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Interindividual variability in the glucuronidation and sulphation of ethinyloestradiol in human liver

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1 Glucuronidation and sulphation of ethinyloestradiol (EE_2) was studied in human liver. Microsomal glucuronyltransferase activity was measured in 110 livers whose donors were 71 women and 39 men. Enzyme activity ranged between 12.6 and 242 pmol min^{-1} mg^{-1} protein, i.e. over a 19-fold range and the mean (± s.d.) glucuronyltransferase activity was 96.8 ± 47.9 pmol min^{-1} mg^{-1} protein.

2 Cytosolic sulphotransferase activity was measured in 138 livers whose donors were 90 women and 48 men. Enzyme activity ranged between 14.4 and 98.2 pmol min^{-1} mg^{-1} protein, i.e. over a 7-fold range, and the mean (± s.d.) sulphotransferase activity was 43.7 ± 18.6 pmol min^{-1} mg^{-1} protein.

3 Human liver glucuronyltransferase and sulphotransferase activities showed a unimodal distribution pattern. Enzyme activities were neither sex-related nor age-dependent. Sulphotransferase activity did not correlate with glucuronyltransferase activity (n = 80) suggesting that the two enzymes are independently regulated. The ratio of specific glucuronyltransferase to sulphotransferase activity ranged between 0.15 and 8.0 (mean ± s.d., 2.44 ± 1.51) and was unimodally distributed.

Keywords ethinyloestradiol sulphotransferase glucuronyltransferase liver humans

Introduction

Most studies of the interindividual variability of drug metabolism have dealt with phase I enzymes (Alvan *et al.*, 1990), whereas little is known about the phase II enzymes with the exception of acetyltransferase (Price-Evans, 1989; Weber & Hein, 1985) and methyltransferase (Weinshilboum, 1989). Ethinyloestradiol (EE_2) is a synthetic oestrogen widely used in humans. It is extensively metabolized, being hydroxylated and also conjugated with glucuronic acid and sulphate (Bolt, 1979). A large interindividual variability in the metabolism of this drug has been described (Orme *et al.*, 1989). The causes of such variability are not yet understood because of the complex metabolism of EE_2. In the present study, we have measured the activities of glucuronyltransferase and sulphotransferase in human liver to establish whether these enzymes are subject to a similar or different interindividual variability.

Methods

Chemicals

Radioactive (6,7-[^{3}H](N))-ethinyloestradiol (EE_2) (59.2 Ci $mmol^{-1}$, radiochemical purity 99%) was purchased from Du Pont de Nemours, NEN Division (Florence, Italy). Unlabelled EE_2, 3′-phosphate-5′-phosphosulphate (PAPS), uridine 5′-diphosphoglucuronic acid (UDPGA), glycine, 2-mercaptoethanol, trichloroacetic acid and Tris (tris-(hydroxymethyl)aminomethane) were obtained from Sigma (St Louis, MO, USA).

Biological material

Wedge human liver specimens were obtained at laparotomy for hepatic surgery from 96 women aged between 25 and 78 years and from 48 men aged between 23 and 81 years, and would otherwise have been discarded. None of the patients was receiving chronic treatment with any drug known to induce drug metabolizing enzymes such as anticonvulsants. Liver specimens were frozen at −80° C within 3 min of sampling. Tissue specimens with normal cell architecture only were used. Biopsies were homogenized in 5 volumes of 0.25 M sucrose using a glass-teflon homogenizer. The homogenates were centrifuged at 12,000 *g* for 15 min at 4° C and the supernatant was centrifuged at 105,000 *g* for 60 min at 4° C. The supernatants were divided into aliquots, stored at −80° C and investigated as the cytosolic fraction. The pellets were resuspended in 0.1 mM Tris-HCl (pH 7.4)

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containing 30% v/v glycerol, divided into aliquots and stored at −80° C. Protein concentration was measured as described by Lowry *et al.* (1951).

Enzyme assays

Glucuronyltransferase assay was as previously described (Pacifici & Back, 1988). The incubation mixture, final volume 0.2 ml, consisted of 0.1 M Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.2 mM EE_2 (500,000 d min^{-1}), 5 mM UDPGA and an aliquot of the microsomal suspension to give a final protein concentration varying between 0.1 and 0.4 mg ml^{-1}. The reaction was started by the addition of UDPGA, carried out at 37° C for 20 min and was stopped by the addition of 0.8 ml of a mixture containing 0.4 M TCA and 0.6 M glycine. Each sample was assayed in duplicate along with two blanks whose composition was the same as the active samples except that UDPGA was replaced by water. Sulphotransferase assay was as previously described (Pacifici & Back, 1988). Briefly, the final incubation volume was 100 μl and consisted of 0.1 M Tris-HCl (pH 7.4), 5 mM 2-mercaptoethanol, 0.025 mM EE_2 (150,000 d min^{-1}) added in 1 μl of acetone, 0.1 mM PAPS and an aliquot of the cytosolic fraction to give a final protein concentration varying between 1.0 and 1.8 mg ml^{-1}. The reaction was started by the addition of PAPS, carried out at 37° C for 20 min and stopped by the addition of 0.9 ml of a mixture containing 0.4 M TCA and 0.6 M glycine. Each sample was assayed in duplicate along with two blanks whose composition was the same as the active samples except that PAPS was replaced by water.

Unreacted EE_2 was removed from the reaction mixture by extraction into diethylether as previously described for glucuronyltransferase and sulphotransferase assays (Pacifici & Back, 1988). The radioactivity was measured in an aliquot (0.5 ml) of the aqueous residue, and the activities of both sulphotransferase and glucuronyltransferase were measured on the basis of the specific radioactivity of EE_2, after correction for blanks. It was assumed that all residual activity remaining in the aqueous phase after correction for the appropriate blanks, represented either the sulphate or glucuronide conjugate. The activities of the two enzymes are expressed as pmol min^{-1} mg^{-1} protein.

Results

The contents (average ± s.d.) of microsomal and cytosolic protein were 21.4 ± 8.8 and 59.9 ± 18.9 mg g^{-1} liver, respectively. The rate of EE_2 glucuronidation was measured in 110 liver specimens and the results are shown in Figure 1a. Glucuronyltransferase activity ranged between 12.6 and 242 pmol min^{-1} mg^{-1} protein with a mean (± s.d.) of 96.8 ± 47.9 (all patients, n = 110), 94.2 ± 38.4 (men, n = 39) and 98.3 ± 52.4 (women, n = 71) pmol min^{-1} mg^{-1} protein. There was no significant difference between the values obtained in men and women. The percentage coefficient of variation was 49.5% (all patients), 40.7% (men) and 53.3% (women). There was no correlation between glucuronyltransferase activity and liver donor's age. The enzyme activity was also expressed on the basis of g liver equivalent and the mean (± s.d.) of all samples was 1.85 ± 1.09 nmol min^{-1} g^{-1} liver.

The rate of EE_2 sulphation was measured in 138 specimens and the activity of individual specimens is shown in Figure 1d. The sulphotransferase activity ranged between 14.4 and 98.2 pmol min^{-1} mg^{-1} protein with a mean (± s.d.) activity of 43.7 ± 18.6 (all patients), 44.8 ± 19.3 (women, n = 48) and 41.6 ± 17.1 (men, n = 90) pmol min^{-1} mg^{-1} protein. There was no significant difference between the values obtained in men and women. The coefficient of variation was 41.1% (all patients), 43.1% (women) and 41.6% (men). There was no correlation between sulphotransferase activity and the age of the liver donor. The enzyme activity of all samples was also expressed on the basis of g liver equivalent and the mean ± s.d. was 2.63 ± 1.48 nmol min^{-1} g^{-1} liver.

The duration of storage at −80° C did not influence the activities of either glucuronyltransferase or sulphotransferase (Figure 1, b, e).

A unimodal distribution was observed for both glucuronyltransferase and sulphotransferase activities (Figure 1, c, f).

Figure 2a, b shows the relationship between the glucuronyltransferase to sulphotransferase ratio and liver donor age when the enzyme activities were expressed on the basis of mg protein. The ratio ranged between 0.15 and 8.0, with the mean (± s.d.) and the coefficient of variation being 2.44 ± 1.51 and 61.8%, respectively. There was no correlation between this ratio and liver donor age. When the enzyme activities were expressed on the basis of g liver the ratio ranged between 0.05 and 2.92 (Figure 2, c, d) with the mean (± s.d.) and the coefficient of variation being 0.86 ± 0.61 and 70.9, respectively. There was a significant correlation ($P < 0.05$) between this ratio and liver donor age. The glucuronyltransferase to sulphotransferase ratio was not different in women (2.37 ± 1.47) and men (2.54 ± 1.59) and it was unimodally distributed (Figure 2d).

Discussion

The results show that the variability in the rate of EE_2 glucuronidation is greater than that of EE_2 sulphation in human liver. We have also shown that the activities of glucuronyltransferase and sulphotransferase towards EE_2 are neither sex-related nor age dependent.

Glucuronidation and sulphation of EE_2 have been previously studied in human liver and the present data are in accord with the published data (Pacifici & Back, 1988; Pacifici *et al.*, 1988). Under the appropriate storage conditions both glucuronyltransferase and sulphotransferase activities are very stable. Powis *et al.* (1988) have observed that the duration of storage at −80° C has no effect on the activities of the human liver phenolsulphotransferase and thiopurinemethyltransferase at least for up to 3–4 years.

The activity of glucuronyltransferase is more susceptible than that of sulphotransferase to enzyme-inducing agents (Pacifici & Back, 1988; Viani *et al.*, 1990). The greater interindividual variability observed in glucu-

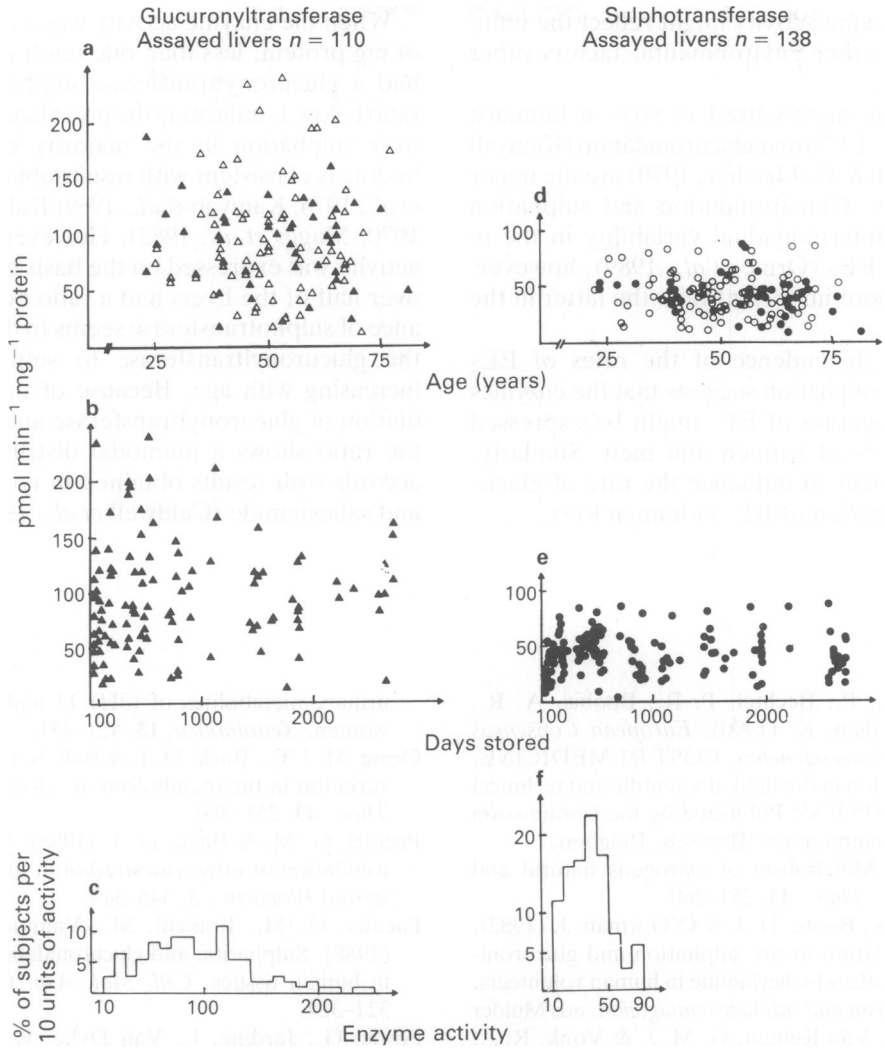

Figure 1 Glucuronyltransferase and sulphotransferase activity towards ethinyloestradiol in human liver. Glucuronyltransferase activity is plotted against liver donor age (a) and the duration of storage at −80° C (b). (c) Frequency distribution of glucuronyltransferase activity in human liver. Sulphotransferase activity is plotted against liver donor age (d) and the duration of storage at −80° C (e). (f) Frequency distribution of sulphotransferase activity in human liver. (a and b) Filled and unfilled symbols refer to men and women, respectively.

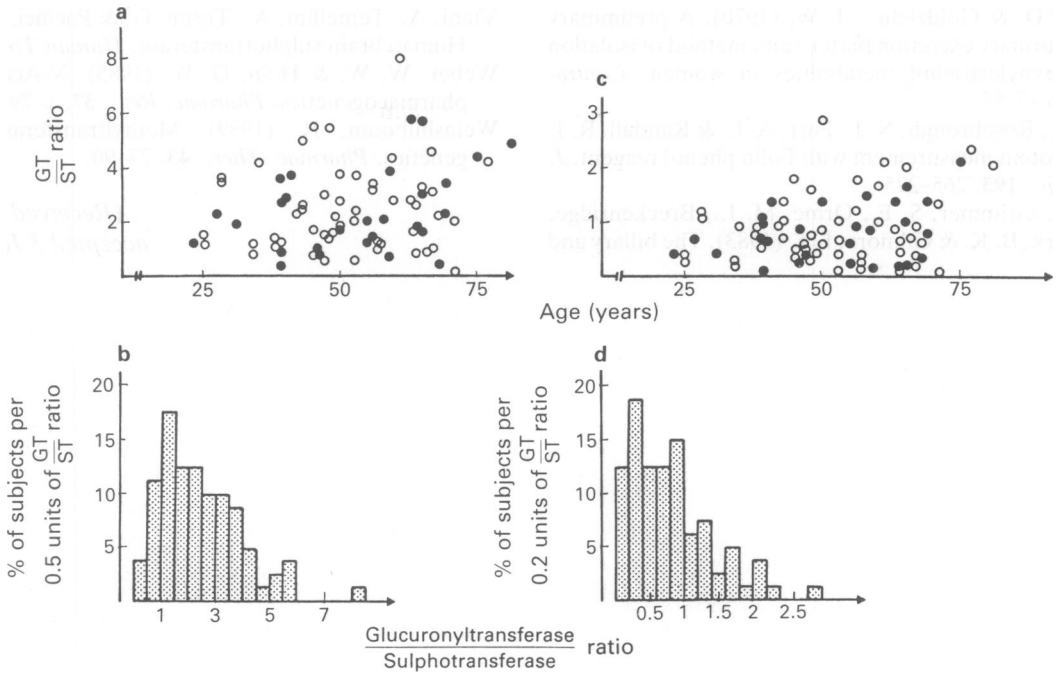

Figure 2 Glucuronyltransferase to sulphotransferase ratio in 80 human livers. The glucuronyltransferase and sulphotransferase activities are expressed on the basis of mg protein (a, b) and on the basis of g liver equivalent (c, d). (a, c) The ratio is plotted against liver donor age. Filled and unfilled circles refer to men and women, respectively. (b, d) Frequency distribution histogram of glucuronyltransferase to sulphotransferase ratio in human liver.

ronyltransferase enzyme activity might reflect the influence of drugs and other environmental factors other than genetic factors.

EE_2 is extensively metabolized *in vivo* in humans; hydroxylation (Bolt, 1979) and glucuronidation (Kamyab *et al.*, 1969; Kulkarni & Goldzieher, 1970) are the major metabolic pathways. Glucuronidation and sulphation may contribute to interindividual variability in the *in vivo* metabolism of EE_2 (Orme *et al.*, 1989), however, the former seems more important than the latter in the liver.

The lack of sex dependence of the rates of EE_2 glucuronidation and sulphation suggests that the enzymes catalyzing the conjugation of EE_2 might be expressed similarly in the livers of women and men. Similarly, ageing does not appear to influence the rate of glucuronidation and sulphation of EE_2 in human liver.

When the enzyme activity was expressed on the basis of mg protein, less than one tenth of the assayed livers had a glucuronyltransferase/sulphotransferase activity ratio below 1, indicating the prevalence of glucuronidation over sulphation in the majority of individuals. This finding is consistent with results obtained *in vivo* (Helton *et al.*, 1976; Kamyab *et al.*, 1969; Kulkarni & Goldzieher, 1970; Maggs *et al.*, 1983). However, when the enzyme activity was expressed on the basis of g liver equivalent, over half of the livers had a ratio below 1. The importance of sulphotransferase seems to decrease with ageing the glucuronyltransferase to sulphotransferase ratio increasing with age. Because of the independent regulation of glucuronyltransferase and sulphotransferase, the ratio shows a unimodal distribution. This finding accords with results obtained *in vivo* with paracetamol and salicylamide (Caldwell *et al.*, 1982).

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