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GLUTATHIONE S-TRANSFERASE ACTIVITIES IN RAT AND MOUSE SPERM AND HUMAN SEMEN

Hasan Mukhtar, Insu P. Lee, and John R. Bend

Laboratory of Pharmacology, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709

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SUMMARY:

Glutathione S-transferase activity was found in sperm of the rat and DBA/2J and C57 BL/6J mice. In rat sperm activities with benzo(a)pyrene 4,5-oxide, styrene 7,8-oxide, and 1-chloro-2,4-dinitrobenzene were 0.88, 1.07, and 26.1 nmoles/min/mg protein, respectively. Δ^5-3-Ketosteroid isomerase activity of rat sperm was 4.9 nmoles/min/mg protein. These specific glutathione S-transferase and Δ^5-3-ketosteroid isomerase activities in sperm represent 0.4-4.1% of rat liver cytosol values. Human semen also contained significant glutathione S-transferase activity. It is postulated that these enzymes could function in the metabolism and detoxification of certain electrophilic xenobiotics, if present in sperm.

INTRODUCTION:

The glutathione S-transferases, a family of cytosolic enzymes, play an important role in the biotransformation and detoxification of many xenobiotics. Enzymic reactions involving conjugation of glutathione with a variety of electrophiles have been described (1-5). Glutathione S-transferase activity is widely distributed in living organisms, being present in hepatic and most extrahepatic tissues (1-5) including ovary (6), testes (7), and serum (8) of all mammalian species studied. Recently, Δ^5-3-ketosteroid isomerase activity of rat liver was shown to be principally associated with glutathione S-transferase B (9, 10), which was previously identified as ligandin (11). The glutathione transferases, including ligandin, bind many organic anions including bilirubin, metabolites, hormones, dyes, and drugs noncovalently, and covalently

Abbreviations used: SO, styrene 7,8-oxide, 4,5-BPO, benzo(a)pyrene, 4,5-oxide; CDNB, 1-chloro-2,4-dinitrobenzene; HEPES, N-2-hydroxypiperazine-N'-2-ethane sulfonic acid; PBS (0.01 M sodium phosphate, 0.145 M sodium chloride, pH 7.2); CTAB, cetyltrimethylammonium bromide.

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bind several carcinogens and/or their metabolites. The role of the glutathione S-transferases in the detoxification of carcinogenic aminoazo dyes, polycyclic aromatic hydrocarbons, aromatic amines, and other electrophiles including epoxides has recently been reviewed (5, 12). Our laboratory is interested in the biotransformation of alkene and arene oxides by glutathione S-transferases in hepatic and extrahepatic tissues of mammalian and aquatic species.

To our knowledge enzymes involved in the biotransformation of xenobiotics have not been previously reported in sperm. Here we describe the presence of glutathione S-transferase activities in rat and mouse sperm and human semen.

MATERIALS AND METHODS:

Vasa deferentia were dissected from male Sprague-Dawley rats (300-350 g body weight), DBA/2J, and C57 BL/6J mice (30-40 g body weight). Sperms in the vas deferens of rats were gently flushed out by washing with ice-cold PBS with a syringe fitted with a 23-gauge needle. For collection of sperm from mice, vas deferens were squeezed gently with the help of a fine forceps. The collected sperms were washed twice with PBS by centrifugation at 1000xg for 15 min at 4°C. To remove the small amount of red blood cell contamination, the sperm preparation was subjected to hypotonic shock for 20 sec, using distilled water. The osmolarity of the sperm suspension was immediately readjusted to 300 mOsm using 0.45 M NaCl. The sperm preparation was then repeatedly washed by resuspension (three times) and sedimentation at 1500xg for 15 min to remove cellular contaminants. Finally the sperms were resuspended in PBS containing 0.1% CTAB and sonicated for 15 sec with a Branson 20 KHz sonicator equipped with a microprobe (setting of 40). The sonicated sperm suspensions were used as the enzyme source. Identical volumes of 0.1% CTAB in PBS were added to blank assay tubes and to tubes used to determine nonenzymatic reaction rates. Samples of human semen were obtained from normal healthy volunteers (29-38 years of age). Glutathione S-transferase activities with SO, 4,5-BPO, or CDNB as substrate were assayed according to procedures described earlier (6, 8). Δ^5-3-Ketosteroid isomerase activity was determined spectrophotometrically (9). The cuvette (in a final volume of 3.0 ml) contained: 24 mM Tris base, 12.5 mM K_2HPO_4, sufficient H_3PO_4 to attain a pH of 8.5, 100 µM GSH, 68 µM Δ^5-androstene-3,17-dione (in 20 µl of methanol) and enzyme source (200 µl) which was used to initiate the reaction. The absorbance of the sample cuvette was recorded at 25°C in a UV vis Gilford spectrophotometer at 248 nm. One unit of enzyme is defined as the amount causing the isomeration of 1 nmol of Δ^5-androstene-3,17-dione per minute to Δ^4-androstene-3,17-dione. The activities were calculated from $\varepsilon M = 16000\ M^{-1}\ cm^{-1}$. All reaction rates reported have been corrected for the nonenzymatic contribution.

All chemicals used were of the highest purity available commercially and were obtained from the sources described previously (8). Δ^5-Androstene-3,17-dione (m.p. 155-157°) was prepared by Dr. J. H. Maguire according to the procedure of Jones and Gordon (13).

RESULTS AND DISCUSSION:

Specific rat sperm glutathione S-transferase activities with 4,5-BPO, a K-

region arene oxide, and with SO, a mutagenic alkene oxide, were 0.88 and 1.07 nmoles/min/mg protein, respectively. Activity with the more commonly used aryl halide substrate CDNB was 26.1 nmoles/min/mg protein (Table 1). These sperm glutathione S-transferase activities represent 1.9, 0.4, and 1.4 percent of specific hepatic cytosolic activities in the rat. The differences in these ratios may be relevant since the various (total) glutathione S-transferases present in rat sperm may differ qualitatively and/or quantitatively from those of hepatic and extrahepatic tissues. Studies by Jakoby and his associates (1, 11) earlier demonstrated the presence of seven different glutathione S-transferases in rat liver with overlapping substrate specificities. Similarly, with partially purified glutathione S-transferases from rabbit liver and lung, it was shown in our laboratory (14) that SO was not a substrate for all glutathione S-transferases whereas 4,5-BPO was. The specific Δ^5-3-ketosteroid isomerase activity in sonicated rat sperm was 2.46 nmoles/min/mg protein, which represents 4.1% of the hepatic activity (Table 1).

Specific glutathione S-transferase activities with 4,5-BPO and CDNB in C57 BL/6J and DBA/2J mice sperm are given in Table 2. The activities in mouse sperm were slightly higher with CDNB than those observed in rat sperm, although activities were very similar in rats and mice with BPO as substrate. Glutathione S-transferase activity in human semen is given in Table 3. Semen from four healthy individuals had glutathione S-transferase activity ranging from 47-112 nmoles/min/ml ejaculate and 1.0-3.2 nmoles/min/ml ejaculate, respectively, with CDNB and 4,5-BPO as substrates. In the sperm preparations we were unable to detect cytochrome P-450, benzo(a)pyrene hydroxylase, or epoxide hydrase activity.

The origin and functional role of glutathione S-transferase and Δ^5-3-ketosteroid isomerase activity in sperm are uncertain. It is not known whether the mature spermatozoa, obtained from the vas deferens, acquired these enzymes from the reproductive tract after cell differentiation or if these enzymes were retained during spermatogenic cell differentiation. The presence of gluta-

TABLE 1

SPECIFIC GLUTATHIONE S-TRANSFERASE AND Δ^5-KETOSTEROID ISOMERASE ACTIVITIES IN RAT SPERM

Substrate	nmoles product/min/mg protein
Benzo(a)pyrene 4,5-oxide	0.88 ± 0.27 (45.6)
Styrene 7,8-oxide	1.07 ± 0.32 (275)
1-chloro-2,4-dinitrobenzene	26.05 ± 2.54 (1832)
Δ^5-androstene-3,17-dione	2.46 ± 0.48 (58.9)

Data represent mean ± S.D. of three determinations. Corresponding hepatic values are given in parentheses for comparison.

TABLE 2

SPECIFIC GLUTATHIONE S-TRANSFERASE ACTIVITIES IN SPERM FROM C57 BL/6J AND DBA/2J MICE

Mouse Strain	nmoles product/min/mg protein 4,5-BPO	CDNB
C57 BL/6J	0.97,0.71	74,52
DBA/2J	0.92,0.64	58,39

Individual values represent sperm pooled from 25 mice.

thione S-transferase activity in human semen suggest that it may assist in the detoxification of electrophilic metabolites of environmental chemicals.

It is interesting to note that relatively large amounts of some drugs including methadone (18, 19), thalidomide (20), and phenytoin (5,5-diphenylhydantoin (21, 22) are excreted in high concentrations in the semen of rabbit

TABLE 3

SPECIFIC GLUTATHIONE S-TRANSFERASE ACTIVITIES IN HUMAN SEMEN WITH BENZO(a)PYRENE 4,5-OXIDE (4,5-BPO) OR 1-CHLORO-2,4-DINITROBENZENE (CDNB) AS SUBSTRATE

Subject	Age (Years)	Semen Volume ml	nmoles product/min Per ml 4,5-BPO	Per ml CDNB	Per ejaculate 4,5-BPO	Per ejaculate CDNB
A	29	4.4	1.0	47	4.4	207
B	32	2.9	1.3	64	3.8	186
C	36	1.8	3.2	112	5.8	202
D	38	5.3	1.4	49	7.4	260
	Mean ± S.D.		1.7 ± 1.0	68.0 ± 30.3	5.4 ± 1.6	214 ± 32

and man. Recently chlordecone (Kepone), a polychlorinated pesticide, was also found in sperm of dosed rats (23). We have earlier demonstrated that a cytosolic fraction of rat testicular germ cells had significantly higher (2.5-fold) glutathione S-transferase activity than a similar fraction of interstitial cells (7). Failure to detect epoxide hydrase (or cytochrome P-450) in rat sperm suggests that the major protective enzymatic pathway for alkene or arene oxide biotransformation in rodent sperm is conjugation with glutathione.

Human seminal fluid has previously been shown to contain γ-glutamyl transpeptidase activity (15), a key enzyme in glutathione synthesis. Seminal fluid is known to contain other enzymes such as acid phosphatase, lactate dehydrogenase, malate dehydrogenase, and transaminases (16, 17).

The biological significance of these enzymes in sperm and human semen was not assessed in the present investigation. However, it may be of interest to determine the relationship between glutathione S-transferase and Δ^5-3-ketosteroid isomerase activities and the functionality of sperm in animals that have been pretreated with chemicals known to be localized in sperm, particularly if these compounds have electrophilic intermediary metabolites.

REFERENCES

1. Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974) J. Biol. Chem., 249, 7130-7139.
2. Mukhtar, H. and Bresnick, E. (1976) Biochem. Pharmac., 25, 1081-1084.
3. James, M. O., Fouts, J. R., and Bend, J. R. (1976) Biochem. Pharmac., 25, 187-193.
4. Hayakawa, T., Udenfriend, S., Yagi, H., and Jerina, D. M. (1975) Arch. Biochem. Biophys., 170, 438-451.
5. Jerina, D. M. and Bend, J. R. (1977) Glutathione S-Transferases. In Biological Reactive Intermediates (Jollow, D., Kocsis, J. J., Snyder, R., and Vainio, H., eds.) pp. 207-236, Plenum Press, New York.
6. Mukhtar, H., Philpot, R. M., and Bend, J. R. (1978) Biochem. Biophys. Res. Commun., 81, 89-98.
7. Mukhtar, H., Lee, I. P., Foureman, G. L., and Bend, J. R., Chem.-Biol. Interact. In press.
8. Mukhtar, H. and Bend, J. R. (1977) Life Sci., 21, 1277-1286.
9. Benson, A. M. and Talalay, P. (1976) Biochem. Biophys. Res. Commun., 69, 1073-1079.
10. Benson, A. M., Talalay, P., Keen, J. H., and Jakoby, W. H. (1977). Proc. Natl. Acad. Sci., U.S.A., 74, 158-162.
11. Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M., and Jakoby, W. B. (1974) Proc. Natl. Acad. Sci. U.S.A., 71, 3879-3882.
12. Smith, G. H., Ohl, V. S., and Litwack, G. (1977) Cancer Res., 37, 8-14.
13. Jones, J. B. and Gordon, K. D. (1972) Can. J. Chem., 50, 2712-2718.
14. Maguire, J. H., Fouts, J. R. and Bend, J. R. (1977) Fed. Proc., 36, 951.
15. Krishnaswamy, P. R., Tate, S. S., and Meister, A. (1977) Life Sci., 20, 681-686.
16. Cannon, D. C. (1974) In Clinical Diagnosis by Laboratory Methods, pp. 1300-1306, W. B. Saunders, Philadelphia.
17. Eliasson, R. (1973) In Human Reproduction, pp. 39-51, Harper and Row, New York.
18. Gerber, N. and Lynn, R. K. (1976) Life Sci., 19, 787-792.
19. Swanson, B. N. and Gerber, N. (1977) Proc. West. Pharmacol. Soc., 20, 477-482.
20. Lutwak-Mann, C., Schmid, K. and Keberle, H. (1967) Nature, 214, 1018-1020.
21. Leger, R. M., Swanson, B. N., and Gerber, N. (1977) Proc. West. Pharmacol. Soc., 20, 69-72.
22. Swanson, B. N., Leger, R. M., Gordon, W. P., Lynn, R. K., and Gerber, N. (1978) Drug Metab. Dispos., 6, 70-74.
23. Egle Jr., J. L., Fernandez, B. S., Guzelian, P. S., and Borzelleca, J. F. (1978) Drug Metab. Dispos., 6, 91-95.