# THE FUNCTIONAL STATE OF THE XENOBIOTIC METABOLIZING SYSTEM IN RAT LIVER FOLLOWING CHRONIC ADMINISTRATION OF DIETHYLNITROSAMINE OR ITS PRECURSORS

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**SUMMARY**

The effect of chronic administration of 0.002% N-nitrosodiethylamine (DENA), 0.002% diethylamine (DEA) and 0.0005% sodium nitrite (SN) on the functional state of the xenobiotic metabolizing system in rat liver was investigated. Administration of DEA and DENA increased concentration of cytochromes P-450 and $b_5$. SN did not affect the enzymes of the monooxygenase system. Coadministration of DEA and SN maximally increased the concentration of cytochrome P-450. It is not possible to explain the phenomenon of combined administration of SN and DEA by simple summation of the effects caused by them separately. The activity of microsomal glutathione S-transferase did not change when DEA and SN were given together, yet increased when they were administered separately. The maximum increase of the total activity of cytosol glutathione S-transferases was observed following DENA. In all four experimental groups a decrease of isoenzyme 5-5 activity was observed. Investigation of Se-independent glutathione peroxidase activity showed the multivariance of response of the glutathione S-transferase family to the compounds studied. The concentration of hepatic free SH-groups increased following administration of DENA and decreased dramatically when SN and DEA were coadministered. When they were given separately the concentration remained at control level.

**KEY WORDS:** sodium nitrite, diethylamine, N-nitrosodiethylamine, cytochromes P-450 and $b_5$, glutathione S-transferases

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## INTRODUCTION

A decisive factor in the realization of the toxic, cancerogenic and mutagenic effects of many chemicals is the xenobiotic metabolizing system /1,2/. It is evident that the efficiency of detoxication of compounds is determined by the balance of the processes involved in phase 1 and 2 biotransformation of xenobiotics. The cancerogenic nitrosamines may be synthesized in the stomach directly from nitrites and the corresponding amines /3/. Therefore it is of interest to study the effect on the organism not only of nitrosamines but also of their possible precursors.

We compared responses of certain components of the two phases of xenobiotic metabolism to chronic administration of sodium nitrite, diethylamine and N-nitrosodiethylamine to rats.

## MATERIALS AND METHODS

### Chemicals

N-Nitrosodiethylamine was synthesized at R.E. Kavetsky Institute for Oncology Problems (Kiev, Ukraine). Other reagents used were of analytical grade.

### Scheme of administration of compounds to rats

The experiments were carried out on non-inbred male white rats (weight 200-210 g), supplied by the Experimental Animal Center "Rappolovo" of the Academy of Sciences of Russia. The animals were divided into five groups: the first consisted of controls; the second, third and fourth groups were given 0.002% DEA, 0.002% DENA and 0.0005% SN solutions for 30 days, respectively. The fifth group received 0.002% DEA simultaneously with 0.0005% SN. All the chemicals were given in drinking water. The control group received drinking water without xenobiotics.

### Preparation of microsomal and cytosol liver fractions

Animals were killed by decapitation between 09:00 and 10:00 h. The livers were perfused with ice-cold 0.9% NaCl and homogenized in ice-cold 25 mM Tris buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA. Microsomes and cytosol were prepared by sequential

centrifugation of homogenate (20%) at 18,000 *g* for 20 min and at 105,000 *g* for 1 h. To remove cytosolic contamination, the microsomes obtained were washed with Tris buffer (pH 7.4) by centrifugation (105,000 *g*, 1 h). After being washed, the microsomes were resuspended in the same buffer and stored under liquid nitrogen until assayed.

**Enzyme assay**

The total content of cytochromes P-450 and $b_5$ in the microsomal fraction was determined according to the method of Omura and Sato /4/.

Glutathione S-transferase activity was measured spectrophotometrically with 1-chloro-2,4-dinitrobenzene and p-nitrophenethyl bromide as substrates /5/. The activity of microsomal glutathione S-transferase was determined according to Habig *et al.* /5/, using 1 mM 1-chloro-2,4-dinitrobenzene and 5 mM reduced glutathione. The activity of Se-dependent glutathione peroxidase was determined using $H_2O_2$ as a substrate /6/. The activity of Se-independent glutathione peroxidase was determined by the difference of the reduction rates of t-butyl hydroperoxide and $H_2O_2$. The amount of non-protein SH-groups was estimated as described by Sedlak and Lindsay /7/.

The enzymatic activity was expressed in nmoles/min per mg protein. The concentration of cytochromes and SH-groups unbound to protein was expressed in nmoles per mg protein.

**Protein determination**

The total concentration of protein in cytosol and microsomal fractions was determined by the method of Lowry *et al.* /8/ and Peterson /9/ with bovine serum albumin as a standard.

**Statistical analysis**

The obtained results are presented as means ± SE. The means were compared using Student's t-test.

## RESULTS

As can be seen from Table 1, chronic administration of DEA and DENA to rats increased the total content of cytochromes P-450 by

**TABLE 1**

Functional state of the xenobiotic metabolizing system in rat liver following chronic administration of 0.002% DENA, 0.002% DEA, 0.0005% SN or SN + DEA (mean ± SE, n = 6)

| Parameter | Control | SN | DEA | DENA | SN + DEA |
|---|---|---|---|---|---|
| Cytosolic glutathione S-transferase[a] (1-chloro-2,4-dinitrobenzene) | 482.10±15.7 | 472.00±23.3 | 494.70±14.4 | 616.60±21.0** | 416.00±22.0* |
| Cytosolic glutathione S-transferase[a] (p-nitrophenethyl bromide) | 86.40±11.0 | 60.80±5.3* | 53.30±5.4* | 59.60±4.6* | 65.40±4.2 |
| Microsomal glutathione S-transferase[a] | 22.80±5.6 | 42.60±6.3* | 31.10±7.4 | 33.70±4.6 | 18.60±3.6 |
| Se-dependent glutathione peroxidase[a] | 36.90±4.7 | 77.20±8.4* | 26.10±3.0 | 19.30±2.1* | 39.90±3.4 |
| Se-independent glutathione peroxidase[a] | 27.20±3.7 | 60.30±7.2* | 67.70±7.2** | 23.20±1.9 | 19.60±2.7 |
| Non-protein SH-groups[b] | 23.00±1.9 | 17.20±4.8 | 21.20±4.9 | 37.30±5.9 | 4.40±1.4** |
| Cytochrome P-450[b] | 0.54±0.04 | 0.51±0.1 | 0.68±0.09** | 0.83±0.09*** | 0.97±0.09*** |
| Cytochrome $b_5$[b] | 0.31±0.02 | 0.37±0.03 | 0.37±0.02 | 0.42±0.03** | 0.42±0.01** |

*p<0.05, **p<0.01, ***p<0.001
[a]nmoles/min per mg protein
[b]nmoles per mg protein

26% and 54%, respectively. In the case of SN intoxication no statistically significant change of P-450 concentration was observed. Coadministration of SN and DEA resulted in an 80% increase of cytochrome P-450 content. In animals receiving separately DEA and SN a 19% increase of cytochrome $b_5$ was observed, whereas coadministration of DEA and SN resulted in a 36% increase of cytochrome $b_5$. Intoxication with DENA increased the $b_5$ content by 35%.

The activity of microsomal glutathione S-transferase increased to varying degrees when the xenobiotics were given separately. In the case of coadministration of DEA and SN it remained at the control level. The maximum increase of activity was 87%, due to SN.

The total activity of cytosolic glutathione S-transferases (with dinitrochlorobenzene as a substrate) showed a 28% increase after administration of DENA. The coadministration of DEA and SN decreased the enzymatic activity insignificantly. If DEA and SN were given separately, the activity of glutathione S-transferases remained at the control level.

In all four experimental groups the activity of glutathione S-transferases decreased when p-nitrophenethyl bromide, specific for isoenzyme 5-5 /10/, was used as a substrate. The maximum suppression of activity (48%) was observed with DEA. When DEA was coadministered with SN, the activity decreased by 24% of the control values. Administration of SN and DENA decreased the enzyme activity by 30% and 31%, respectively.

Increase of Se-dependent glutathione peroxidase activity was observed only after administration of SN (109%). DEA and DENA decreased the reduction rate of $H_2O_2$ by 29% and 48%, respectively. Coadministration of SN and DEA did not affect the activity of the enzyme.

When administered separately, SN and DEA increased Se-independent glutathione peroxidase activity by 122% and 149%, respectively. When SN and DEA were coadministered, they decreased this activity by 28%. The administration of DENA did not change this activity compared to the control level.

Administration of DENA increased the concentration of non-protein SH-groups by 62%. The concentration of free SH-groups was 5-fold less after the combined effect of DEA and SN, yet practically unchanged when these xenobiotics were given separately.

## DISCUSSION

In rats receiving DENA the increased enzyme content of the monooxygenase system proceeded against a background of rising activity of microsomal glutathione S-transferases, total activity of the cytosol glutathione S-transferases and an increase in the content of non-protein bound SH-groups.

The DEA-intoxication of rats resulted in a slight increase of the main components of the first phase of xenobiotic biotransformation; it also resulted in an increase in the activity of Se-independent glutathione peroxidase which is capable of decomposing organic hydroperoxides and thereby partially compensating for the inhibition of Se-dependent glutathione peroxidase activity. However, in the present case there is no reason to suggest a full compensation, since Se-independent glutathione peroxidase $K_m$ values for organic hydroperoxides are much higher than those of Se-dependent glutathione peroxidase /11/.

The administration of SN did not affect the concentration of cytochromes P-450, while resulting in a considerable increase of both Se-dependent and Se-independent glutathione peroxidase activity and stimulating the activity of membrane-bound glutathione S-transferase.

Investigation of Se-independent glutathione peroxidase in this study confirms the complexity of the response of the multifunctional family of glutathione S-transferases to the incorporation of the compounds administered. Taking into account the identity of Se-independent glutathione peroxidase with class alpha isoenzymes and with isoenzyme 5-5 from the family of glutathione S-transferases /12/, as well as the lack of activity of isoenzyme 5-5 /10/ toward t-butyl hydroperoxide, we can suppose that the induction of individual isoenzymes of the alpha class was the reason for the stimulation of Se-independent glutathione peroxidase in animals intoxicated by SN and DEA. Following coadministration of DEA and SN an increase in the content of the main components of the first phase of xenobiotic metabolism paralleled the decreased activities of glutathione S-transferases and Se-dependent and Se-independent glutathione peroxidase, as well as a dramatic decrease of non-protein sulfhydryl groups.

The various changes in the activity and contents of the components of the xenobiotic metabolizing system and exhaustion of the non-protein-SH pool significantly increase the probability of development

of pathological processes in a cell as a result of the accumulation of metabolites of DEA and DENA. Evidently the response of individual components of the monooxygenase system to intoxication by DEA simultaneously with SN cannot be understood by simple addition of the effects seen when the two compounds are given separately. Taking into account published data on the possibility of endogenous synthesis of nitrosamines from exogenous precursors /3/ and an increase in cytochrome P-450 and $b_5$ concentration on administration of DENA (Table 1), we suggest that the observed phenomenon of the combined effect of DEA and SN is to some extent a consequence of the formation of endogenous nitrosamine. The observed differences in the effects caused by DENA and DEA might be due to the occurrence of the N-nitroso group within the DENA molecule.

## REFERENCES

1. Ketterer B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mutat Res 1988; 202: 343-361.
2. Guengerich FP. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer Res 1988; 48: 2946-2954.
3. Bartsch H. N-Nitroso compounds and human cancer: where do we stand? Relevance to human cancer of N-nitroso compounds, tobacco smoke and mycotoxins. Proc 10th Int. Symp. N-Nitroso Compounds, Lyon, 25-27 Sept., 1989 / Lyon 1991; 1-10.
4. Omura T, Sato RJ. The carbon monoxide binding pigment of liver microsomes. 11. Solubilization, purification and properties. J Biol Chem 1964; 239: 2379-2385.
5. Habig WH, Pabst MJ, Jacoby WB. Glutathione S-transferases. J Biol Chem 1974; 240: 7130-7139.
6. Hafeman DG, Sunde RA, Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 1974; 104: 580-587.
7. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192-205.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
9. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977; 83: 346-356.
10. Ketterer B. Detoxication reaction of glutathione and glutathione transferases. Xenobiotica 1986; 16: 957-973.
11. Ketterer B, Meyer DJ. Glutathione transferase: A possible role in the detoxication and repair of DNA and lipid hydroperoxides. Mutat Res 1989; 214: 23-40.

12. Ketterer B, Meyer DJ, Coles B, Taylor JB, Pemble S. Glutathione transferases and carcinogenesis. In: Shankel DM, Hartman PE, Kada T, Hollaender A, eds, Antimutagenesis and Anticarcinogenesis Mechanisms. New York: Plenum Press, 1986; 103-128.