Biochemical Pharmacology, Vol. 25, pp. 1935–1937. Pergamon Press, 1976. Printed in Great Britain.

COMMENTARY

METABOLIC TRANSFORMATION OF CLINICALLY USED DRUGS TO EPOXIDES: NEW PERSPECTIVES IN DRUG–DRUG INTERACTIONS

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The work of several laboratories has demonstrated that epoxides derived from carcinogenic polycyclic hydrocarbons irreversibly bind to DNA, RNA and protein, cause bacterial and eucaryotic mutations, transform cells in culture and are actually produced from polycyclic hydrocarbons by microsomal monooxygenases (for reviews see [1–5]). However, it was not known whether such metabolically formed epoxides contributed significantly to such effects which could possibly also be produced by a great number of reactive metabolites other than epoxides.

As a specific probe for the relative importance of epoxides, epoxide hydratase was purified to homogeneity [6]. Glutathione-*S*-epoxide transferases which are also involved in epoxide transformation are not specific for epoxides but also catalyze the transfer of glutathione to a great number of electrophilic substrates other than epoxides [7]. The purified epoxide hydratase was homogeneous by gel electrophoretic, ultracentrifugal and immunological criteria [6]. C- and N-terminal amino acid analysis confirmed the homogeneity of the preparation [8].*

The homogeneous epoxide hydratase was now used as a tool to probe the relative importance of epoxides amongst possibly a great number of reactive metabolites other than epoxides. The mutagen precursor, benzo(a)pyrene, did not revert the histidine-dependent *Salmonella typhimurium* mutants TA 98, TA 100, TA 1537 or TA 1538 to histidine autotrophy. However in the presence of liver microsomes (from untreated C3H mice) and NADPH, benzo(a)pyrene was metabolized to species very active in reverting all these strains. This mutagenicity was dramatically potentiated by epoxide hydratase inhibitors [10, 11] and completely prevented by the homogeneous epoxide hydratase [12]. Thus, epoxides (or unknown reactive species non-enzymically arising therefrom) are the sole species responsible for the mutagenicity of benzo(a)pyrene in this system.†

This critical importance of metabolically produced epoxides in mediating adverse biological effects aroused concern about clinically used drugs known to be metabolized to epoxides. Drugs possessing structural features prone to metabolic epoxidations are abundant. Metabolically produced epoxides have been reported for several of them such as allobarbital, secobarbital and alphenal [13], protriptyline [14], carbamazepine [15], cyproheptadine [16–18] and phylloquinone [19]. Moreover, terminal metabolites which implicate metabolism via an epoxide intermediate have been observed with numerous drugs such as diethylstilbestrol [20], diphenylhydantoin [21], phensuximide [22], phenobarbital and mephobarbital [23], metaqualone [24], lorazapam [25], imipramine [26] and acetanilide [27]. However, it is important to realize that the alarming biological effects of some epoxides do not automatically imply that all epoxides have similar effects. Epoxides vary greatly amongst themselves in molecular geometry, stability, electrophilic reactivity [28] and relative activity as substrates of epoxide-transforming enzymes [1–5]. Firm predictions of biological effects of a given epoxide based on any of these factors are, as yet, not possible. For instance, planarity is not an absolute prerequisite for frameshift mutagenesis to occur: 7,12-dimethylbenz(a)anthracene-5,6-oxide (severely distorted with the angle between the outer six-membered rings close to 35°! [29]) proved to be a potent mutagen for TA 1537 [11]. Moreover, the observation that the alkene oxide 4-phenylstyrene-7,8-oxide is mutagenic to the two strains TA 1537 and TA 1538 but the K-region arene oxide derived from 7,12-dimethylbenz(a)anthracene is inactive for the latter strain [11], indicates that epoxidation of an aromatic double bond of a polycyclic hydrocarbon is neither a necessary nor a satisfying condition for frameshift mutagenesis to occur.

We have therefore studied the 10,11-epoxides which are known in several of the species investigated to represent major metabolites of the three medical drugs carbamazepine [15], cyproheptadine [16–18] and cyclobenzaprine [30]. These epoxide metabolites proved mutagenically fully inactive for *Salmonella typhimurium* TA 1537 and TA 1538 [11] which, on the other hand, were very readily mutated by epoxides derived from carcinogenic polycyclic hydrocarbons such as benzo(a)pyrene-4,5-oxide and benz(a)anthra-

* At about the same time a different procedure also leading to a gel electrophoretically homogeneous hydratase preparation was reported [9].

† Liver microsomes from "normal (i.e. non-induced) mice were used for these studies. After induction by 3-methylcholanthrene (high monooxygenase levels favoring further metabolism of metabolites by oxidative rather than other routes) the contribution of the mutagenicity of metabolically activated dihydrodiol to the overall mutagenicity of metabolically activated benzo(a)pyrene is significant and the influence of homogeneous epoxide hydratase is weaker, and more complex (Glatt and Oesch, unpublished results).

cene-5,6-oxide [11]. Moreover, the former were also not cytotoxic to these strains over the entire concentration range investigated [11]. Although these results do not rule out possible adverse biological effects of the epoxides metabolically produced from the three medical drugs carbamazepine, cyproheptadine and cyclobenzaprine towards other organisms than the ones we used as tester strains, the greatly differing effects of the epoxides derived from the carcinogenic polycyclic hydrocarbons very clearly show that one epoxide cannot simply be equated with any other. Thus the present epoxide hysteria which, admittedly but quite unintentionally, our work may have helped to create, rests on an unjustified extrapolation of known adverse properties of some epoxides to epoxides in general.

On the other hand, it must be realized that biological effects of metabolically produced epoxides may not be confined to those exerted directly by the epoxide in question. Potentiation of adverse effects of other epoxides metabolically produced from ubiquitous environmental compounds such as benzo(a)pyrene may play a major role. Since the levels of the latter compounds in our tissues is normally very low, alterations of cellular macromolecules by covalent binding of epoxides metabolically produced from them may normally be so minimal that repair and defense mechanisms can easily cope with them and no adverse biological effects become apparent. However, this situation may drastically change upon administration of a therapeutic drug if a major metabolite of it is an epoxide. Epoxides are metabolized further by epoxide hydratase [2] and glutathione *S*-epoxide transferase [31]. These enzymes have recently been purified to apparent homogeneity [6, 7, 9] and the pure enzymes were shown to possess very broad substrate specificities [6, 7, 12, 32]. Thus, epoxides differing very widely in chemical structures may effectively compete with each other for inactivating systems. With respect to epoxides metabolically produced from carcinogenic polycyclic hydrocarbons the most critical system may be epoxide hydratase since, in contrast to the cytoplasmic glutathione *S*-epoxide transferases [31], it is localized in the microsomal fraction [2] where the epoxides are formed and where the epoxides derived from polycyclic hydrocarbons due to their predominantly lipophilic character would be expected to linger. Studies on substrate specificities of the various main and branching-off fractions during purification of epoxide hydratase, relative potencies of inhibitors in the various fractions and the effect of antibodies raised against the homogeneous enzyme on pure and crude fractions indicated that one single enzyme in microsomal membranes is involved in hydrating epoxides chemically as different as the alkene oxide styrene oxide and the K-region arene oxide benzo(a)pyrene-4,5-oxide [32]. Thus drug–drug interactions between epoxides metabolically formed from clinically used drugs and from ubiquitous environmental compounds may frequently occur via inhibition of this critical enzyme. Dramatic potentiation of the mutagenic effects of metabolically activated benzo(a)pyrene by 1,1,1-trichloropropene-2,3-oxide [11] and cyclohexene oxide [12] have been observed. Table 1 shows that the 10,11-epoxides metabolically produced from the 3 medical drugs, the direct biological effects of which were discussed in the preceding paragraph, all inhibit the hydration of styrene oxide and benzo(a)pyrene-4,5-[K-region-]oxide [33]. These results refer to experiments using the 10,11-epoxides of the medical drugs at concentrations equal to and four times greater than that of benzo(a)pyrene 4,5-(K-region)-oxide. In a therapeutic situation the concentrations of the epoxides metabolically produced from these medical drugs would be expected to be much higher than those of epoxides metabolically produced from the low levels of ubiquitous environmental polycyclic hydrocarbons. Thus the actual inhibition of hydration of epoxides derived from polycyclic hydrocarbons may be even more pronounced than reflected in the data given in Table 1. Interestingly, the inhibitory potencies of the epoxides derived from the three medical drugs are very different from one another. Thus, it is readily apparent from the data in Table 1 that the potential for adverse drug–drug interactions, in the sense discussed in this paragraph, is much less for carbamazepine than for cyproheptadine.

In summary, the work of several teams over the last few years strongly implicates metabolically pro-

Table 1. Inhibition of epoxide hydratase*

Inhibitor		% Inhibition of hydration of		
		Styrene oxide		Benzo(a)pyrene-4,5-oxide
		0.5 mM	2 mM	0.5 mM
Carbamazepine-10,11-oxide	0.5 mM	11	N.S.	N.S.
	2 mM	13	N.S.	N.S.
Cyclobenzaprine-10,11-oxide	0.5 mM	19	—	20
	2 mM	21	—	26
Cyproheptadine-10,11-oxide	0.5 mM	67	27	—
	2 mM	83	57	45

* Epoxide hydratase was assayed with styrene oxide [34] and benzo(a)pyrene-4,5-(K-region-)oxide [35] as substrate as described. Liver microsomes of adult male Sprague–Dawley rats were used [33]. Numbers represent values significantly ($P < 0.01$) different from controls. N.S. = not significant.

duced epoxides as the species responsible for mutagenic, carcinogenic, allergenic and cytotoxic effects of several aromatic and olefinic compounds. Yet, adverse effects of some epoxides do not automatically imply that all epoxides have such effects. Some data are discussed which very clearly discriminate between individual epoxides with respect to some adverse effects. However, the enzymes catalyzing the further metabolism of epoxides have very low specificity. Precursors of opoxides which do exert potent adverse biological effects are ubiquitous yet normally occur at such low levels that repair and defense systems may be able fully to cope with them. In this situation inhibition of epoxide metabolizing enzymes by epoxides derived from pharmaceuticals may lead to potentiation of such effects. The potency of such inhibition will depend on the structure of the epoxide in question. The safety evaluation of new drugs would be greatly increased by addition of some simple test(s) to assess this parameter.

Acknowledgements—The author wishes to thank his collaborators for their work quoted in this Commentary and the Deutsche Forschungsgemeinschaft for financial support.

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