proteins, creatinine and electrolytes. No subject was taking regular medication and, in particular, none was taking the oral contraceptive pill. Nine frail elderly patients (one man) aged over 60 years (82 ± 2), receiving continuous hospital care for chronic disabling conditions such as cerebrovascular and musculoskeletal disease were also recruited. Although all were physically frail, none was suffering

from acute illness and all had normal haematological and biochemical indices of renal and hepatic function. None was receiving any medication known to influence hepatic glucuronide or sulphate conjugation; two were receiving small doses of trifluoperazine, one ibuprofen and one a combination of levodopa and carbidopa. Three of the young volunteers and one of the frail group, smoked cigarettes. All gave informed consent; informed consent was also received from the next of kin of the frail elderly subjects. The study had the approval of the Newcastle Joint Ethics Committee.

Liver volume was estimated by a modification of the method of Carr *et al.* [14], as previously described [13]. All scans were performed on fasted subjects in the early afternoon. The images were recorded on videotape and volumes subsequently calculated using a computer graphics tablet.

Sterile ampoules of paracetamol (20 mg/ml) dissolved in distilled water containing 15% v/v ethanol were prepared. After an overnight fast, 500 mg paracetamol solution was given intravenously to each subject over 10 min. Food and drink were allowed after 2 h. Venous blood was taken at 0, 15, 30, 45, 60, 75 and 90 minutes and 2, 3, 4, 5, 6 and 7 hours. Urine was collected throughout. Plasma and an aliquot of the urine sample were stored at −20°C until the time of analysis.

Plasma paracetamol levels were measured by pressure liquid chromatography (HPLC). A 15-cm FSA steel column (internal diameter 4.6 mm) containing Spherisorb ODS 5 μm with a pre-column was fitted to a Kontron Autoanalyser system with the UV detector set at 254 nm. The mobile phase was weak phosphoric acid (600 μl per litre, pH adjusted to 3.0 with sodium hydroxide) with methanol 80% and 20%, respectively. At a flow rate of 1.6 ml/min pressures were around 210 bars. Samples were prepared by precipitating the protein with 15% perchloric acid and centrifuging after addition of beta-hydroxy ethyl theophylline as internal standard. Retention time for paracetamol was around 2.8 min and for the internal standard about 5.7 min. A standard curve with concentrations of paracetamol in plasma from 0 to 40 μg/ml was used. The coefficients of variance for the analyses with two successive quality controls were 4.5% and 3.3%.

Urinary paracetamol glucuronide and sulphate levels were measured using a modified HPLC ion-pair system [15]. Separation was achieved with a 25-cm FSA steel column containing Spherisorb ODS 5 μm with a precolumn. The mobile phase consisted of 1% acetic acid, 500 μmol/l tetrabutyl ammonium phosphate (TBAP) as ion-pairing agents, 5 mmol/l potassium nitrate as modifier, and 22% methanol. Flow rate was 1.2 ml/min, with a pressure of 180 bar. Retention times, using pure conjugates (Dr R. Andrews, Sterling Winthrop, personal gift) were around 5 min for the glucuronide, 19 min for the sulphate and 13 min for the internal standard (β-hydroxyethyltheophylline). A standard curve of paracetamol from 0 to 50 μg/ml was constructed for each analytical run. The amount of paracetamol excreted as the glucuronide and sulphase conjugates was calculated by use of molar extinction coefficients.

Groups were compared by one-way analysis of variance (ANOVA) followed by the Scheffe test. The significance of differences between the subgroups of fit elderly and frail elderly subjects was determined using the Mann–Whitney U test.

Results

Mean liver volumes were lower in the fit and frail elderly than in the young subjects both when expressed in absolute terms and when adjusted for body weight. Although the volume of the central compartment (Vc) and the steady state volume of distribution (Vss) were lowest in the frail elderly, differences between the groups were not significant. Elimination half-life was significantly longer in the frail elderly when compared to the other two groups. Paracetamol clearance expressed in terms of body weight was significantly lower ($p < 0.01$) in the healthy elderly (3.7 ± 0.2 ml/min/kg) compared with young subjects (4.7 ± 0.2 ml/min/kg) and was least in the frail patients (2.5 ± 0.1 ml/min/

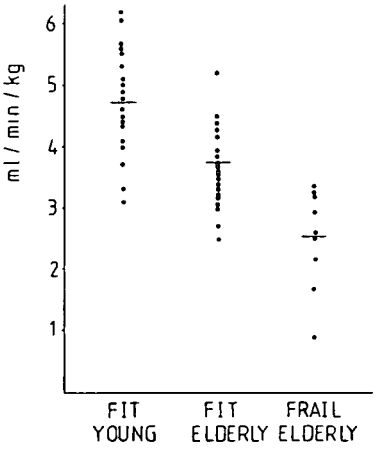

Figure 1. Paracetamol clearance expressed in terms of body weight in the three groups ($p < 0.01$ ANOVA).

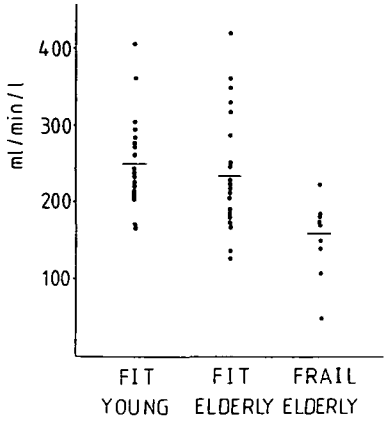

Figure 2. Paracetamol clearance per unit volume of liver in the three groups ($p < 0.01$ ANOVA).

kg). (Figure 1). Total paracetamol clearance per unit volume of liver was similar in the fit young and elderly subjects (251 ± 13 ml/min/l) *vs.* (234 ± 18 ml/min/l) but was significantly ($p < 0.01$) diminished in the frail elderly patients (157 ± 17 ml/min/kg) (Figure 2). The physical characteristics of the subjects and the pharmacokinetic data obtained are summarized in Table I.

In order to examine the possibility that the reduced paracetamol clearance per unit volume of liver of the hospitalized elderly subjects was related to their greater mean age, rather than to their frailty, paracetamol clearance was calculated in a subgroup of the eldest eight fit and the youngest seven frail elderly subjects. Although mean ages were now similar (79 years), the

Table I. Physical characteristics and paracetamol pharmacokinetics in the three groups of subjects

	Fit young	Fit elderly	Frail elderly
Age (years)	25 ± 1	73 ± 1	82 ± 2
Weight (kg)	59 ± 2	66 ± 2	53 ± 4*
Liver volume (ml)	1124 ± 40	1091 ± 43	843 ± 50*†
Liver volume/body weight (ml/kg)	19.2 ± 0.7	16.8 ± 0.9*	15.9 ± 0.5*
Vc (ml/kg)	232 ± 43	334 ± 34	199 ± 29
Vss (ml/kg)	770 ± 41	739 ± 27	614 ± 25
$t_{\frac{1}{2}}$ (min)	123 ± 6	144 ± 4	226 ± 42*†
CL (ml/min/kg)	4.7 ± 0.2	3.7 ± 0.2*	2.5 ± 0.1*†
CL (ml/min/l liver)	251 ± 13	234 ± 18	157 ± 17*†

ANOVA values = mean ± SEM.
$p < 0.01$ compared to * young, † fit elderly subjects.

Table II. Paracetamol clearance per unit body weight and per unit liver volume in subgroups of the oldest eight fit elderly and the youngest seven frail elderly subjects

	Fit elderly	Frail elderly
No. of subjects	8	7
Age (years)	79 ± 1	79 ± 2
Paracetamol clearance/body weight (ml/min/kg)	3.8 ± 0.3	2.3 ± 0.3†
Paracetamol clearance/liver volume (ml/min/l)	254 ± 34	144 ± 17†

† $p < 0.01$ compared to fit elderly subjects. Mann–Whitney U test.

Table III. Urinary clearance of paracetamol and its major metabolites in the three groups of subjects

	Fit young	Fit elderly	Frail elderly
CLG			
(ml/min/kg)	2.5 ± 0.1	2.1 ± 0.1*	1.0 ± 0.1*†
(ml/min/l liver)	134 ± 10	128 ± 11	67 ± 10*†
(mg)	140 ± 17	163 ± 17	95 ± 16
CLS			
(ml/min/kg)	1.8 ± 0.1	1.2 ± 0.1*	1.1 ± 0.1*
(ml/min/l liver)	92 ± 6	77 ± 8	67 ± 9
(mg)	95 ± 13	102 ± 13	99 ± 14
CLR			
(ml/min/kg)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0
(mg)	9 ± 3	15 ± 1	11 ± 3

$p < 0.01$ compared to * young, † fit elderly subjects.

lower clearances per unit volume of liver were still present in the frail elderly subjects (Table II).

In keeping with the effect of age upon total paracetamol clearance, the partial metabolic clearance to paracetamol sulphate (CLS) was lower in the fit elderly in absolute terms but not when expressed per unit liver volume. Similar changes were noted in the partial metabolic clearance to paracetamol glucuronide (CLG). In contrast however, although clearance to sulphate fell only in terms of body weight in the frail elderly, clearance to glucuronide was markedly lower, not only in terms of body weight but also per unit volume of liver. No differences in the renal clearance of unchanged drug (CLR) were noted between the three groups: absolute amounts excreted unchanged were very small. Inter-individual variations in the amounts of paracetamol and its major conjugates recovered in the 7-h urine collection were large, and thus there were no significant differences in the absolute amounts collected in the three groups. These data are summarized in Table III.

Discussion

Paracetamol is a useful model substrate of hepatic conjugation, being extensively metabolized, primarily to the glucuronide and sulphate conjugates, prior to excretion in the urine [16]. As systemic availability is variable, which precludes the accurate estimation of clearance after oral administration [17], paracetamol was here given intravenously.

Clearance of paracetamol expressed per unit body weight was 21% lower in the fit elderly than in the young subjects. This is of the same order [18–21] as the reported 20–45% fall with age in the clearance of drugs whose metabolism is capacity limited (such as imipramine, antipyrine, theophylline and acetanilide). Furthermore, it is entirely consistent with the reported 19% fall with age in the clearance of the anticonvulsant agent, lamotrigine, a drug which is mainly cleared by glucuronidation [4]. The preservation of clearance rates per unit volume of liver suggests that, as had been noted in the case of acetanilide [21], the fall in paracetamol clearance with age *per se* is related to the fall in liver volume rather than to a decline in the specific activities of conjugating enzymes.

Observations such as the impairment of salicylate elimination and reduced levels of aspirin esterase activity in frail, rather than healthy elderly people have underlined the importance of the influence of ill health on drug metabolism in ageing man [22, 23]. Clearance of paracetamol by our frail elderly subjects was lower than

in young and elderly healthy subjects with both a 47% fall per unit body weight and 37% fall per unit liver volume when compared to young subjects. Thus, in contrast with the situation in the fit elderly subjects, the reduction in paracetamol clearance in the frail elderly is not completely accounted for by a fall in liver volume. The significant reduction in partial metabolic clearance to paracetamol glucuronide per unit volume of liver, suggests that an impairment of glucuronidation provides an additional explanation in these patients. Chronic but stable disease states must thus be added to the list of influences such as smoking and concurrent drug therapy [24, 25] as factors to be controlled for when investigating drug metabolism in aged humans.

The mechanism underlying these changes is uncertain. The effect of age upon the *in vitro* activities of conjugating enzymes in human liver has not been reported and no consistent effects of ageing in rats have been noted. Uridine diphosphate glucuronyl transferase activities have been shown to be variably affected by ageing [26, 27], although the important influences of species, tissue and diseases must be underlined [28]. A reduction in sulphotransferase activity with reduced excretion of sulphate and increased excretion of glucuronide conjugates has been reported in aged male rats [29], although our data show that this does not occur in frail elderly humans.

Dietary factors are important influences of hepatic conjugation reactions. Thus, as well as producing glucuronic acid in smaller amounts than well-fed animals, uridine diphosphate dehydrogenase activity is significantly lower in the liver of fasted than fed animals [30]. Furthermore, dietary ingestion of, for example, cruciferous vegetables enhances glucuronidation probably because of induction by certain indoles [31]. Although a detailed comparison of the diets of subjects was not undertaken, food intake of the hospitalized frail subjects will have differed from those of the community-dwelling young and fit elderly subjects both in quality and quantity and this may be of critical importance in explaining the difference in hepatic clearance to glucuronide. An alteration in renal function in the frail elderly subjects such as the selective saturation of the mechanism of active tubular secretion of the glucuronide conjugate is a theoretical explanation of our data. Saturation of the renal clearance of the sulphate rather than the glucuronide conjugate, however, has been reported in man [15].

In summary, this study suggests that the fall in liver volume with age is the major contributor to the decline in the clearance of paracetamol in healthy elderly subjects. Conjugation by glucuronidation is impaired per unit volume of liver in frail, hospitalized elderly subjects, perhaps owing to changes in the availability of substrates or in the activities of conjugating enzymes. The results confirm the important influence of the patient's physical state upon the changes which occur in drug metabolism with age.

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