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EPOXIDE HYDROLASE ACTIVITY IN HUMAN SKIN

VERONICA A. O'NEILL, M.D. RAWLINS & P.H. CHAPMAN

Wolfson Unit of Clinical Pharmacology,
The University, Newcastle upon Tyne

1 Epoxide hydrolase (EH) activity was measured in biopsied skin (n = 42) using 7-[H^3]-styrene oxide as substrate, and separation of the products by high performance liquid chromatography.

2 EH activity (mean ± s.d.) was present in separated epidermis (139 ± 105 pmol glycol formed mg^{-1} min^{-1}) and dermis (165 ± 120 pmol glycol formed mg^{-1} microsomal protein min^{-1}).

3 Whole skin EH activity (mean ± s.d.) varied widely (433 ± 254 pmol glycol formed mg^{-1} microsomal protein min^{-1}).

4 No significant difference in EH activity was observed in skin from breast, penis and leg.

5 Skin EH activity does not appear to contribute significantly to the systemic metabolism of epoxide, but may be important in determining the effects of epoxides formed within the epidermis.

Introduction

Microsomal mono-oxygenases form electrophilically reactive epoxides, which are metabolised to trans dihydrodiols by epoxide hydrolase (EH), E.C. 3.3.2.3) (Daly, Jerina & Witkop, 1972; Oesch, 1973; Jerina & Daly, 1974). Epoxides may undergo conjugation with glutathione (Jerina *et al.*, 1970; Nemoto *et al.*, 1975) or form phenols non-enzymatically (Daly *et al.*, 1972). Alternatively, epoxides may bind with subcellular macromolecules resulting in cytotoxicity, mutagenicity and carcinogenicity (Daly *et al.*, 1972; Oesch, 1973; Jerina *et al.*, 1974; Wiebel, Whitlock & Gelboin, 1974). While EH inactivates epoxides, it also participates in the formation of carcinogenic dihydrodiol epoxides such as 7,8-dihydroxy- 7,8-dihydrobenzo(a)pyrene 9,10-oxide (Malaveille *et al.*, 1975). EH activity appears to be ubiquitous in rat tissue (Oesch, Glatt & Schmassmann, 1977) and has been observed in human adult (liver, lung, cultured lymphocytes and skin) and foetal (kidney and adrenal) tissue (Grover, Hewer & Sims, 1973; Juchau & Namkung, 1974; Kellerman & Shaw, 1976; Oesch, Thoenen & Fahrlaender, 1974; Kapitulnik *et al.*, 1977; Oesch, Schmassmann & Bentley, 1978; Glatt, Kaltenback & Oesch, 1980). Because of the potential importance of EH in human skin, a detailed study of its properties, distribution and activity has been undertaken.

Methods

Human skin was obtained at surgery, and included breast skin (21), foreskin (10), leg (7), axillary skin (1), facial skin (1) and abdominal skin (2) from patients whose ages ranged from 8 weeks to 80 years. The skin was dissected from underlying fat and subcutaneous tissue immediately after its removal, and placed in ice cold buffer (1.15% potassium chloride, 10 mM potassium phosphate pH 7.4). After transfer to the laboratory, the tissue was weighed and finely chopped with a scalpel before homogenisation. In some experiments epidermis and dermis were separated by stretching and scraping off the epidermis (Van Scott, 1952).

Microsomal preparation

Homogenisation (I.L.A. Homogeniser) was performed in a minimum of 15 ml phosphate buffer (10 ml buffer/g tissue) at 3–4 × 20 s periods, with cooling for approximately 2 min between each period. The homogenate was filtered through gauze and then centrifuged for 10 min at 600 g, and then 15 min at 10,000 g to minimize cosedimentation of microsomes with larger particles (Schmassmann, Glatt & Oesch, 1976). The supernatant was transferred to clean tubes and centrifuged at 100,000 g for 60 min, and the microsomal pellet resuspended in phosphate buffer (pH 7.4) to give a final protein concentration of 0.4 to 1.8 mg protein ml^{-1}. Protein concentration was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Epoxide hydrolase (EH) assay

EH activity was estimated by measuring the rate of conversion of styrene oxide to styrene glycol. 7-[H^3]-styrene oxide (specific activity 33 miCi $mmol^{-1}$,

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Radiochemical Centre Amersham) was purified by solvent extraction prior to use (Seidegard de Pierre *et al.*, 1977) and at 3–4 monthly intervals thereafter. Incubations were carried out in duplicate in 13 ml ground glass stoppered tubes containing 75 μl microsomal suspension, 25 μl 0.5 M Tris HCl (Sigma), and 4 μl of [H^3]-styrene oxide (6.0 nmol) at 37°C for 45 min. The reaction was stopped by the addition of 200 μl of ice cold acetone and the incubation tubes placed on ice before centrifugation at 4°C for 2 min (3,000 *g*) to remove microsomal debris. In all experiments parallel tubes containing boiled microsomes (30 min at 100°C) from the same tissue samples, as well as the other reagents, were used as controls. [H^3]-styrene glycol concentrations were either estimated immediately, or after storage at −20°C, by high performance liquid chromatography (HPLC). Preliminary studies showed that storage for up to 3 weeks did not result in any change in glycol levels. HPLC separation of the glycol was performed using a Laboratory Data Control chromatograph with an ODS column (5 μm). A methanol:water (50:50, v/v) solvent system was employed at a flow rate of 1.66 ml min^{-1}. With this system the retention times of styrene glycol and styrene oxide was 2.4 min and 4.6 min respectively (Figure 1).

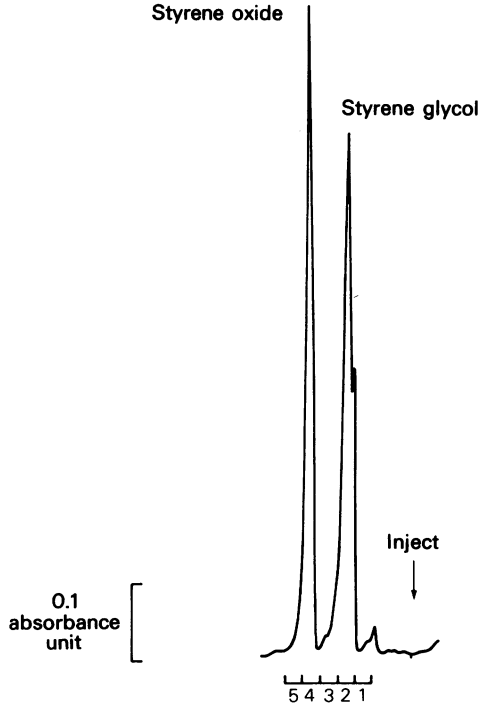

Figure 1 Sample HPLC trace (wavelength 219 nm) using cold styrene oxide and glycol (50 mM). First peak is an acetone solvent peak. Fraction 1–blank, 2–glycol, 3–blank, 4–oxide, 5–blank.

An aliquot (20 μl) of the incubation supernatant was injected onto the column and 90 s later five fractions were collected at 1 min intervals, the effluent corresponding to the glycol and oxide fractions were thus collected separately. Radioactivity was determined by liquid scintillation spectrometry (Intertechnic) with background subtraction and quench correction. The results were expressed as pmol glycol formed mg^{-1} microsomal protein min^{-1} (pmol mg^{-1} min^{-1}).

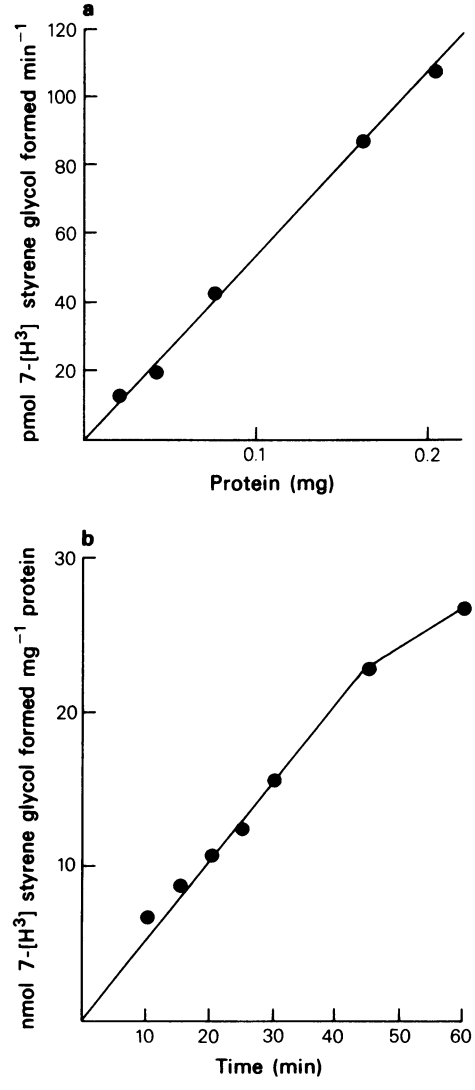

Figure 2a Limit of linearity of the epoxide hydrolase assay with respect to protein concentration.
b Limit of the linearity of the epoxide hydrolase assay with respect to time.

Results

Characteristics of EH activity

Under the assay conditions described, the rate of formation of [H³]-styrene glycol was linearly related (Figure 2a) to protein concentration up to 2.7 mg ml^{-1} (or 200 μg per assay), and to the incubation time up to 45 min (Figure 2b). Varying the pH of the incubation medium over the range pH 6 to pH 11 was not associated with significant changes in EH activity. The relationship between 7-[H³]-styrene oxide concentration and the rate of formation of [H³]-styrene glycol is shown in Figure 3 up to a substrate concentration of 360 μM.

The activity of whole skin stored in phosphate buffer (pH 7.4) at −20°C fell to 90% of original activity after 1 week, 80% after 1 month and less than 50% after 2 months. Skin microsomes stored at −20°C also showed a reduction of EH activity to 80% after 1 month.

Subcellular distribution of EH activity

The activity of EH in the 10,000 *g* pellet, the 100,000 *g* pellet, and the 100,000 *g* supernatant is shown in Table 1. The highest specific activity was observed in the 100,000 *g* pellet, but some activity was also present in the 100,000 *g* supernatant

Microsomal EH activity in human skin

EH activity was observed in the microsomal fractions of separated dermis and epidermis. Epidermis EH activity (mean ± s.e. mean) (139 ± 32 pmol mg^{-1} min^{-1}) was usually lower than dermal activity (165 ± 36 pmol mg^{-1} min^{-1}).

Whole skin EH activity varied from 92 pmol mg^{-1} min^{-1} to 948 pmol mg^{-1} min^{-1}. In three experiments the K_m and V_{max} were determined using the direct linear plot method (Eisenthal, 1974) and observed to be (mean ± s.d.) 2.45 ± 0.82 nM and 510 ± 40.5 pmol mg^{-1} min^{-1} respectively. Whole skin EH activity from various sites is shown in Table 2. Analysis of variance showed no significant difference between breast, foreskin, and leg and there was no significant correlation with age.

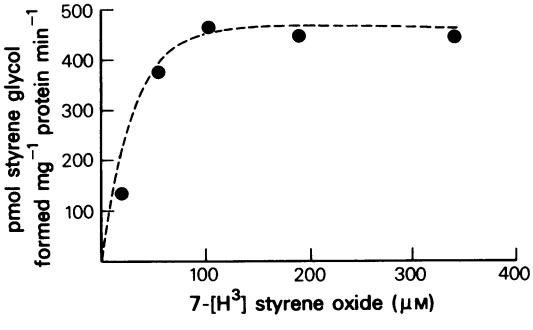

Figure 3 Effect of epoxide hydrolase activity in increasing substrate concentration.

Table 1 Subcellular distribution of epoxide hydrolase activity (n = 3)

	Specific activity (pmol mg^{-1} protein min^{-1})
Whole skin homogenate	50 ± 7
10,000 *g* pellet	17.5 ± 4.2
100,000 *g* pellet	511 ± 23
100,000 *g* supernatant	38 ± 10

Discussion

Several methods for measuring EH activity, using a variety of epoxides as substrate, have been published. The present assay using [H³]-styrene oxide as substrate, separating the glycol by HPLC, and quantitation by liquid scintillation spectrometry, provides a technique suitable for measuring the low activity of EH present in human skin. The EH activity in human skin reported in this paper is similar to that previously described (Oesch *et al.*, 1978).

Activity in human skin is low (mean ± s.e. mean 433 ± 39 pmol mg^{-1} min^{-1}) when compared to that reported (Kapitulnik *et al.*, 1977) in human liver (mean 18.7 ± 3.5 nmol mg^{-1} min^{-1}), and both the apparent K_m (2.4 nM and 380 μM for skin and liver respectively) and V_{max} (0.510 and 62.1 nmol mg^{-1} min^{-1} (for skin and liver respectively) are substantially different (Oesch *et al.*, 1974).

EH activity was observed to be mainly present in the microsomal fraction of human skin, with some activity in the 100,000 *g* supernatant. Cytosolic EH activity has been described in mouse liver (Gill & Hammock, 1980) but as with human skin it appears to make only a minor contribution to the total activity (Oesch & Golan, 1980). This present study confirms that EH is present in both human epidermis and dermis (Oesch *et al.*, 1978) and this distribution contrasts with that of aryl hydrocarbon hydroxylase which is almost exclusively confined to the epidermis (Chapman, Rawlins & Shuster, 1979). Dermal EH may thus play a role in the detoxification of epoxides formed within the epidermis. An increase of liver EH activity with age has been reported in the rat (Birnbaum & Baird, 1979) and the mouse (Gill & Hammock, 1980). Human breast skin aryl hydrocarbon hydroxylase activity is also positively correlated with age (Chapman *et al.*, 1979). However, we have been unable to observe an age-related change in

Table 2 Epoxide hydrolase activity (mean ± s.d.) in skin microsomes from various sites

Site	*Number of samples*	*Epoxide hydrolase activity (pmol mg^{-1} protein min^{-1})*
Breast	21	440 ±220
Foreskin	10	367 ± 282
Leg	7	582 ± 176
Abdomen	2	138, 614
Face	1	307
Auxillary	1	794

EH activity in human skin. The present study suggests that EH activity is similar in breast skin, foreskin and leg skin, but there is insufficient evidence to conclude that there is no dependence on either site or sex.

The significance of EH activity in human skin is as yet uncertain. It is unlikely to make an important contribution to the total systemic metabolism of epoxides since we calculate that total skin EH activity only contributes 0.48% of total liver EH activity by comparison with the data of Kapitulnik *et al.* (1977). However, skin EH may play an important part in preventing the systemic absorption of epoxides formed within the epidermis, as well as determining the toxicity of foreign compounds within the skin.

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