Acta Chemica Scandinavica B 36 (1982) 255–279

Short Communications

A Comparison of Induction of Microsomal Glutathione S-transferase Activity in the Liver of the Mouse and Rat by Dietary 2(3)-*tert*-Butyl-4-hydroxyanisole (BHA)*

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Glutathione *S*-transferases (EC 2.5.1.18) (for a review, see Ref. 1) are a family of enzymes involved in the detoxification of numerous mutagenic, carcinogenic and pharmacologically active substances.[2] These enzymes, as well as others involved in drug metabolism, can be induced by different compounds, notably phenobarbital, 3-methylcholenthrene, *trans*-stilbene oxide[3,4] and 2(3)-*tert*-butyl-4-hydroxyanisole.[5,6] The glutathione *S*-transferase activity in rat liver microsomes is, however, not significantly affected by these inducers.[7,8] The highest induction of glutathione *S*-transferase activity reported to data is about 10-fold in mouse liver cytosol when the animals received BHA in their diet.[5] We therefore decided to investigate whether this species demonstrates a liver microsomal glutathione *S*-transferase activity which can be distinguished from its cytosolic counterpart by *N*-ethylmaleimide (MAlNEt)-activation[9] and also whether this *in vitro* activity can be elevated by treatment with BHA. A parallel study was made on the rat.

Experimental. Female NMRI mice (25–30 g) and male Sprague-Dawley rats (180–200 g) were used in these studies. Mice and rats were fed a diet containing 0.75 g BHA/kg for 2 weeks. Control animals received the same diet without BHA. Microsomes and cytosol were prepared according to Ref. 10 except that microsomes were washed twice in 0.15 M Tris-Cl, pH 8, in order to remove cytosolic contaminations. Glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate was measured as in Ref. 11. Activation of microsomal activity with 1 mM MalNEt was carried out as described previously.[7] Protein was determined by the method of Lowry *et al.*[12] with bovine serum albumin as the standard. All chemicals were obtained from common commercial sources and were of the highest purity available.

The results in Table 1 show that glutathione S-transferase activity is found in mouse liver microsomes. The activity towards CDNB is nearly

*Communication at the Meeting of the Swedish Biochemical Society in Stockholm, 26–27th November, 1981.

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Table 1. Glutathione *S*-transferase activity towards CDNB in rat and mouse liver.[a]

	Cytosol	Microsomes + MalNEt	
Rat			
Control	1170 ± 110[b]	140 ± 20	600 ± 58
BHA treated	1990 ± 350	167 ± 10[c]	667 ± 28
Mouse			
Control	2050 ± 270	147 ± 35	689 ± 140
BHA treated	15500 ± 3600	251 ± 66[d]	995 ± 230[d]

[a] Expressed as (nmol/min) (mg protein). [b] $\bar{x} \pm$ SD $n = 9$ (mice), 6 (rats) and 3 (rat microsomes + MalNEt). [c] Significantly different $p < 0.005$. [d] Significantly different $p < 0.001$; students *t*-test was used.

0302-4369/82/040255-02$02.50

the same as in rat. In addition, activation with MalNEt occurs to about the same extent in both species. Because this SH reagent stimulatable activity is located on the endoplasmic reticulum,[7] one can study the effect of inducers without (or allowing for) cytosolic interference. As can be seen in the table, mouse microsomal glutathione *S*-transferase activity is significantly increased by BHA both in untreated (171%) and MalNEt treated (144%) microsomes. The higher figure in untreated microsomes is probably due to some increase in cytosolic contamination. This is not unexpected, because of the large increase in the soluble activity (756%). In the parallel study with the rat, a small but significant increase (119%) is seen in the microsomal activity. However, MalNEt-treated microsomes reveal no significant difference in activity between microsomes from BHA-treated and control animals. Hence the small elevation might be due to increased cytosolic contamination. Both the mice and rat cytosol induction agrees with previous data.[5,6,13] In conclusion we have shown the presence of microsomal glutathione *S*-transferase activity in mouse liver and shown it to be inducible by BHA. This could be important concerning drug biotransformation in the mouse and the protective effect exerted by BHA in connection with carcinogenesis.

Acknowledgements. This work was supported by Grant Number 1 RO1 CA 26261-02 awarded by the National Cancer Institute, Department of Health, Education and Welfare and a grant from the Swedish Medical Research Council B81-O3X-05918-01.

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Received December 17, 1981.