Valpromide inhibits human epoxide hydrolase

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1 The effect of antiepileptic drug valpromide (VPM) on the activity of epoxide hydrolase was studied in human adult and foetal liver, kidneys, lungs, intestine and in placenta. The activity of the epoxide hydrolase was measured with both styrene oxide and benzo(a)pyrene-4,5-oxide as substrates.

2 VPM inhibited the epoxide hydrolase obtained from all organs studied. The degree of inhibition was independent of the substrate used.

3 A lowering of the epoxide hydrolase activity by 50% was observed when the concentration of VPM was similar to that of the substrates.

4 VPM competitively inhibited the activity of adult liver epoxide hydrolase with styrene oxide as substrate.

Keywords valpromide epoxide hydrolase adult subjects foetuses

Introduction

Valpromide (VPM) administered in combination with carbamazepine to epileptic patients increases the plasma concentration of the major carbamazepine metabolite, carbamazepine-10, 11-epoxide (Meijer *et al.*, 1984). The main metabolic pathway of this epoxide is hydration to carbamazepine-10, 11-dihydrodiol (Eichelbaum *et al.*, 1985). It was suggested that VPM might inhibit this reaction. Support for this hypothesis was obtained using Rhesus monkey liver with styrene oxide and benzo(a)pyrene-4, 5-oxide as substrates (Pacifici *et al.*, 1985a). We now report on the effect of VPM on epoxide hydrolase from human liver. Since liver is not the only site of metabolism of epoxides we extended the investigation to include non-hepatic tissues from adult subjects. Furthermore, since the teratogenicity of carbamazepine has been ascribed to its epoxide (Lindhout *et al.*, 1984) we also tested the effects of VPM on the epoxide hydrolase from human foetal tissues.

Methods

Chemicals

(7-³H)Styrene-7,8-oxide (99.6 mCi mmol⁻¹) was purchased from The Radiochemical Centre (Amersham, England). Benzo(a)pyrene-4, 5-epoxide and 4,5-dihydrobenzo(a)pyrene-4, 5-oxide(G-³H) (382 mCi mmol⁻¹) were from the NCI chemical carcinogen Reference Standard Repository, a section of the Division of Cancer Cause and Prevention, NCI, NIH (Bethesda, MA, USA). The radiochemical purity of both substrates was checked by thin layer chromatography and it was higher than 99%. Unlabelled styrene oxide and styrene glycol were obtained from Aldrich (Beerse, Belgium).

Biological material

Human adult liver was obtained as the surplus of tissue material for histological analysis obtained at laparotomy. Hepatic wedge biopsies were

frozen on dry ice within 5 min after sampling. Specimens from the kidneys, lungs and intestine were obtained after resection of the organ for therapeutic purposes. A normal part of the tissue next to the tumour was made available for our studies. In some cases histological analysis of the tissue specimens used as the enzyme source was performed. Tissues were free from pathological changes. The mucosa of the intestine was isolated at room temperature. The procedure of isolation required about 15 min. Tissues were frozen as soon as possible (usually within 20 min) and stored at -80°C .

Human foetal tissues were obtained at legal abortion performed for socio-medical reasons. The age of the foetuses was estimated from the crown-rump length. The foetal liver and the other tissues were excised as soon as possible (usually within 20 min after the abortion) and immediately frozen on dry ice.

The clinical data of the patients and the age of the foetuses included in the study are summarized in Table 1.

Assays

An aliquot of human adult liver (0.2–0.3 g) and other tissues (1–3 g) was homogenized in 3 vol-

umes of 0.25 M sucrose, adjusted to pH 7.4 with Tris buffer. The homogenates were centrifuged at 9,000 g for 15 min. The resulting supernatant was centrifuged again at 105,000 g for 1 h. The pellet residue were resuspended in 0.05 M Tris-HCl (pH 7.4) containing 30% v/v glycerol and investigated as the microsomal fraction.

Epoxide hydrolase activity was measured with 1 mM styrene oxide or 0.2 mM benzo(a)pyrene-4,5-oxide, as described by Jerina *et al.* (1977). The final volume of the incubation mixture was 0.1 ml and its pH was 8.7. When the kinetics of the enzyme were studied the concentration of the substrate (styrene oxide) ranged between 0.12 and 2.0 mM. The microsomal protein concentration ranged between 0.9 and 1.3 mg ml⁻¹ (adult liver); 1.3 and 2.1 mg ml⁻¹ (foetal liver); 1.8 and 3.5 mg ml⁻¹ non-hepatic tissues of adult and foetal origin. The incubation time was 10 min (human adult liver) or 30 min (foetal liver and non-hepatic tissues of adult and foetal origin) when styrene oxide was the substrate. It was 4 min when benzo(a)pyrene-4,5-oxide was the substrate. VPM was added in 1 µl of dimethylsulphoxide. The controls contained 1 µl of dimethylsulphoxide. Reactions were started by the addition of styrene oxide or benzo(a)pyrene-4, 5-oxide in 5 µl of acetonitrile. Each sample

Table 1 Clinical data of the foetuses and patients included in the study

Foetus	Age (weeks)		
1	13		
2	15		
3	16		
4	17		
5	21		
Liver donors	Age (years)	Sex	Diagnosis
1	40	F	Cholelithiasis
2	55	F	Cholelithiasis
3	57	F	Cholelithiasis
4	60	M	Cholelithiasis
Kidney donors			
1	32	F	Renal carcinoma
2	40	F	Hydropnephrosis
3	64	M	Renal carcinoma
4	75	M	Renal carcinoma
Lung donors			
1	57	M	Lung carcinoma
2	59	M	Lung carcinoma
3	60	M	Lung carcinoma
4	61	M	Lung carcinoma
Gut donors			
1	31	F	Descending colon carcinoma
2	54	F	Ascending colon carcinoma
3	54	F	Ascending colon carcinoma
4	73	F	Sigmoid colon carcinoma

was assayed in duplicate. Each assay was provided with two blanks which had the same content as the samples except that the fresh microsomal protein was replaced by boiled protein. Reactions were stopped by addition of 20 μ l of tetrahydrofuran containing unlabelled styrene glycol or benzo(a)pyrene-4,5-dihydrodiol. Each incubation mixture (30 μ l) was applied to the loading zone of a 19-channel LK5DF Whatman t.l.c. plate. Chromatography was performed with chloroform/ethyl acetate (8/2 v/v) when styrene oxide was the substrate and benzene/methanol (93:7 v/v) when benzo(a)pyrene-4,5-oxide was the substrate. The spots representing styrene glycol and benzo(a)pyrene-4,5-dihydrodiol were located under UV light and scraped into scintillation vials containing 2 ml of methanol. Scintillation mixture (10 ml) was added and the radioactivity was measured in a liquid scintillation system. The radioactivity of the sample was at least twice as high as that of the blank. The data reported were obtained under conditions of linearity with respect to time and protein.

Protein concentration was measured according to Lowry *et al.* (1951).

Calculations

The significance of differences between controls and 'VPM samples' was determined by Student's *t*-test using $P = 0.05$ as the criterion. Kinetic parameters ($V_{\max}$ and K_M) of the epoxide hydrolase were measured after construction of Eadie-Hofstee plots. The apparent K_i was measured after construction of plots of $1/V$ vs the

concentrations of the inhibitor (VPM) at various concentrations of styrene oxide.

Results

The effects of different concentrations of VPM on the activity of the epoxide hydrolase were first studied in human foetal and adult liver microsomal preparations (Figure 1). Inhibition of the epoxide hydrolase activity by 50% was observed at a concentration of VPM between 1 and 2 mM when styrene oxide was the substrate, and at about 0.2 mM when benzo(a)-pyrene-4,5-oxide was the substrate. The concentrations of 2 and 0.25 mM VPM were then used to test the inhibitory effect of VPM on the epoxide hydrolase from different tissues towards styrene oxide and benzo(a)pyrene-4,5-oxide, respectively (Figure 2).

A significant inhibition of the epoxide hydrolase was observed in each tissue except for the intestinal mucosa from adult subjects with benzo(a)pyrene-4,5-oxide as substrate. The type of inhibition exerted by VPM on the epoxide hydrolase was studied in three adult livers using styrene oxide as substrate. The inhibition was found to be competitive. The results of a representative experiment are summarized in Figure 3. The kinetic parameters, $V_{\max}$ and K_M , for three livers are given in Table 2. The K_i of VPM varied between 0.36 and 0.65 mM (Table 2).

Discussion

Our findings show that VPM inhibits the epoxide hydrolase of various tissues from human foetuses and adult subjects.

Several endogenous compounds (Rane & Gufstasson, 1973; Disse *et al.*, 1980; Vogel-Bindel *et al.*, 1982; Meyer & Ketterer, 1982), drugs (Frigerio *et al.*, 1976; Eichelbaum *et al.*, 1985) and chemicals (Daly *et al.*, 1972; Jerina & Daly, 1974) are biotransformed into epoxides as intermediate metabolites. The formation of epoxides is catalyzed by cytochrome P-450 (Daly *et al.*, 1972) and they are further metabolized into glycols or dihydrodiols by epoxide hydrolase (Oesch, 1979). Epoxides may also be conjugated to glutathione by glutathione S-transferases (Arias & Jacoby, 1976). Therefore, the hydration of epoxides represents an important physiological function of xenobiotic metabolism, being a mechanism of clearing some drugs or drug metabolites from the body and a system of biochemical defence. Interactions with epoxide hydrolase may thus result in an alteration of one or more of these functions.

Figure 1 Effects of various concentrations of VPM on the activity of liver microsomal epoxide hydrolase towards styrene oxide and benzo(a)pyrene-4,5-oxide. The liver donor was a 55-year-old man. The foetal liver was from a 16-week-old foetus. Styrene oxide: ●, adult liver; ○, foetal liver. Benzo(a)pyrene-4,5-oxide: ▲, adult liver; △, foetal liver.

Figure 2 Effects of VPM on the activity of the epoxide hydrolase from various tissues. Columns refer to the mean values. The concentrations of VPM were 0.25 and 2.0 mM when the substrates were benzo(a)pyrene-4,5-oxide and styrene oxide, respectively. Vertical bars refer to the s.e. mean. The stars refer to the level of significance of the difference between controls (□) and VPM samples (▨). One, two and three stars refer to a level of significance of 0.05; 0.01 and 0.002 respectively. Li, Liver; Lu, lung; K, kidney; G, gut; P, placenta; RC, renal cortex; RM renal medulla.

The activity of epoxide hydrolase towards styrene oxide in foetal and adult tissues accords with results from this and other laboratories (Kapitulnik *et al.*, 1977; Pacifici & Rane, 1982; Pacifici *et al.*, 1981; 1984; 1985b). The availability of information on the epoxide hydrolase towards benzo(a)pyrene-4,5-oxide in human tissues is sparse. Data for human adult liver (Kapitulnik *et al.*, 1977) and foetal hepatic and extrahepatic tissues (Pacifici *et al.*, 1985b) are consistent with the present results.

Microsomal epoxide hydrolase activity in human liver represents a family of different isoenzymes (Guengerich *et al.*, 1979a,b; Levin *et al.*, 1978). Thus it has been shown that the hydration of styrene oxide and benzo(a)pyrene-4,5-oxide may be catalyzed by different forms of the microsomal epoxide hydrolase in human adult liver (Lu *et al.*, 1979; Guengerich *et al.*, 1979a). Indirect evidence for the presence of

Figure 3 Effects of VPM on the activity of epoxide hydrolase towards styrene oxide. The liver donor was 60-year-old man. a) The enzyme activity is plotted against the concentration of styrene oxide. b) Eadie-Hofstee plots. ●, VPM 0mM; □, VPM 0.5 mM; ○, VPM 2 mM.

Table 2 Enzymatic kinetic constants of epoxide hydrolase in preparations of human adult liver microsomes in the presence or absence of valpromide

Liver donor	V_{max} (nmol min ⁻¹ mg ⁻¹)			K_M (mM)			K_i (mM)
	Valpromide concentration (mM)			Valpromide concentration (mM)			
	0	0.5	2.0	0	0.5	2.0	
55 F	24.80	21.17	25.58	0.14	0.56	1.65	0.40
40 F	16.67	14.53	14.37	0.08	0.47	1.22	0.36
60 M	16.02	16.91	15.62	0.04	0.49	0.91	0.65

such isoenzymes in foetal liver has also been presented (Peng *et al.*, 1984).

VPM is capable of lowering the rate of hydration of both styrene oxide and benzo(a)pyrene-4,5-oxide. However, VPM is not an epoxide. Our previous work with Rhesus monkey liver has shown that VPM, but not valproic acid, inhibits epoxide hydrolase. This finding with valproic acid has been confirmed using human adult liver (unpublished results). Therefore, the substitution of the acid group in the valproic acid molecule with the amide group seems necessary for an inhibitory effect on the enzyme.

Although VPM is converted into valproic acid largely in the gut (Pisani *et al.*, 1981; 1982), some of the drug may enter the liver and then inhibit hepatic epoxide hydrolase. This hypothesis may explain the interaction between VPM and carbamazepine-10,11-epoxide described in humans (Meijer *et al.*, 1984). Because of the low plasma concentrations of VPM, after oral administration to humans (Pisani *et al.*, 1981), the

interaction of VPM with the epoxide hydrolase of non-hepatic tissues may not be of clinical relevance. However, we investigated the effects of VPM on the epoxide hydrolase in several tissues to observe whether a tissue-difference existed. The epoxide hydrolases from foetal and adult tissues were similarly inhibited by VPM. This drug must therefore act on a common 'site' in the foetal as well as the adult enzyme and in hepatic as well as non-hepatic tissues.

It may be significant that the teratogenicity of carbamazepine has been found to be greater in mothers taking VPM in multiple combinations (Lindhout *et al.*, 1984). It is also possible that other drugs or chemicals in addition to VPM produce an inhibition of epoxide hydrolase.

This work was supported by The Cilag Foundation and The Italian Association for the Study of Cancer, the Swedish Medical Research Council (14X-04496) and the Expressen Prenatal Research Foundation.

References

- Arias, J. M. & Jacoby, W. B. (1976). *Glutathione: metabolism and function*. New York: Raven Press.
- Daly, J. W., Jerina, D. M. & Witkop, B. (1972). Arene oxides and the NIH shift: the metabolism, toxicity and carcinogenicity of aromatic compounds. *Experientia*, **28**, 1129–1164.
- Disse, B., Sieckmann, L. & Breuer, H. (1980). Biosynthesis of 16- α , 17- α -epoxy-4-androsteron-3-one in rat liver microsomes. *Acta Endocrinol.*, **95**, 58–66.
- Eichelbaum, M., Tomson, T., Tybring, T. & Bertilsson, L. (1985). Carbamazepine metabolism in man: Induction and pharmacogenetic aspects. *Clin. Pharmacokin.*, **10**, 80–90.
- Frigerio, A., Cavo-Briones, M. & Belvedere G. (1976). Formation of stable epoxides in the metabolism of tricyclic drugs. *Drug Metab. Rev.*, **5**, 197–218.
- Guengerich, F. P., Wang, P., Mitchell, M. B. & Mason, P. S. (1979a). Rat and human liver microsomal epoxide hydratase. Purification and evidence for the existence of multiple forms. *J. Biol. Chem.*, **254**, 12248–12254.
- Guengerich, F. P., Wang, P., Mason, P. S. & Mitchell, M. B. (1979b). Rat and human microsomal epoxide hydratase. Immunological characterization of various forms of the enzyme. *J. Biol. Chem.*, **254**, 12255–12259.
- Jerina, D. M. & Daly J. W. (1974). Arene oxides: A new aspect of drug metabolism. *Science*, **185**, 573–582.
- Jerina, D. M., Dansette, P. M., Lu, A. Y. H. & Levin W. (1977). Hepatic microsomal epoxide hydrolase: a sensitive radiometric assay for hydration of arene oxides of carcinogenic aromatic hydrocarbons. *Mol. Pharmacol.*, **13**, 342–351.
- Kapitulnik, J., Levin, W., Lu, A. Y. H., Morecki, R., Dansette, P. M., Jerina, D. M. & Conney, A. H. (1977). Hydration of arene and alkene oxides by epoxide hydration in human liver microsomes. *Clin. Pharmac. Ther.*, **21**, 158–165.
- Levin, W., Thomas, P. E., Korzeniowski, D., Seifried, H., Jerina, D. M. & Lu, A. Y. H. (1978). Liver microsomal epoxide hydrolase: activation, inhibition and immunological properties of the purified enzyme. *Mol. Pharmacol.*, **14**, 1107–1120.
- Lindhout, D., Hoppener, R. J. E. A. & Meinardi, H. (1984). Teratogenicity of antiepileptic drug combinations with special emphasis on epoxidation (of carbamazepine). *Epilepsia*, **25**, 77–83.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. &

- Randall, R. J. (1951). Protein measurement with folin reagent. *J. biol. Chem.*, **193**, 265–275.
- Lu, A. Y. H., Thomas, P. E., Ryan, D., Jerina, D. M. & Levin, W. (1979). Purification of human liver microsomal epoxide hydrolase. Differences in the properties of the human and rat enzymes. *J. biol. Chem.*, **254**, 5878–5881.
- Meijer, J. W. A., Binnie, C. D., Debets, R. M. C., Van Parys, J. A. P. & De Beer-Pawlikowski, N. K. B. (1984). Possible hazard of valpromide-carbamazepine combination therapy in epilepsy. *Lancet*, **i**, 802.
- Meyer, D. J. & Ketterer, B. (1982). 5 α -6 α -Epoxycholestan-3 β -ol(cholesterol- α -oxide): A specific substrate for rat liver glutathione transferase B. *FEBS Letters*, **150**, 499–502.
- Oesch, F. (1979). Epoxide hydratase. In *Progress in Drug Metabolism*, Vol. 3, eds Bridges, J. W. & Chasseaud, F. L., pp. 253–301. Chichester: John Wiley.
- Pacifici, G. M., Boobis, A. R., Brodie, M. J., MacManus, M. E. & Davies, D. S. (1981). Tissue and species differences in enzymes of epoxide hydrolase. *Xenobiotica*, **11**, 73–79.
- Pacifici, G. M., Colizzi, G., Giuliani, L. & Rane, A. (1984). Nuclear epoxide hydrolase in the human fetal and adult liver. *Pharmacology*, **28**, 321–329.
- Pacifici, G. M., Glaumann, H. & Rane, A. (1985b). Isolation of nuclear fractions from human fetal tissues. Ultrastructure and presence of epoxide hydrolase and aryl hydrocarbon hydroxylase. *Pharmacology*, **30**, 188–196.
- Pacifici, G. M. & Rane, A. (1982). Metabolism of styrene oxide in different human fetal tissues. *Drug Metab. Disp.*, **10**, 302–305.
- Pacifici, G. M., Tomson, T., Bertilsson L. & Rane, A. (1985a). Valpromide/carbamazepine and risk of teratogenicity. *Lancet*, **i**, 397.
- Peng, D. R., Pacifici, G. M. & Rane, A. (1984). Human fetal liver cultures: basal activities and inducibility of epoxide hydrolase and aryl hydrocarbon hydroxylase. *Biochem. Pharmacol.*, **33**, 71–77.
- Pisani, F., Fario, A., Oteri, G. & Di Perri, R. (1981). Dipropylacetic acid levels: Diurnal fluctuations during chronic treatment with dipropylacetamide. *Ther. Drug Monitoring*, **3**, 297–301.
- Pisani, F., Fario, A., Oteri, G. & Di Perri, R. (1982). A study on the metabolism of dipropylacetamide to dipropylacetic acid in rats. *J. Pharm. Pharmacol.*, **34**, 45–46.
- Rane, A. & Gustafsson, J. A. (1973). Formation of a 16,17-trans-glycolic metabolite from a 16-dehydro-androgen in human fetal liver microsomes. *Clin. Pharmac. Ther.*, **14**, 833–839.
- Vogel-Bindel, U., Bentley, P. & Oesch, F. (1982). Endogenous role of microsomal epoxide hydrolase. *Eur. J. Biochem.*, **126**, 425–431.

(Received 24 January 1986,
accepted 2 April 1986)