METABOLIC EPOXIDATION OF trans-4-ACETYLAMINOSTILBENE: A PROTECTIVE MECHANISM AGAINST ITS ACTIVATION TO A MUTAGEN

H.R. Glatt, M. Metzler*, H.-G. Neumann*, and F. Oesch

Institute of Pharmacology, University, Obere Zahlbacher Strasse 67, D-6500 Mainz, and (*) Institute of Pharmacology and Toxicology, University, Versbacher Landstrasse 9, D-8700 Würzburg, Federal Republic of Germany

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SUMMARY: Trans-4-acetylaminostilbene is activated by liver preparations to mutagens for Salmonella typhimurium. Since this compound is metabolized to the trans-α,β-epoxide and since many epoxides are ultimate mutagens, this epoxide was tested for direct mutagenicity. It was, however, found to be non-mutagenic, and, in contrast to the parent compound, the epoxide was no longer activated by liver preparations to mutagens. The same was found for the β-ketone and for the threo-α,β-dihydrodiol, which are formed metabolically from trans-4-acetylaminostilbene and from its α,β-epoxide. 4-Acetylaminobibenzyl showed a very weak mutagenic activity in the presence of the liver preparation.

Thus, it is important to realize that where epoxides are formed from compounds which are known to be metabolized to mutagens, they are not necessarily responsible for the mutagenicity. Epoxidation may even prevent the possibility of bioactivation to mutagens.

Introduction

Epoxides are electrophilically reactive and can bind to DNA, RNA and proteins both in vivo and in vitro (1-4). Many epoxides, derived from aromatic or olefinic compounds, are mutagenic for microorganisms (5-11) or mammalian cells in culture (11-13). Some epoxides also transform cells in culture to malignancy (14-16). Therefore, the observation of metabolic epoxidation of a chemical compound in the human environment should lead us to test this compound for biological effects such as carcinogenicity or mutagenicity. However, indiscriminately extrapolating the established adverse biological effects of the more thoroughly investigated epoxides to epoxides in general is unjustified. We have found that many epoxides, including those metabolically produced from clinically used drugs (e.g. carbamazepine), and photochemically produced from the endogenous steroid cholesterol, did not mutate any of the Salmonella typhimurium strainsTA 1535, 1537, 1538, 98, 100, whilst under the same conditions epoxides derived from carcinogenic polycyclic hydrocarbons were mutagenic (8 and unpublished results). Even in cases where

Table 1. Mutagenicity in the absence of an activating system

	µg/plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
Control	-	29(26-32)	8(3-11)	24(20-27)	36(29-43)	161(139-188)
Trans-4-acetylaminostilbene	0.3-100	-	9(2-14)	24(18-29)	35(28-39)	158(128-186)
Trans-4-acetylaminostilbene α,β-oxide	0.3-100	29(24-36)	6(3-12)	27(22-30)	37(31-41)	159(137-176)
Cis-4-acetylaminostilbene α,β-oxide	0.3-100	26(19-33)	9(8-10)	25(24-27)	36(30-41)	172(157-190)
Threo-α,β-dihydroxy-4-acetylaminobibenzyl	0.3-100	-	6(2-11)	26(19-31)	37(27-45)	149(116-175)
β-Keto-4-acetylaminobibenzyl	0.3-100	-	7(4- 9)	24(20-27)	36(22-40)	157(135-171)
4-Acetylaminobibenzyl	0.3-100	-	8(6-13)	25(18-30)	38(28-46)	167(129-190)
Benzo(a)pyrene 4,5-oxide	0.8	30,31	559,586	220,238	1070,1098	1320,1401

The test compounds and the histidine dependent bacteria were added to histidine poor agar plates as described by Ames et al. (19). Six different concentrations of the test compounds were used. Values shown are means and ranges (in brackets) of his^+ revertants per plate after incubation for 2 days at 37° C. Benzo(a)pyrene 4,5-oxide, which served as a positive control, reverted TA 1537, TA 1538, TA 98, TA 100 with linear dose dependence when less than 1.5 µg were added per plate.

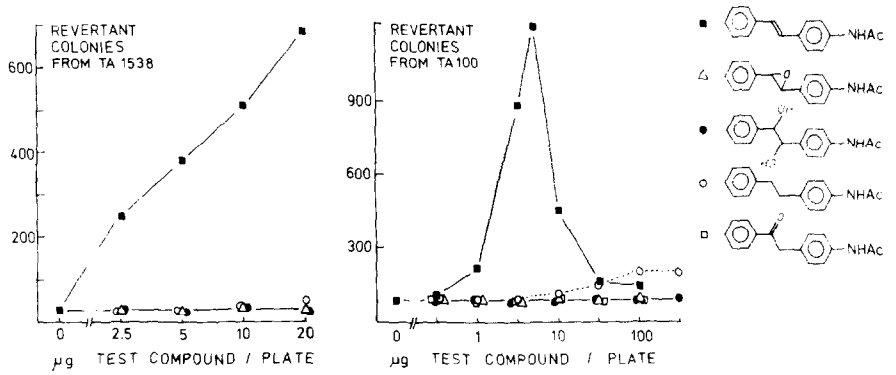

Fig. 1 Mutagenicity of 4-acetylaminostilbene derivatives in the presence of an activating system: The test compound and the bacteria were incubated together with mouse liver 10'000 g supernatant fraction and a NADPH generating system as described (19). Revertant colonies were counted after incubation for 2 days at 37° C. Values represent means (n=3). The standard deviation was always less than 15 % of the means.

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a compound exerts adverse biological effects such as mutagenicity and where metabolic epoxidation does occur, the epoxide may not be responsible for these effects. Epoxidation may actually prevent the compound from being biotransformable to a mutagenically reactive metabolite. We wish to report here a striking example for this concept observed with the carcinogen (17,18) trans-4-acetylaminostilbene.

Materials and Methods

Mutagenicity was tested as described in detail elsewhere (19). In brief, histidine dependent Salmonella typhimurium and the test compound were added to a histidine poor agar plate. Histidine prototroph mutant colonies were counted after incubation for 2 days at 37° C. Where indicated, liver homogenate from adult male C57BL/6J mice and a NADPH generating system were added to the bacteria together with the test compound. Trans-4-acetylaminostilbene and 4-acetylaminobibenzyl, trans- and cis-4-acetylaminostilbene α,β-oxide, β-keto-4-acetylaminobibenzyl, threo-α,β-dihydroxy-4-acetylaminobibenzyl (20), and benzo(a)pyrene 4,5-oxide (21) were synthesized as described earlier.

Results and Discussion

Trans-4-acetylaminostilbene by itself, i.e. in the absence of an activating system was not mutagenic (Table 1). However, it was activated to a mutagen in the presence of liver homogenate and a NADPH generating system (Figure 1). Since trans-4-acetylaminostilbene is metabolically

transformed to the trans-α,β-epoxide (20) it was tempting to speculate that this epoxide represented an ultimate mutagen.

Table 1 shows, however, that trans-4-acetylaminostilbene α,β-oxide as well as its cis-isomer did not revert any of the five Salmonella strains in the absence of an activating system. No indications of toxicity such as reduced background lawn of his-bacteria or a decrease in the number of spontaneous revertant colonies below the control level were observed. Thus mutagenicity cannot have been overshadowed by toxicity. Benzo(a)pyrene 4,5-oxide which was used as a positive control reverted 4 of the 5 strains at very low concentrations (Table 1).

In addition to being non-mutagenic trans-4-acetylaminostilbene α,β-oxide was, in contrast to the parent compound trans-4-acetylaminostilbene, no longer activated by liver homogenate and NADPH to a mutagen reverting any of the Salmonella strains used (Figure 1). β-Keto- and threo-α,β,dihydroxy-acetylaminobibenzyl, which are formed metabolically by rearrangement and hydration of the epoxide respectively, both lacked direct mutagenicity (Table 1) and were not activated to a mutagen in this system (Figure 1).

4-Acetylaminobibenzyl (Table 1, Figure 1), a non-carcinogenic (17,22) compound, which differs from trans-4-acetylaminostilbene only by saturation of the α,β-bond, was not mutagenic itself, but showed slight activity in the presence of liver homogenate. This mutagenic activity was 30 to 100 times lower than that of trans-4-acetylaminostilbene. Thus, the olefinic double bond which conjugates the two benzene rings seems to be very important for mutagenic activation of trans-4-acetylaminostilbene.

In conclusion, although epoxidation often represents an activation of a biologically rather inert molecule to a reactive metabolite, it is important to realize that this does not necessarily convey a toxic potential. Epoxides represent a heterogeneous class of compounds varying in many parameters which in some cases influence biological activities more decisively than does the epoxide moiety. Thus, metabolic formation of a toxic metabolite as well as metabolic formation of an epoxide does not imply their identity. For the compound under investigation in the present study, trans-4-acetylaminostilbene, which is metabolically activated to a potent mutagen for several Salmonella strains, epoxidation at the α,β-position actually produces a molecule which no longer possesses this activatability.

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