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INFLUENCE OF DISULFIRAM ON GLUTATHIONE, GLUTATHIONE-S-TRANSFERASE, AND ON NITROSAMINE-DEALKYLASES OF LIVER, KIDNEY AND ESOPHAGUS OF THE RAT.

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INTRODUCTION

Disulfiram (DSF) is described to be an anticarcinogen in animal experiments (Wattenberg 1978). It reduces or even inhibits the carcinogenic effects of chemical carcinogens. In certain cases, however, a shift in organotropy was observed; e.g. when nitrosodiethylamine (NDEA) is combined with DSF treatment, liver cancer is reduced from 90% to 31%, but development of esophageal tumors rises from 29% to 81%. When nitrosodimethylamine (NDMA) is combined with DSF, liver tumors occur in 3% yield instead of 51%, and 59% of paranasal tumors instead of 0% (Schmähl et al., 1976). Experiments have been carried out to clarify whether this shift is due to changes in in vivo metabolic processes such as enzymatic nitrosamine activation and the detoxifying system glutathione/glutathione-S-transferase (GSH/GST).

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 200 ± 10 g, were used in the experiment. Twelve animals received DSF-enriched food (2 g/kg) for two weeks, twelve animals served as control. The animals were killed by cervical dislocation and livers, kidneys and esophagi were removed. Cytosol and microsomes of these organs were assayed for glutathione (GSH), glutathione-S-transferase (GST, 2,4-dinitrochlorobenzene as co-substrate), and dealkylases of NDMA, NDEA and nitrosodibutylamine (NDBA) according to methods described by Ellmann (1959), Habig (1974) and Farrelly (1980), respectively. Triplicate assays from liver and kidney microsomes and cytosolwere carried out from seven individual animals, whereas the esophagi were pooled (three times four animals).

RESULTS AND DISCUSSION

Protein content in the different organs is not changed by DSF treatment (Table 1). The esophagus shows the lowest GSH/GST content/activity of the organs examined, which, together with its lack in DNA repair enzymes, could explain its high rank as target organ in experiments with chemical carcinogens. Disulfiram exerts an enhancement on both GSH and GST in all organs, the increase being higher in liver and esophagus than in the kidney.

The highest dealkylating activities are located in the liver, whereas in the kidney these enzyme activities are low. It is widely accepted that a correlation exists between enzyme activity and tumor formation for N-nitrosamines. Our data apparently support this hyphothesis because NDMA, NDEA and high concentrations of NDBA cause predominantly liver cancer.

NDEA was the best substrate for dealkylation in the esophagus, whereas NDBA was debutylated at a much faster rate than the other nitrosamines in the liver. DSF treatment led to an inhibition of NDMA-demethylation in the liver. No effect of DSF on the in vitro metabolism of the other two nitrosamines in those two organs was observed. Due to the high variability in the activities of the nitrosamine meta-

bolizing enzymes in the esophagus, no statistically significant influence of DSF can be discerned. Nevertheless an inhibition of NDEA-deethylation in the esophagus and a slight activation of debutylation were observed after DSF-treatment. The later observation would fit into findings of Schweinsberg et al. (1982), who reported that the combined application of NDBA plus DSF led to a protection of the liver but neither of the esophagus nor the bladder.

CONCLUSIONS

GSH and GST are known to perform several detoxicating functions. Recently we demonstrated in vitro and in vivo that this system prevents covalent binding of NDMA metabolites to rat liver macro-molecules (Frei et al., 1982). It is shown here, that DSF induces GSH and GST especially in liver and esophagus. Simultaneously, DSF inhibits the NDMA demethylating enzymes in the liver.

The results suggest that in the case of NDMA the protective effect of DSF on the development of liver tumors could indeed be caused by an inhibition of the nitrosamine activating enzymes on one hand and on an induction of a detoxicating system on the other. The fact that GSH/GST in the liver was enhanced but NDEA deethylase was not seriously affected by DSF, could explain that the liver is only partially protected against NDEA tumor formation.

In the esophagus obviously other mechanisms are responsible for the effects observed in the carcinogenesis experiments. Two explanations may be given for this observation. First esophagus shows a higher tendency to incorporate intact NDEA after DSF pretreatment followed by NDEA than other organs with or without DSF. (Frank et al., 1983). A second explanation may be that at least in the liver further changes in the parameters determined occur during prolonged treatment with DSF, as we observed in experiments to be published.

		liver	kidney	esophagus
control	Protein (microsomes)§	16.1 ± 0.5	8.5 ± 1.5	2.26 - 3.5
	Protein (cytosol)§	61.3 ± 9	47.4 ± 3.1	11.8 - 19
	Glutathione-S-transferase*	1102 ± 100	310 ± 39	52 -172
	Glutathione (nMol/mg prot.)	49.1 ± 3.3	39.4 ± 2	0 - 13.6
	NDMA-demethylase*	0.92 ± 0.1	0.1 ± 0.06	0 - 0.16
	NDEA-deethylase*	0.9 ± 0.35	0.14 ±0.04	0.09- 1.36
	NDBA-debutylase*	2.09 ± 0.4	0.18 ±0.04	0.08-0.24
DSF	Protein (microsomes)§	17.3 ± 1.3	6.9 ± 0.8	1.6 - 3.35
	Protein (cytosol)§	64.1 ± 3.3	47.3 ± 6.8	10.9 -16.5
	Glutathione-S-transferase*	1963 ± 180[a]	439 ±84[b]	189 -388
	Glutathione (nMol/mg prot.)	64.9 ± 7.7[a][c]	44.2 ± 3.2[a]	30.8 -49.3
	NDMA-demethylase*	0.53 ± 0.18	0.07 ± 0.05	0 - 0.2
	NDEA-deethylase*	0.76 ± 0.18	0.15 ± 0.1	0 - 0.34
	NDBA-debutylase*	2.38 ± 0.3	0.18 ± 0.05	0 - 0.45

Table1: Influence of a 2 weeks treatment with disulfiram (2g/kg food) on glutathione and glutathione-S-transferase and on different nitrosamine-dealkylating enzymes in the rat. Mean ± s.d.
§ = mg/g organ, * = nMol/mg protein · min, a = $p < 0.001$, b = $p < 0.005$
c = $p < 0.01$ (Wilcoxon rank-sum-test). Statistical evaluation of values obtained from the esophagus has not been carried out (see Materials & Methods), only lowest and highest values are given.

REFERENCES

G.L. Ellmann, Archives of Biochem. Biophys. 82, 70 (1959)
G. Farrelly, M.L. Stewart, J.E. Saavedra and W. Lijinsky, Cancer Res. 42, 2105 (1982
N. Frank, D. Hadjiolov and M. Wießler, Proceedings of the 8th International Meeting on Nitroso Compounds, Banff, Canada, 1983
E. Frei, B. Bertram and M. Wießler, Naturwiss. 70, 42 (1983)
W.H. Habig, M.J. Pabst and W.B. Jakoby, J. Biol. Chem. 249, 7130 (1974)
D. Schmähl, F.W. Krüger, M. Habs and B. Diehl, Z. Krebsforsch. 85, 271 (1976)
F. Schweinsberg, I. Weissenberger and V. Bürkle, IARC Sci. Publ. No. 11, Lyon 1982
L.W. Wattenberg, J. Natl. Cancer Inst. 60, 11-18 (1978)