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The effect of age on glucuronidation and sulphation of paracetamol by human liver fractions

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Glucuronidation and sulphation were studied *in vitro* in human liver samples from 22 subjects aged 40–89 years using paracetamol as substrate. There was no significant correlation with age for the activity of either enzyme pathway. These results provide further evidence that age *per se* does not have a major effect on the activities of hepatic drug metabolising enzymes.

Keywords ageing human glucuronidation sulphation paracetamol

Introduction

Adverse drug reactions are generally held to be more frequent in the elderly (Williamson & Chopin, 1980), one reason being the decreased hepatic clearance of some drugs. Studies of drug metabolism in humans have concentrated largely on oxidised drugs and have tended to show a fall in the rate of metabolism (Schmucker, 1985). This observation was initially thought to be due to decreased activities of hepatic microsomal monooxygenase enzymes; a finding which was first demonstrated in rats (Kato *et al.*, 1964). However, *in vitro* studies of drug oxidation in man, examining both the metabolism of several probe substrates and the concentration of specific cytochrome P450 isoenzymes, have shown no significant fall with age (Schmucker *et al.*, 1990; Woodhouse *et al.*, 1984). Further work has suggested that much of the decreased hepatic drug clearance with age can be accounted for by a decline in liver volume (Wynne *et al.*, 1989).

The effect of age on conjugation pathways of drug metabolism has been much less extensively studied. Both *in vivo* and *in vitro* work has been contradictory. Our earlier study of the effect of age and of frailty on paracetamol conjugation (Wynne *et al.*, 1990) found that although there was a decrease in both sulphation and glucuronidation, this was proportional to the decrease in liver size in fit, old subjects. A further decrease in paracetamol clearance was observed in frail subjects who should be considered as an entirely separate group. These results suggested that, as has been found with Phase I enzymes, specific enzyme activity is not age-related. It should be noted that no direct measurements of specific activities of the enzymes of conjugation have been made in human liver with respect to age—and that rat studies have given differing results depending on sex, species and substrate studied (van Bezooijen, 1984). We therefore set out to examine glucuronidation and sulphation using paracetamol as the model drug; firstly because it is extensively conjugated, about 60% as glucuronide and 30% as sulphate (Prescott, 1980), and secondly, to enable us to relate *in vitro* results to our previous *in vivo* work. Activities of the paracetamol sulphotransferases and glucuronyltransferases were measured *in vitro* using liver samples from individuals over a wide age range.

Methods

Subjects

Wedge biopsies of liver were obtained with informed consent from 22 patients undergoing elective cholecystectomy. Approval for the study was obtained from the Newcastle Joint Ethics Committee. Only samples from those patients with benign, non-obstructing lesions who had normal liver function tests were used in the study. All subjects had normal biochemistry and haematology. Alcohol consumption was noted and no patient consumed more than four units of alcohol per day, most consuming very little. Smoking status and use of medication were recorded also: four subjects smoked and four subjects were taking ranitidine. Patients taking drugs known to affect drug-metabolising enzymes were not included in the study.

Laboratory methods

Liver samples were placed on ice and stored immediately at −80° C until use. At this temperature the enzymes

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under consideration are stable (Powis *et al.*, 1988; von Bahr *et al.*, 1980). Liver tissue (weight 85–400 mg) was minced with scissors and then homogenised in ice-cold phosphate buffer (0.25 M potassium phosphate, 0.15 M potassium chloride, pH 7.25) using a hand held glass homogeniser. The homogenate was centrifuged for five minutes at 100 *g* and then for 10 min at 18,000 *g*. An aliquot of the supernatant (the post mitochondrial fraction, consisting of all subcellular fractions except mitochondria) was removed and stored at −80° C. The remainder was centrifuged for 1 h at 120,000 *g*. The microsomal pellet was resuspended in buffer, ultracentrifuged for a further hour and then the pellet was resuspended in 30% v/v glycerol buffer before storing at −80° C. Protein concentrations were measured using a modified method of Lowry *et al.* (1951).

Concentrations of substrate and cofactor were found which achieved maximal activity for each enzyme system. Glucuronidation was studied in microsomes. Sulphation, which is cytosolic, was studied in the post mitochondrial fraction. Conditions used (final concentrations) for glucuronidation were: protein 1–2 mg ml^{-1}, uridine diphosphoglucuronic acid (UDPGA) 10 mM, Triton X-100 0.001% and paracetamol 15 mM, and for sulphation: adenosine 3′-phosphate 5′-phosphatosulphate (PAPS) 2 nM and paracetamol 5 mM. Duplicate incubations, at 37° C in a shaking water bath, were stopped at 30 min and 20 min, respectively, when both reactions were still proceeding linearly. The reactions were stopped by adding 40 µl 30% v/v perchloric acid and 40 µl β-hydroxy ethyl theophylline (50 µg ml^{-1}) as internal standard to a 100 µl aliquot. After centrifugation the supernatant was analysed by h.p.l.c. using a Kontron autoanalyser.

The h.p.l.c. assay was adapted from the method of Knox & Jurand (1978). It was an ion-pair method using 0.5 mM tetrabutyl ammonium phosphate in 1% w/v acetic acid with 20 mM potassium sulphate in NANO pure water as the mobile phase. For measurement of the sulphate an isocratic elution with 18% v/v methanol gave good resolution (retention times 5, 9 and 12 min, respectively, for paracetamol, its sulphate and internal standard). For the measurement of the glucuronide a gradient of methanol from 12% to 27% v/v was used (retention times 11, 7 and 20 min, respectively, for paracetamol, its glucuronide and internal standard). Duplicate standard curves of concentrations from 0–50 µM paracetamol, along with a quality control sample, were run with each assay. An aliquot of each sample was injected via a pre-column onto a 25 cm steel 5 µm ODS column. Paracetamol and its metabolites were detected at 254 nm.

Coefficients of variance for the h.p.l.c. method were 4% for both glucuronide and sulphate for five replicate assays. The amounts of conjugate were expressed as paracetamol equivalents as they have molar coefficients very similar to paracetamol (Howie *et al.*, 1977). Reference samples of paracetamol sulphate and glucuronide were kindly supplied by Dr R. S. Andrews of Winthrop Laboratories, Newcastle upon Tyne. Results of repeat incubations of the same liver samples (four for glucuronidation and three for sulphation) were within 10%.

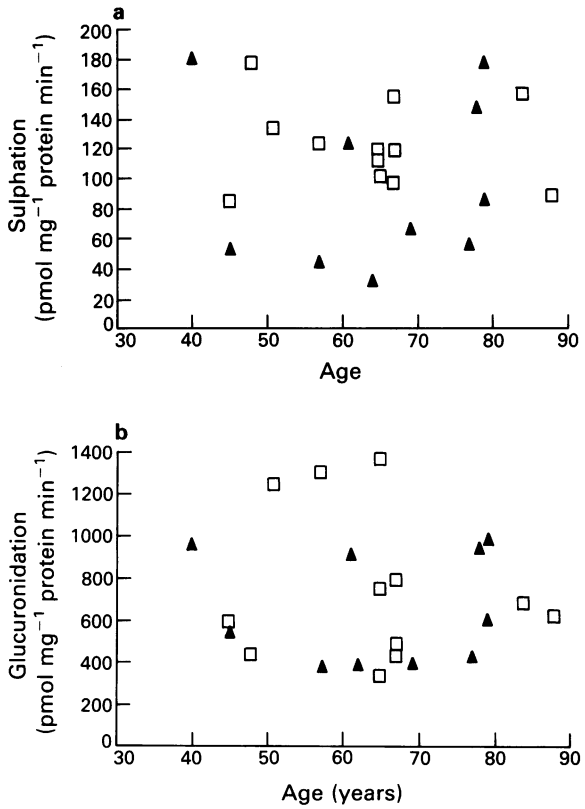

Figure 1 Activities of a) sulphotransferases and b) glucuronosyltransferases with respect to the metabolism of paracetamol by human liver fractions, as a function of age. ▲ females, □ males.

Results

The results are shown in Figure 1. Correlation was assessed using Kendall's rank correlation. There was no correlation between age and enzyme activity for either males or females and no significant sex difference was found. Sub-groups were compared using the unpaired Wilcoxon rank sum test, and the results expressed with accompanying standard deviations. No significant differences were found between non-smokers and smokers for either sulphation (119 ± 9.9 against 77 ± 21 pmol mg^{-1} protein min^{-1}) or glucuronidation (744 ± 78 against 582 ± 114 pmol mg^{-1} protein min^{-1}), but the numbers were small. For glucuronidation, there was no significant difference between results using tissue from patients taking ranitidine and those not (771 ± 200 against 706 ± 72 pmol mg^{-1} protein min^{-1}). Females tended to have lower activities for both glucuronidation (652 ± 83 against 771 ± 104 pmol mg^{-1} protein min^{-1} for males) and sulphation (101.1 ± 18.2 against 122.9 ± 8.1 pmol mg^{-1} protein min^{-1} for males), but these differences did not reach statistical significance.

Discussion

As expected, the results show a wide variation in activities, confirming the findings of previous studies of drug metabolising enzymes in man (Schmucker *et al.*, 1990; Woodhouse *et al.*, 1984). No fall in activity with age was

found. This wide inter-individual variation means that it is impossible to exclude a minor change but this would be unlikely to be of any clincal significance. It should be noted that these results can only be applied to the fit subjects whom we have studied. Tissue from frail subjects was not investigated.

Several factors may influence drug metabolism which were not controlled in our subjects. Sex has been found to affect paracetamol metabolism, clearance being lower in women (Miners *et al.*, 1983). Conversely, women taking oral contraceptive steroids have been shown to have greater clearance, due largely to an increase in glucuronidation (Miners *et al.*, 1986). We found no differences in the data for our subjects with respect to their sex but as only two of our female subjects were less than 50 years old, and hence most were post-menopausal, hormonal effects would be different from those in young women. None of our subjects was takng oral contraceptive steroids.

The effect of smoking is not clear from previous studies and there were too few smokers in our study for any conclusions to be drawn.

Four of our subjects were taking ranitidine which has been shown to inhibit glucuronidation of paracetamol in hepatocytes in a competitive manner (Emery *et al.*, 1985). Our preparation would have removed virtually all drug from microsomes and thus no effect on activities would be expected, and none was found.

In conclusion, our results show that glucuronidation and sulphation of paracetamol by liver fractions *in vitro* do not fall significantly with normal ageing. Therefore, any age-related decrease in paracetamol clearance is likely to reflect reduced liver volume and liver blood flow.

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