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SPECIFICITY OF HUMAN, RAT AND MOUSE SKIN EPOXIDE HYDRATASE TOWARDS K-REGION EPOXIDES OF POLYCYCLIC HYDROCARBONS

FRANZ OESCH,* HANSUELI SCHMASSMANN and PHILIP BENTLEY
Institute of Pharmacology, The University of Mainz, Obere Zahlbacher Straße 67, 6500 Mainz, West Germany

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Abstract Epoxide hydratase activity has been measured in microsomal fractions of skin from mouse, rat and humans. The skin enzyme was able to hydrate all epoxides tested. The specific enzyme activities decreased in the order human > mouse > rat. The relative activity towards K-region epoxides of various polycyclic hydrocarbons in skin microsomal fractions from all three species decreased in the order phenanthrene 9,10-oxide > benz(a)anthracene 5,6-oxide ≃ benzo(a)pyrene 4,5-oxide ≃ 7-methyl-benz(a)anthracene 5,6-oxide > 3-methylcholanthrene 11,12-oxide > dibenz(a,h)anthracene 5,6-oxide. The activity of epoxide hydratase in human skin microsomal fractions showed little pH dependence and was inhibited by small molecular weight inhibitors in a manner similar to that of the liver microsomal enzyme. Interindividual variation of epoxide hydratase activity in skin microsomal fractions from six human subjects was considerable, namely from 175 to 447 pmoles benzo(a)pyrene 4,5-dihydrodiol/min per mg protein. This variation was not due to skin disease or treatment and had no apparent correlation with age or sex. A possible correlation with the part of the body from which the skin sample was taken could not be excluded since the activity in skin samples from the abdomen seemed lower than that in samples from leg or breast.

Aromatic and olefinic compounds, including environmental potent skin carcinogens such as benzo(a)pyrene, can be metabolically transformed by microsomal enzymes to epoxides [1–5]. Such epoxides are electrophilic and can spontaneously form covalent bonds with nucleophilic moieties of tissue macromolecules such as DNA, RNA and protein.

Epoxide hydratase (EC 4.2.1.63) (epoxide hydrase) catalyses the formation of *trans*-dihydrodiols from epoxides and is found in the microsomal fractions of many tissues [2, 6]. The enzymes from the hepatic microsomes of rat, guinea pig and human have been widely studied [7–10] and shown to have a very broad substrate specificity, catalysing the hydration of many epoxides [11] including (as shown for the rat) both K-region and non-K-region epoxides of polycyclic hydrocarbons [12, 13]. Epoxides have received a great deal of attention in the search for the carcinogenic metabolites of polycyclic hydrocarbons, since they have been shown to be highly mutagenic [14–18]. However, a dihydrodiol epoxide has been shown to be responsible for much of the binding of benzo(a)pyrene to DNA *in vitro* [19, 20] and *in vivo* [21], and benzo(a)pyrene 7,8-dihydrodiol (the precursor of the diol epoxide) has been shown to be carcinogenic [22] on mouse skin. Thus epoxide hydratase has a unique role in the metabolism of polycyclic hydrocarbons to carcinogens.

Skin is susceptible to the carcinogenic action of polycyclic hydrocarbons, whilst generally liver is not. Thus we felt it necessary to investigate the activity of epoxide hydratase in skin tissues because as a consequence of the difficulties caused by the very low levels of epoxide hydratase in this organ and the resistence of this organ to ready homogenisation, very little was known about the skin enzyme [23, 24]. We report here some properties of epoxide hydratase in human, rat and mouse skin microsomal fractions and compare them to those of liver preparations.

MATERIALS AND METHODS

[^{3}H]benzo(a)pyrene 4,5-oxide was synthesised by the method of Dansette and Jerina [25]. The other tritium labelled epoxides were a generous gift from Dr. P. Sims, Institute of Cancer Research, Royal Cancer Hospital, London. All of the epoxides were generally labelled, the specific radioactivities used were (Ci/mole) benzo(a)pyrene 4,5-oxide, 1.2; dibenz-(a,h)anthracene 5,6-oxide, 6.6; 3-methylcholanthrene 11,12-oxide, 3.0; phenanthrene 9,10-oxide, 3.8; benz-(a)anthracene 5,6-oxide, 2.2; and 7-methyl-benz(a)-anthracene 5,6-oxide, 10.0. Epoxide hydratase assays were performed as described [13, 23], but when human samples were measured the total volume of the incubations was reduced to 200 μl to allow more assays using the limited amounts of tissue available. In these cases the extraction volumes were reduced by the same factor.

Tissue preparation. Human skin removed from patients during operation was immediately immersed in ice cold 0.15 M KCl containing 10 mM potassium phosphate buffer pH 7.4 and was stored in ice for no longer than 4 hr before preparation. The skin samples were washed with 0.15 M KCl containing 10 mM potassium phosphate buffer pH 7.4. Subcutaneous fat tissue was removed and the remaining tissue was homogenised using an Ultra Turrax homogeniser at full speed for 6 × 20 sec. Samples were cooled in ice for 5 min between each homogenisation

* To whom correspondence should be addressed.

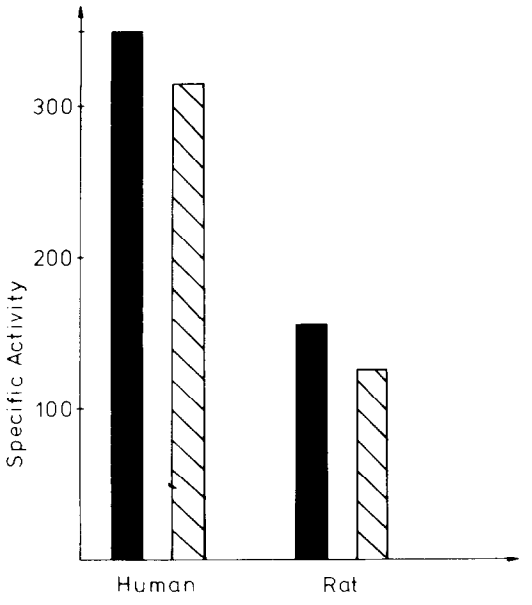

Fig. 1. Distribution of epoxide hydratase in skin. Epidermis and subepidermal fraction were separated and homogenised separately. Microsomes were prepared from each fraction and the hydratase activity determined using benzo(a)pyrene 4,5-oxide as substrate. The specific activity is expressed as pmoles benzo(a)pyrene 4,5-dihydrodiol per min per mg protein in epidermal fraction (■) and subepidermal fraction (▨).

period. The homogenate was filtered through cheese cloth and the microsomal fraction prepared as described [23]. The human skin samples used in this study were from Caucasian male and female donors aged between 28 and 62 and may be considered as "normal" in that no gros lesions became apparent upon pathological inspection and no drugs had been applied to the skin for at least the last week prior to donation.

Skin was removed from the shaved dorsal region of Sprague–Dawley rats (male, 200 g) and NMRI mice (male, 35 g) and microsomal fractions were prepared as described above. Protein concentrations were determined by the method of Lowry *et al.* [26] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

In initial experiments the epidermis and subepidermal fraction were separated before homogenisation and microsomal fractions were prepared separately. However, as shown in Fig. 1 the specific activity of epoxide hydratase towards benzo(a)pyrene 4,5-oxide as substrate was similar in both layers in tissue from human and rat. Thus in subsequent experiments microsomal fractions from whole skin homogenates were used. The epoxide hydratase of microsomal fraction from human skin was very stable. No decrease in activity was observed during storage for 7 days at −20°. The activity decreased by about 40 per cent if whole skin was maintained at 4° for 4 days.

The specific epoxide hydratase activities of microsomal fractions prepared from skin samples of six human individuals measured with benzo(a)pyrene 4,5-oxide as substrate are shown in Table 1 together with the age and sex of the subjects. The epoxide hydratase activity showed a wide interindividual variation, from 175 to 447 pmoles benzo(a)pyrene 4,5-dihydrodiol per min per mg protein. This variation had no apparent correlation with either the age or sex of the individuals. Since all samples were taken from apparently healthy skin and no drugs had been applied to the skin for at least one week prior to donation, the observed variation cannot be explained by skin disease or treatment. The enzyme activity may be different in skin from different parts of the body, since the activities in the 3 samples from the abdomen were lower (175–255 pmoles benzo(a)pyrene 4,5-dihydrodiol per min per mg protein) than those in the 3 samples from leg and breast (349–447 pmoles per min per mg protein). However, since only 6 human skin samples were available, this generalization cannot be made with certainty.

The activity of one skin sample (R.G.) was measured with several epoxides derived from polycyclic hydrocarbons as substrates. These measurements are shown in Table 2, together with the activities of mouse and rat skin microsomal fractions. The results show that skin epoxide hydratase was able to hydrate all epoxides tested as substrates and that the activity towards all substrates was higher in the human sample than in the mouse and rat skin microsomal fractions. The human skin sample which was chosen for this study because greater quantities were available, happened to be the one with the highest specific activity amongst all human samples studied. However, calculation shows that this general statement of a higher epoxide hydratase activity in human skin as compared to rat and mouse may be expected to apply to all other samples. The specific activity with benzo(a)pyrene 4,5-oxide as substrate was much lower

Table 1. Hydration of benzo(a)pyrene 4,5-oxide by human skin microsomal fractions

Individual	Sex	Age (year)	Part of body*	Specific activity†
H.R.	Female	28	Breast	349
A.M.	Female	48	Abdomen	220
R.G.	Female	51	Breast	447
F.H.	Male	38	Leg (thigh)	402
H.M.	Male	58	Abdomen	255
R.H.	Male	62	Abdomen	175

* Part of body from which the skin sample was taken.

† Specific activity expressed as pmoles benzo(a)pyrene 4,5-dihydrodiol produced per min per mg protein.

Table 2. Substrate specificity of epoxide hydratase in skin microsomal preparations*

Substrate	Human		Rat		Mouse	
	Protein	Sp. Act.	Protein	Sp. Act.	Protein	Sp. Act.
Phenanthrene 9,10-oxide	0.47	2530	0.8	808	0.23	1578
Benz(a)anthracene 5,6-oxide	0.95	526	1.06	110	0.23	129
Benzo(a)pyrene 4,5-oxide	0.95	447	1.06	119	0.23	172
7-Methylbenz(a)anthracene 5,6-oxide	0.47	384	0.26	119	0.47	159
3-Methylcholanthrene 11,12-oxide	0.95	59	1.06	4	0.47	23
Dibenz(a,h)anthracene 5,6-oxide	1.42	21	1.06	1.5	0.94	3.9

* Specific activities are given as pmoles diol formed per min per mg protein. Assays were performed at 37 using at least two different protein concentrations, one being twice as high as the other. The highest concentrations of microsomal protein used are given in the table as mg/ml. The human skin sample was donated by R.G.

in the microsomal fractions from skin as compared to those from liver. The ratio (liver:skin) was about 50:1 in the rat and 5:1 in the mouse. The relationship between the structure of the substrate and the specific activity of skin microsomal fractions was similar with all three species. The rate of hydration decreased in the order of phenanthrene 9,10-oxide > benz(a)anthracene 5,6-oxide ≃ benzo(a)pyrene 4,5-oxide ≃ 7-methylbenz(a)anthracene 5,6-oxide > 3-methylcholanthrene 11,12-oxide > dibenz(a,h)anthracene 5,6-oxide. Data on the substrate specificity of human liver epoxide hydratase towards epoxides derived from polycyclic hydrocarbons is not available. However, the same relationship between the rates of hydration of the substrates was obtained with mouse and rat liver microsomes and the enzyme purified to homogeneity from rat liver [13] as with the skin microsomal fractions.

The rate of hydration of benzo(a)pyrene 4,5-oxide by human skin microsomal fractions was remarkably unaffected by pH. Measurements made over a pH range from 7–10 in three different buffers showed no variation outside the error of the measurement. This is in contrast to the enzyme in rat skin microsomal fractions which showed a clear optimum at pH 9.0, although the variation between pH 7.0 and pH 9.5 was only 1.6-fold. This relative insensitivity of membrane bound epoxide hydratase towards pH has also been observed with the rat liver enzyme, although once the enzyme was solubilised a marked pH dependence was observed.

The epoxide hydratase of human skin was tested for inhibition by three epoxides known to have different effects upon the activity of epoxide hydratase in liver microsomal fractions. Table 3 shows that the enzyme was inhibited strongly by 1,1,1-trichloro 2,3-propene oxide, inhibited to a lesser extent by cyclohexene oxide and only inhibited by trans-stilbene oxide at very high concentrations. These results are very similar to what was found with epoxide hydratase in rat and human liver fractions and with pure enzyme isolated from rat liver [10, 27].

The results in this paper show that epoxide hydratase in mouse, rat and human skin is present at levels which can be accurately measured. The specificities towards various epoxides derived from polycyclic hydrocarbons and the effect of small molecular weight inhibitors were similar to those of liver preparations. This suggests that the enzyme found in skin microsomal fractions is intrinsically similar to that found in liver microsomal fractions. Thus investigations of the role of epoxide hydratase in the activation and inactivation of polycyclic hydrocarbons which use enzyme purified from the liver are most probably applicable to the situation in skin, a tissue which is sensitive to polycyclic hydrocarbon carcinogens. Thus, if differences in susceptibility to the carcino-

Table 3. Effect of small molecular weight inhibitors on human skin epoxide hydratase*

Inhibitor	Concentration (mM)	% Inhibition
1,1,1-Trichloropropene oxide	2	100
	0.2	100
	0.1	100
	0.05	96
Cyclohexene oxide	4	84
	2	75
	0.5	63
	0.2	51
Trans-stilbene oxide	10	38
	5	7
	2.5	4

* Assays were performed using benzo(a)pyrene 4,5-oxide (50 μM) as substrate in a final volume of 200 μl. Substrate and inhibitors were added to incubations in 10 μl of acetonitrile. Results are expressed as percent inhibition compared to control assays which contained 10 μl of acetonitrile. This control activity was 402 pmoles benzo(a)pyrene 4,5-dihydrodiol/min/mg protein.

genic action of polycyclic hydrocarbons between liver and skin are in part caused by differences in the epoxide hydratase, these differences are quantitative rather than qualitative.

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