Oxford Biomedical Research

SUPERIOR SCIENCE. RELIABLE RESULTS.™

Preclinical and Clinical Investigations of the Expression of Glutathione *S*-transferase Isozymes

Thomas Primiano, Ph.D.
Department of Environmental Health Sciences
Johns Hopkins University
Baltimore, Maryland

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

P.O. Box 522 • Oxford, MI 48371 • tel: 800.692.4633 • fax: 248.852.4466 • hppt://www.oxfordbiomed.com

Introduction

The glutathione *S*-transferases (EC 2.5.1.18) (GSTs) are a group of inducible enzymes important in the detoxication of many different xenobiotics in mammals (1,2). The GSTs achieve detoxication by catalyzing the conjugation of reduced glutathione to various electrophilic substrates. Glutathione (GSH), as the chief intracellular non-protein thiol compound, functions as a cellular storage pool of reduced thiols. GSH conjugation is the first step in mercapturic acid synthesis which aids in the protection of the cell by enhancing the excretion of toxic metabolites for both animals and humans. Therefore, the levels of GSTs has been suggested as important determinant of the susceptibility of organisms or tissues to pharmacological or physiological challenges.

To date, the superfamily of mammalian cytosolic GST isozymes have been defined genetically to consist of four classes, termed Alpha, Mu, Pi and Theta (3). These classes differ in their tissue-specific expression and distribution within tissues. The GSTs exist as homo-or hetero-dimeric combinations of subunits. Two types of Alpha, three Mu, and one Pi class GST subunit have been found to exist in human tissues. GSTs are abundant in mammalian liver comprising 2-10% of the soluble hepatic protein. The Alpha GSTs are very basic enzymes that represent the most abundant liver isozymes in humans and are broadly expressed throughout the lobule (4-6). The Alpha GSTs are also highly expressed in the proximal tubule of the kidney, testis, and adrenal glands. The Mu class GSTs have been found in high concentrations in the

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

brain, muscle, liver, kidney and lung (5,6). The Pi class GST is a highly acidic enzyme that is widely distributed except in adult liver, where it is localized to the biliary epithelium (5-8). The Theta class have only recently been described enzymatically in liver (9,10), and details of its expression in other tissues is forthcoming.

The classes of GST isozymes differ in their specificity toward xenobiotic or endogenous substrates. The substrates which are conjugated to GSH include a variety of electrophilic chemicals such as 1-chloro-2,4-dinitrobenzene which undergoes nucleophilic displacement of the chloro moiety by GSH. This reaction is catalyzed by all classes of GSTs except theta. The substrates *trans*-4-phenyl-3-buten-2-one and 4-hydroxynon-2-enal which undergo nucleophilic addition at an _,_-unsaturated ketone and 1,2-epoxy-3(p-nitrophenoxy)propane which undergoes nucleophilic attack on a strained oxirane ring are preferentially catalyzed by Mu and Theta class GSTs, respectively. The Alpha class GSTs possess organic hydroperoxidase and prostaglandin isomerase activities (11,12). These varied activities to conjugate GSH to exogenous or endogenous organic electrophiles suggests that increased expression of GSTs may enhance the metabolism and disposition of pharmaceuticals, components of the diet, and environmental toxicants, which has been the subject of considerable experimental studies (13).

Preclinical Investigations

Tissues highly expressing activities of GSTs are protected from cytotoxic damage elicited by electrophiles for which the conjugation to GSH is readily catalyzed. Purified GST Mu and

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

Theta have high activities against polycyclic aromatic hydrocarbon epoxide metabolites that may be generated from constituents of tobacco smoke (14-16). Additionally, over-expression of Alpha GSTs in cells enhances their protection from necrotic toxicity produced by cyclophosphamide or diethylnitrosamine (17,18). Elevated levels of Alpha, Mu and Pi GSTs have been associated with protection of tissues from cytotoxicity produced by acetaminophen, carbon tetrachloride and aflatoxin B_1 (19,20). The GSTs also have been shown to detoxify cyclophosphamide (21), nitroglycerin (22), L-phenylalanine mustard (23), and chlorambucil (24). Therefore, measurement of the concentrations and activities of GSTs, and their modulation in tissues that are subjected to physiological or environmental stimuli, may provide researchers with an important tool in monitoring the detoxication potential of cellular systems. For example, the GST isozymes are inducible to varying degrees by a number of xenobiotics, including phenobarbital, 3-methylcholanthrene, phenolic antioxidants, azo dyes, flavonoids, TCDD, diphenols, thiocarbamates, 1,2-dithiol-3-thiones, isothiocyanates, cinnamates, coumarins, and _-naphthoflavone in mice (25-29). Induction of GST isozymes has been monitored at the protein level using enzymatic assays, SDS-PAGE/immunoblot analyses, as well as enzyme-linked immunosorbant assays (ELISA) assays. Analyzing GST isozyme induction using enzymatic assays is complicated by the overlapping substrate specificity associated with the GST isozymes, making immunoblot analysis or ELISA techniques essential for identification of the GST isozymes or classes of GST isozymes which may be affected by xenobiotic treatment. Thus, measurement of GST isozyme profiles in preclinical rodent model scan rapidly provide clues to potential drug interactions or modification in the detoxication of environmental contaminants.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

Clinical Investigations

In addition,altered expression of the GSTs have been measured in mammalian tissues and in human serum, plasma and urine is observed. For example, increased levels of Alpha GST isozymes in plasma or serum have been related to acute or chronic liver disease (30). Similarly, the levels of urinary Alpha GSTs relate to renal damage (31-33). Increased GST plasma levels measured using radioimmunoassay or ELISA have been observed following hepatocellular damage elicited by acetaminophen (34), alcohol (35), or halothane (36). The Alpha GST serum half life is short (less than 1 hr), and because of its high concentration in liver, its broad lobular distribution and rapid release from tissues, the Alpha GSTs are considered to be more sensitive indicators of hepatocellular injury than the standard serum transaminases. The may also give important information about the course of liver disease and their subsequent management. Measurement of Alpha GSTs in serum and urine were found to be better indicators than the serum transaminases in early prediction of rejection following liver or kidney transplantation, respectively (37). The Alpha GSTs were also a better measure of successful intervention to alleviate rejection following transplantation, thereby demonstrating the important clinical use of monitoring GST levels in biological tissues (37). Similarly, the plasma levels of Alpha GSTs were correlated with onset and therapeutic suppression of chronic or acute hepatitis infection (38). Elevated Alpha GSTs have been found in plasma of patients with hepatocellular damage caused by hypoglycemia (39), birth asphyxia (40), or autoimmune chronic hepatitis (41). These results indicate that

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

measurement of Alpha GST expression in biological fluids can be an important clinical tool to monitor the dynamic impact of stresses on liver and kidney.

Deficiencies in expression of GSTs in humans have been documented (For review, see Board *et al.*, 42). Approximately half of the population lacks GST Mu expression, termed the GST M1 null genotype (43-44). Epidemiological evidence supports the conclusions that persons possessing this genotype are predisposed to a number of cancers including breast, prostate, liver and colon cancers (45-47). Measurement of a lack of GST Mu class isozymes in human polymorphonuclear cells by ELISA methods has been associated with increased predisposition to liver and colon cancers (44,47). GST Pi is an acidic isozyme which is expressed in high concentrations in chemically-induced preneoplastic rat hepatocyte nodules (48-50) and in rat primary hepatomas (51). The human ortholog, GST P1-1 (52), is also expressed in many human tumors, including colonic (53), hepatic (54), prostate (55), lung (56), breast, ovarian, gastric, and renal cell (57,58) carcinomas, as well as melanoma, uterine adenocarcinoma, and mesothelioma (57,59). In contrast, GST 7-7 is present in only trace amounts (6-8) in normal rat liver, and is expressed primarily in the bile duct epithelium. GST 7-7 has also been induced *de novo* in rat hepatic tissue by treatment with *trans*-stilbene oxide (60), lead nitrate (61), and pyrrole (62). Elevated expression of GST P1-1 has been found in a number of human multi-drug resistant cell lines and tumors (63-65) and GST P1-1 has been associated with the resistance of these cells and tumors towards chemotherapeutic agents. Therefore, measurement of GST Alpha, Mu and Pi class expression may have clinical benefit for monitoring therapeutic progression of cancerous disease or identification of populations susceptible to chemotherapeutic interventions.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

Conclusions

The GSTs constitute a primary pathway for the detoxication of cellular electrophiles generated endogenously or form xenobiotic administration or exposure. Preclinical studies have correlated enhanced metabolism of electrophiles with increased levels of GST isozymes within various tissues. Expression of GSTs within individuals can then provide an indicator the metabolic potential of their tissues and possible deficiencies in their susceptibility to dietary or environmental carcinogens. GSTs are over-expressed in certain tumor types, and measurement of GSTs in serum or in pathological specimens can be used to follow the course of disease and the success of intervention. Release of GSTs from damaged tissues can also be monitored by rapid ELISA or western blotting techniques to inform clinicians of acute toxicity related to tissue rejection after transplantation, chemical insult or viral infection. Readily available ELISA or western blot kits are then extremely useful in the preclinical or clinical laboratory to measure modulated GST expression in experimental or clinical specimens.

References

1. Mannervik, B. (1985) Adv. Biochem. Mol. Biol. 57, 357-417.
2. Tsuchida, S. and Sato, K. (1992) Crit. Rev. Biochem. Mol. Biol. 27, 337-384.
3. Board, P.G., Coggan, M., Johnston, P., Ross, V., Suzuki, T., and Webb, G. (1990) Pharmacol Ther. 48, 357-369.
4. Strange, R.C., Howie, A.F., Hume, R., Matheroo, B., Bell, J., Hiley, C., Jones, P., and Beckett, G.J. (1989) Biochim. Biophys. Acta 993, 186-190.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

5. Hayes, J.D. and Mantle, T.J. (1986) Biochem J. 233, 779-788.

6. Hayes, P.C., Portman, B., Aldis, P.M., Williams, R., and Hayes, J.D. (1987) In Glutathione S-transferases and Carcinogenesis (T.J. Mantle, C.B. Pickett, and J.D. Hayes, Eds.), pp. 175-187,Taylor and Francis, London.

7. Sato, K., Kitahara, A., Satoh, K., Ishikawa, T., Tatematsu, M., and Ito, N. (1984) Jpn. J. Cancer Res. 75, 199-202.

8. Satoh, K., Kitahara, A., Soma, Y., Inaba, Y., Hatayama, I., and Sato, K. (1985) Proc. Natl. Acad. Sci. USA 82, 3964-3968.

9. Meyer, D.J., Coles, B., Pemble, S.E., Gilmore, K.S., Fraser, G.M., and Ketterer, B. (1991) Biochem. J. 274, 409-414.

10. Hiratsuka, A., Sebata, N., Kawashima, K., Okuda, H., Ogura, K., Watabe, T., Satoh, K., Hatayama, I., Tsuchida, S., Ishikawa, T., and Sato, K. (1990) J. Biol. Chem. 265, 11973-11981.

11. Ketterer, B., Tan, K.H., Meyer, D.J., and Coles, B. (1987) In Glutathione S-transferases and Carcinogenesis (T.J. Mantle, C.B. Pickett, and J.D. Hayes, Eds.), pp. 149-164, Taylor and Francis, London.

12. Burgess, J.R., Yang, H., Chang, M., Rao,M.K., Tu, C.P-D., and Reddy, C.C. (1987) Biochem. Biophys. Res. Commun. 142, 441-447.

13. Primiano, T., Sutter, T.R., and Kensler, T.W. (1996) Adv. Pharmacol., in press.

14. Warholm, M, Guthenberg, C., and Mannervik, B. (1983) Biochemistry 22, 3610-3617.

15. Robertson, I.G.C., Guthenberg, C., Mannervik, B., and Jernström, B. (1986) Cancer Res. 46, 2220-2224.

16. Hussey, A.J. and Hayes, J.D. (1992) Biochem. J. 286, 929-935.

17. Tew, K.D. (1994) Cancer Res. 54, 4314-4320.

18. Manoharan, T.H., Puchalski, R.B., Burgess, J.A., Pickett, C.B., and Fahl, W.E. (1987) J. Biol. Chem. 262, 3739-3745.

19. Ansher, S.S., Dolan, P., and Bueding, E. (1983) Hepatology 3, 932-940.

20. Kensler, T.W., Enger, P.A., Dolan, P.M., Groopman, J.D., and Roebuck, B.D., (1987). Cancer Res. 47, 4271-4277.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

21. Coles, B., Wilson, I., Wardman, P., Hinson, J.A., Nelson, S.D., and Ketterer, B. (1988) Arch. Biochem. Biophys. 264, 253-260.

22. Tsuchida, S., Maki, T., and Sato, K. (1990) J. Biol. Chem. 265, 7150-7157.

23. Bolton, M.G., Colvin, O.M., and Hilton, J. (1991). Cancer Res. 51, 2410-2415. 24.Ciaccio, P.J., Tew, K.D., and LaCreta, F.P. (1990) Cancer Commun. 2, 279-285.

25. Kaplowitz, N., Kuhlenkamp, J., and Clifton, G., (1975). Bioch. J., 146, 351-356.

26. Hales, B.F. and Neims, A.H., (1977). Biochem. Pharmacol., 26, 555-556.

27. Igarashi, T., Irokawa, N., Ono, S., Ohmori, S., Ueno, K., Kitagawa, H., and Satoh, T., (1987). Xenobiotica, 17, 127-137.

28. Primiano, T. and Novak, R.F. (1992). Toxicol. Appl. Pharmacol. 113, 64-73.

29. Prochaska, H. and Talalay, P. (1988) Cancer Res. 48, 4776-4782.

30. Beckett, G.J. and Hayes, J.D. (1993) Adv. Clin. Chem. 30, 281-380.

31. Feinfeld, D.A., Fleischner, G.M., and Arias, I.M. (1981) Clin. Sci. 61, 123-125.

32. Feinfeld, D.A., Fleischner, G.M., Goldstein, E.J., Levine, R.D., Levine, S.D., Auran, M.M. and Arias, I.M. (1979) Curr. Probl. Clin. Biochem. 9, 273-280.

33. Bass, N.M., Kirsch, R.E., Tuff, S.A., Campbell, J.A.H., and Saunders, S.J. (1979) Clin. Sci. 56, 419-426.

34. Beckett, G.J., Chapman, B.J., Dyson, E.H., and Hayes, J.D. (1985) Gut 26, 26-31.

35. Hayes, P.C., Hayes, J.D., Hussey, A.J., Bouchier, I.A.D., and Beckett, G.J. (1990) Clin. Chem. Enzyme Commun. 2, 189-194.

36. Hussey, A.J., Aldridge, L.M., Paul, D., Ray, D.C., Beckett, G.J., and Allan, L.G. (1988) Br. J. Anaeth. 60, 130-135.

37. Trull, A.K., Facey, S.P., Rees, G.W., Wight, D.G.D., Nobile-Jamieson, G., Joughin, C., Friend, P.J., and Alexander, G.J.M. (1994) Transplantation 58, 1345-1351.

38. Vaubourdolle, M., Chazouilleres, O., Briaud, I., Legendre, C., Serfaty, L., Poupon,R., and Giboudeau, J. (1995) Clin. Chem. 41, 1716-1719.

39. Howie, A.E., Patrick, A.W., Fisher, B.M., Collier, A., Frier, B.M., and Beckett, G.J. (1989) Diabetic Med. 6, 224-227.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

40. Beckett, G.J., Hussey, A.J., Laing, I., Howie, A.F., Hayes, J.D., Strange, R.C., Faulder, C.G., and Hume, R. (1989) Clin. Chem. 35, 995-999.

41. Hayes, P.C., Hussey, A.F., Keating, J., Bouchier, I.A.D., Williams, R., Beckett, G.J., and Hayes, J.D. Clin. Chim. Acta 172, 211-216.

42. Board, P.G. (1981) An. J. Hum. Genet. 33, 36-43.

43. Seidegård, J., and Pero, R.W. (1985) Hum. Genet. 69, 66-68.

44. Seidegård, J., Pero, R.W., Miller, D.G., and Beattie, E.J. (1986) Carcinogenesis 7, 751-753.

45. Zhong, S., Wyllie, A.H., Barnes, D., Wolf, C.R., and Spurr, N.K. (1993) Carcinogenesis 14, 1821-1824.

46. Brockmoller, J., Kerb, R., Drakoulis, N., Staffeldt, B., and Roots, I. (1994) Cancer Res. 54, 4103-4111.

47. Seidegård, J., Pero, R.W., Markowitz, M.M., Roush, G., Miller, D.G., and Beattie, E.J. (1990) Carcinogenesis 11, 33-36.

48. Kitahara, A., Satoh, K., Nishimura, K., Ishikawa, T., Ruike, K., Sato, K., Tsuda,H., and Ito, N. (1984) Cancer Res. 44, 2698-2703.

49. Jennson, H., Eriksson, C.C., and Mannervik, B. (1985) FEBS Lett. 187, 115-120.

50. Rushmore, T.H., Sharma, R.N.S., Roomi, M.W., Harris, L., Satoh, K., Sato, K., Murray, R.K., and Farber, E.(1987) Biochem. Biophys. Res. Commun.143, 98-103.

51. Meyer, D.J., Beale, D., Tan, K.H., Cole, B., and Ketterer, B. (1985) FEBS Lett. 184, 139-143.

52. Mannervik, B., Awashti, Y.C., Board, P.G., Hayes, J.D., Di Ilio, C., Ketterer, B., Listowsky, I., Morgenstern, R., Muramatsu, M., Pearson, W.R., Pickett, C.B., Sato, K., Widersten, M., and Wolf, C.R. (1992) Biochem. J., 305-306.

53. Kodate, C., Fukushi, A., Narita, T., Kudo, H., Soma, Y., and Sato, K. (1988) Jpn. J. Cancer Res. 77, 226-229.

54. Sherman. M., Campbell, A., Titmuss, S., Kew, M., and Kirsch, D.E. (1983) Hepatology 3, 170-175.

55. Tew, K.D., Clapper, M.L., Greenberg, R., Hoffman, S., Smith, T., and Weese, J.L. Proc. Am. Assoc. Cancer Res. 28, 1149.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.

56. Di Ilio, G., Del Boccio, G., Aceto, A., Casaccia, R., Mucilli, F., and Frederici, G. (1988) Carcinogenesis (Lond.) 9, 335-340.

57. Shea, T.C., Kelley, S.L., and Henner, W.D. (1988) Cancer Res. 48, 527-533.

58. Tsutsuni, M., Sugisaki, T., Makino, T., Miyagi, N., Nakatani, K., Shiratori, T., Takagashi, S., and Konishi, Y. (1987) Jpn. J. Cancer Res. 78, 631-633.

59. Wolf, C.R., Lewis, A.D., Carmichael, J., Ansell, J., Adams, P.J., Hickson, I.J., Harris, A., Balkwill, F.R., Griffin, D.B., and Hayes, J.D. (1987) Glutathione S-transferases and Carcinogenesis (Mantle, T.J., Pickett, C.B., and Hayes, J.D., eds.), pp.199-212,Taylor and Francis, London.

60. Tahir, M.K., Guthenberg, C., and Mannervik, B. (1985) Biochem. J. 257, 215-220.

61. Roomi, M.W., Columbano, A., Ledda-Columbano, G.M., and Sarma, D.S.R. (1987) Toxicol. Pathol. 15, 202-205.

62. Primiano, T. Gandy, J., York, J.L., and Novak, R.F. (1993) Biochem. Biophys. Res. Comm., 190, 1136-1142.

63. Clapper, M.L., Baller, A.C., Smith, T.M., and Tew, K.D. (1987) In Glutathione S-transferases and Carcinogenesis (T.J. Mantle, C.B. Pickett, and J.D. Hayes, Eds.), Taylor and Francis, London pp. 213-224.

64. Duhloff, B., Martinsson, T., Mannervik, B., Jennson, H., and Leron, G. (1987) Anticancer Res. 7, 65-69.

65. Batist, G., Tulpule, A., Sinha, B.K., Katki, A., Meyers, C.E., and Cowan, K.H. (1986) J. Biol. Chem. 261, 15544-15549.

© 1997 Oxford Biomedical Research, Inc. All Rights Reserved.