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Activity of Allelic Variants of Pi Class Human Glutathione *S*-Transferase Toward Chlorambucil

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Clinical efficacy of alkylating anticancer drugs, such as chlorambucil, is often limited by the emergence of drug resistant tumor cells. Increased glutathione (GSH) conjugation (inactivation) of alkylating anticancer drugs or their activated metabolites due to overexpression of the Pi class GSH *S*-transferase (hGSTP1-1) is believed to be an important mechanism in tumor cell resistance to alkylating agents. Interestingly, the *hGSTP1* locus is polymorphic in human populations and involves amino acid residues in positions 104 (isoleucine or valine) and/or 113 (alanine or valine). Here, we report that the allelic variants of hGSTP1-1 significantly differ in their efficiency in catalyzing the GSH conjugation of chlorambucil. Catalytic efficiency of the hGSTP1-1(I104,A113) isoform toward chlorambucil was approximately 2.5-, 7.5- and 15-fold higher compared with I104,V113, V104,A113 and V104,V113 variants of hGSTP1-1, respectively. The results of the present study suggest that hGSTP1-1 polymorphism may be an important factor in GST-mediated tumor cell resistance to some alkylating agents. **© 2000 Academic Press**

Key Words: **glutathione; glutathione *S*-transferase P1-1; polymorphism; chlorambucil; drug resistance.**

Alkylating agents, such as chlorambucil, are extensively used in the treatment of neoplastic diseases, but their effectiveness is often limited by the emergence of drug resistant tumor cells. Several different mechanisms have been identified that mediate tumor cell resistance to alkylating agents (1–4). One such mechanism involves increased inactivation of the alkylating anticancer drugs or their activated metabolites in drug-resistant tumor cells due to elevation of glutathione (GSH) levels and/or overexpression of GSH *S*-transferases (GSTs) (3, 4). GSTs are a superfamily of isoenzymes that can catalyze the addition of GSH to a wide variety of electrophilic compounds generally leading to their detoxification (5, 6). It is believed that the GST-catalyzed GSH conjugation of alkylating agents or their activated metabolites reduces their interaction with DNA, which is a critical event in antineoplastic effects of alkylating agents. Other mechanisms that have been suggested to contribute to alkylating agent resistance include reduced drug uptake, reduced bioactivation of the drug, and increased repair of DNA cross-links (1, 2).

Evidence for the involvement of GSH/GST system in cellular resistance to alkylating agents is provided by studies showing (a) increased GSH conjugation of alkylating agents or their activated metabolites in the presence of GSTs *in vitro* (7–10), (b) overexpression of GSTs in alkylating agent resistant tumors cells (3, 4, 11–13), (c) increased sensitization of tumor cells, including drug-resistant cancer cells, to alkylating agents by depleting cellular GSH levels or by inhibiting cellular GST activity (14–16), and (d) reduction in the cytotoxic activity of alkylating agents in cells transfected with GST genes (17–19).

Mammalian GSTs have been grouped into several classes, such as Alpha, Mu, Pi, Theta, etc. (20, 21), and the human GSTs of the above classes are known to exhibit overlapping but distinct substrate affinities (5, 6, 20). For example, while GSH conjugation of the activated metabolite of ifosfamide is solely catalyzed by the Pi class isoenzyme hGSTP1-1 (10), both hGSTA1-1 (an Alpha class isoenzyme) and hGSTP1-1 are equally

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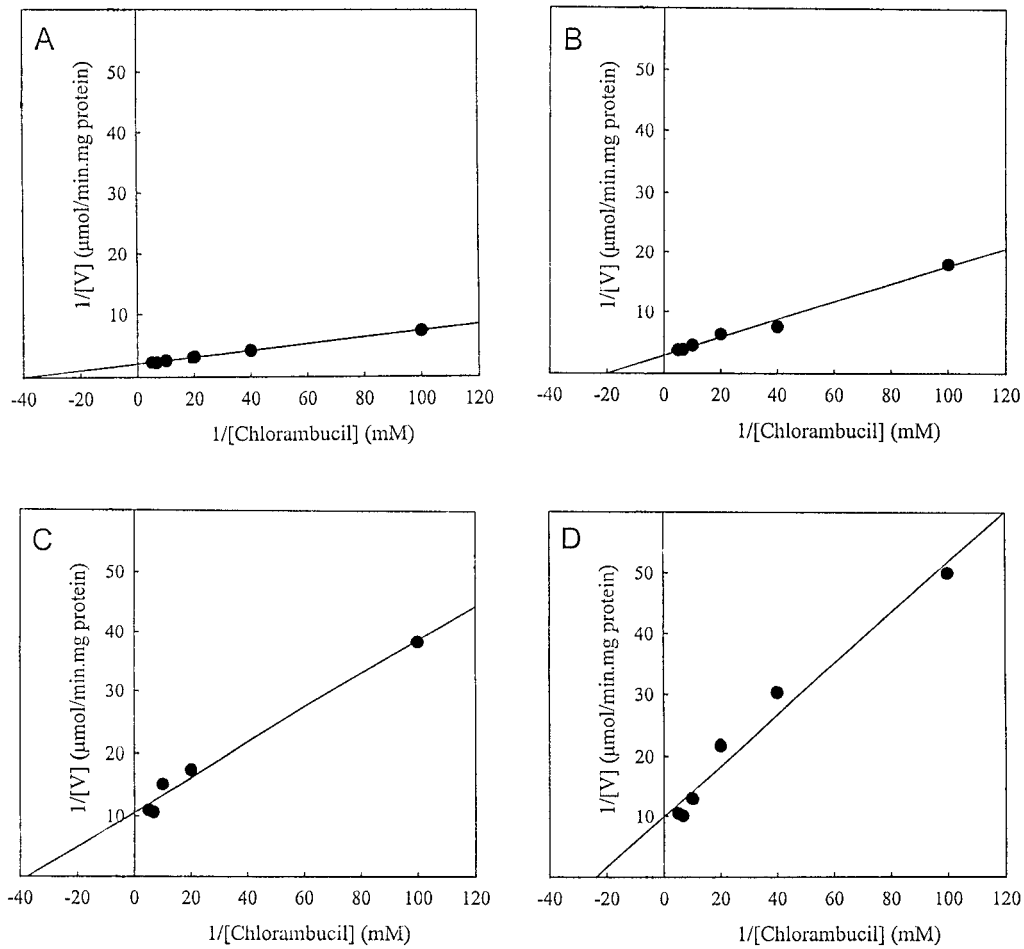

FIG. 1. Lineweaver–Burk plot for hGSTP1-1 variants I104,A113 (A), I104,V13 (B), V104,A113 (C), and V104,V113 (D) in the GSH conjugation of chlorambucil. The concentration of the chlorambucil was varied between 0.01 and 0.2 mM, whereas GSH concentration was kept constant at 2 mM.

effective toward thiotepa (8). Even though Alpha and Mu classes of GSTs can catalyze the addition of GSH to some alkylating agents, GSTP1-1 is likely to play a major role in alkylating agent resistance in humans because (a) the expression of hGSTP1-1 is significantly higher than other classes of GSTs in human cancer cells/tumor tissues, and (b) cellular resistance to alkylating agents in human tumor cells is often associated with the overexpression of hGSTP1-1 rather than other classes of GSTs (3, 4). Interestingly, the *hGSTP1* locus is polymorphic in human populations. The known polymorphisms involve amino acid residues in positions 104 (isoleucine or valine) and/or 113 (alanine or valine) (22–27). However, the physiological significance of hGSTP1-1 polymorphism is not fully understood.

In this communication, we demonstrate that the I104,A113 isoform of hGSTP1-1, which is most frequent in human populations, is significantly more efficient than either V104,A113 or V104,V113 in catalyzing the GSH conjugation of chlorambucil. Our results suggest that hGSTP1-1 polymorphism may be an important factor in GST-mediated tumor cell resistance to some alkylating agents, and that the hGSTP1-1(I104,A113) isoform expressing tumors are likely to be more resistant to the cytotoxic effects of chlorambucil, and possibly other alkylating agents, than tumors expressing other variants of hGSTP1-1.

MATERIALS AND METHODS

Expression and purification of hGSTP1-1 variants. The hGSTP1-1 variants were expressed as described by us previously (28). The hGSTP1-1 variants were purified by GSH affinity chromatography according to the method of Simons and Vander Jagt (29) with some modifications described by us previously (30). Protein content was determined by the method of Bradford (31).

Determination of GST activity toward chlorambucil. The activity of hGSTP1-1 variants toward chlorambucil was determined by the method described by us previously (32). GST activity was measured as a function of varying chlorambucil concentration (0.01–0.2 mM) at a fixed concentration of GSH (2 mM) to determine the kinetic parameters. The K_m and V_{max} values were determined by nonlinear regression analysis of the experimental data points using the Michaelis–Menten (hyperbolic) equation. For calculation of the cat-

TABLE I

Kinetic Constants for hGSTP1-1 Variants in the GSH Conjugation of Chlorambucil

Isoform of hGSTP1-1	V_{max} (nmol · min^{-1} · mg^{-1})	K_m (μM)	Catalytic efficiency (mM^{-1} · s^{-1})
I104,A113	478 ± 10	26 ± 2	15 ± 1.0
I104,V113	319 ± 34*	43 ± 14	6 ± 1.5
V104,A113	110 ± 13*	43 ± 16	2 ± 0.6*
V104,V113	147 ± 25*	91 ± 35	1 ± 0.3*

Note. Activity of the hGSTP1-1 variants was determined at constant GSH (2 mM) and varying (0.01–0.2 mM) chlorambucil concentrations. V_{max} and K_m values were estimated by nonlinear regression fitting of a hyperbola (Michaelis–Menten equation) to the experimental points. Catalytic efficiency was determined by direct fitting of a reparametrized Michaelis–Menten equation (as described under Materials and Methods) rather than by division of the individual kinetic constants. All values are shown ± asymptotic error of the mean obtained from nonlinear regression. *Significantly different from hGSTP1-1(I104,A113), $P < 0.005$.

alytic efficiency, the Michaelis–Menten equation, $v = V_{max} \cdot [S]/(K_m + [S])$, was rearranged to yield $v = K_m \cdot Q \cdot [S]/(K_m + [S])$, where $Q = V_{max}/K_m$. The Q is equivalent to the catalytic efficiency (after appropriate change of units for V_{max}).

RESULTS AND DISCUSSION

As shown in Fig. 1, all four variants of hGSTP1-1 [(I104,A113), (I104,V113), (V104,A113) and (V104,V113)] obeyed Michaelis–Menten equation when GST activity was measured as a function of varying chlorambucil concentration and at a fixed saturating concentration of GSH. Kinetic constants for hGSTP1-1 variants in the GSH conjugation of chlorambucil are summarized in Table I. The hGSTP1-1(I104,A113) variant, which is most frequent in human populations (25–27), was found to be most efficient in catalyzing the GSH conjugation of chlorambucil. The catalytic efficiency of the hGSTP1-1(I104,A113) isoform was about 2.5-fold higher compared with hGSTP1-1(I104,V113) variant. This was mainly due to an approximate 1.7–fold higher K_m and 33% lower V_{max} for the hGSTP1-1(I104,V113) isoform compared with hGSTP1-1 (I104,A113) variant. These results indicate that alanine-to-valine substitution at position 113 in the presence of isoleucine 104 causes a marked decrease in the activity of hGSTP1-1 toward chlorambucil. Interestingly, the isoleucine-to-valine substitution at position 104 results in a much more pronounced decrease in the activity of hGSTP1-1 toward chlorambucil. The catalytic efficiencies of the hGSTP1-1 variants with valine in position 104 were approximately 87–93% lower than that of the hGSTP1-1 (104,A113) isoform.

The results of the present study reveal that the I104,A113 variant of hGSTP1-1 is statistically significantly more efficient (approx. 7.5- to 15-fold) than either V104,A113 or V104,V114 in catalyzing the GSH conjugation of chlorambucil. Since the reaction rate of an enzyme at low substrate concentrations (substrate concentrations $\ll K_m$) is determined by its catalytic efficiency, our results suggest that, at low pharmacologically relevant chlorambucil concentrations, the GSH conjugation of this anticancer drug would be significantly higher in subjects homozygous for the *hGSTP1-1(I104,A113)* allele compared with heterozygotes or *hGSTP1-1(V104,A113)* and *hGSTP1-1(V104,V113)* homozygotes. In a previous study, we have demonstrated that the I104,A113 isoform of hGSTP1-1 is also significantly more efficient than either V104,A113 or V104,V113 in catalyzing the GSH conjugation of thiotepa (33), which is another clinically relevant anticancer agent. However, it is important to point out that the difference in catalytic efficiency between I104,A113 and V104,A113 or V104,V113 is much less pronounced toward thiotepa (1.9- to 2.6-fold) than for chlorambucil (33, present study). Taken together, our results suggest that hGSTP1-1 polymorphism may be an important factor in differential response of individuals to the antineoplastic effects of alkylating agents, and that subjects with the *I104,A113* genotype may be at a greater risk for developing GST-mediated resistance to chlorambucil and thiotepa, and possibly other alkylating agents, than those having *V104,A113* or *V104,V113* allele.

The physiological significance of hGSTP1-1 polymorphism may not be limited to alkylating agent resistance. We have shown previously that the allelic variants of hGSTP1-1 significantly differ in their ability to catalyze the GSH conjugation, and hence detoxification of the ultimate carcinogenic metabolites (diol epoxides) of polycyclic aromatic hydrocarbons (28, 34–36), which are widespread in the environment and suspected human carcinogens. For example, the hGSTP1-1 variants with valine in position 104 (V104,A113 and V104,V113) are about 1.3- to 4.7-fold more efficient than I104,A113 isoform in catalyzing the GSH conjugation of (+)-*anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[α]pyrene [(+)-*anti*-BPDE] (28), which is the activated form of widely spread environmental pollutant benzo[α]pyrene (37). Thus hGSTP1-1 polymorphism may also be an important factor in differential susceptibility of individuals to cancers where polycyclic aromatic hydrocarbons are etiological factors.

The hGSTP1-1 variants are the result of conservative amino acid substitutions; isoleucine versus valine in position 104, and more divergent but still apolar alanine versus valine in position 113. The pronounced effects of these amino acid replacements on catalytic activity of the hGSTP1-1 protein is somewhat surprising. Recently, we have solved the crystal structures of the hGSTP1-1(I104,A113) and hGSTP1-1(V104,A113) variants in complex with GSH conjugate of (+)-*anti*-BPDE (GSBpd) at 2.1 and 2.0 Å resolution, respectively (38). Our studies reveal that the binding mode of the GSBpd is significantly different in hGSTP1-1(I104,A113) compared with hGSTP1-1(V104,A113). For example, the distance between the hydroxyl group

of Y7 and the sulfur atom of the GSBpd is markedly higher in hGSTP1-1(I104,A113) · GSBpd complex (5.9 Å) than in hGSTP1-1(V104,A113) · GSBpd structure (3.2 Å). Second, one of the hydroxyl groups of GSBpd forms a direct hydrogen bond with R13 in hGSTP1-1(V104,A113) · GSBpd complex; in contrast, this hydrogen bond is not observed in the I104 complex (38). In addition, two of the five water molecules in the hydrophilic portion of the H-site are displaced by the GSBpd in V104,A113 complex (38). Even though it is possible that some or all of these interactions may contribute to catalytic differences between hGSTP1-1(I104,A113) and hGSTP1-1(V104,A113) toward chlorambucil and thiotepa, actual crystal structures of allelic variants of hGSTP1-1 in complex with GSH conjugates of chlorambucil and thiotepa are needed to validate this assumption. Furthermore, structural studies with hGSTP1-1(I104,V113) and hGSTP1-1(V104,V113) are also needed to determine the role of residue 113 in the activity of hGSTP1-1 toward alkylating agents.

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REFERENCES

1. Mattern, J., and Volm, M. (1993) Multiple pathway drug resistance (review). *Int. J. Oncol.* **2,** 557–561.
2. Colvin, O. M. (1994) Mechanisms of resistance to alkylating agents. *Cancer Treat. Res.* **73,** 249–262.
3. O'Brien, M. L., and Tew, K. D. (1996) Glutathione and related enzymes in multidrug resistance. *Eur. J. Cancer* **32A,** 967–978.
4. Zhang, K., Mack, P., and Wong, K. P. (1998) Glutathione-related mechanisms in cellular resistance to anticancer drugs (review). *Int. J. Oncol.* **12,** 871–882.
5. Mannervik, B. (1985) The isoenzymes of glutathione transferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **57,** 357–417.
6. Hayes, J. D., and Pulford, D. J. (1995) The glutathione *S*-transferase supergene family: regulation of GST* and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* **30,** 445–600.
7. Ciaccio, P. J., Tew, K. D., and LaCreta, F. P. (1991) Enzymatic conjugation of chlorambucil with glutathione by human glutathione *S*-transferases and inhibition by ethacrynic acid. *Biochem. Pharmacol.* **42,** 1504–1507.
8. Dirven, H. A. A. M., Dictus, E. L. J. T., Broeders, N. L. H. L., van Ommen, B., and van Bladeren, P. J. (1995) The role of human glutathione *S*-transferase isoenzymes in the formation of glutathione conjugates of the alkylating cytostatic drug thiotepa. *Cancer Res.* **55,** 1701–1706.
9. Dirven, H. A. A. M., van Ommen, B., and van Bladeren, P. J. (1994) Involvement of human glutathione *S*-transferase isoenzymes in the conjugation of cyclophosphamide metabolites with glutathione. *Cancer Res.* **54,** 6215–6220.
10. Dirven, H. A. A. M., Megens, L., Oudshoorn, M. J., Dingemanse, M. A., van Ommen, B., and van Bladeren, P. J. (1995) Glutathione conjugation of the cytostatic drug ifosfamide and the role of human glutathione *S*-transferases. *Chem. Res. Toxicol.* **8,** 979–986.
11. Wang, A. L., and Tew, K. D. (1985) Increased glutathione-*S*-transferase activity in a cell line with acquired resistance to nitrogen mustards. *Cancer Treat. Rep.* **69,** 677–682.
12. Tanner, B., Hengstler, J. G., Dietrich, B., Henrich, M., Steinberg, P., Weikel, W., Meinert, R., Kaina, B., Oesch, F., and Knapstein, P. G. (1997) Glutathione, glutathione *S*-transferase alpha and pi, and aldehyde dehydrogenase content in relationship to drug resistance in ovarian cancer. *Gynecol. Oncol.* **65,** 54–62.
13. Gupta, V., Singh, S. V., Ahmad, H., Medh, R. D., and Awasthi, Y. C. (1989) Glutathione and glutathione *S*-transferases in a human plasma cell line resistant to melphalan. *Biochem. Pharmacol.* **38,** 1993–2000.
14. Green, J. A., Vistica, D. T., Young, R. C., Hamilton, T. C., Rogan, A. M., and Ozols, R. F. (1984) Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion. *Cancer Res.* **44,** 5427–5431.
15. Tew, K. D., Bomber, A. M., and Hoffman, S. J. (1988) Ethacrynic acid and piriprost as enhancers of cytotoxicity in drug resistant and sensitive cell lines. *Cancer Res.* **48,** 3622–3625.
16. Hansson, J., Berhane, K., Castro, V. M., Jungnelius, U., Mannervik, B., and Ringborg, U. (1991) Sensitization of human melanoma cells to the cytotoxic effect of melphalan by the glutathione transferase inhibitor ethacrynic acid. *Cancer Res.* **51,** 94–98.
17. Puchalski, R. B., and Fahl, W. E. (1990) Expression of recombinant glutathione *S*-transferase π, Ya, or Ybl confers resistance to alkylating agents. *Proc. Natl. Acad. Sci. USA* **87,** 2443–2447.
18. Nakagawa, K., Saijo, N., Tsuchida, S., Sakai, M., Tsunokawa, Y., Yokota, J., Muramatsu, M., Sato, K., Terada, M., and Tew, K. D. (1990) Glutathione *S*-transferase π as a determinant of drug resistance in transfectant cell lines. *J. Biol. Chem.* **265,** 4296–4301.
19. Manoharan, T. H., Welch, P. J., Gulick, A. M., Puchalski, R. B., Lathrop, A. L., and Fahl, W. E. (1991) Expression of tandem glutathione *S*-transferase recombinant genes in COS cells for analysis of efficiency of protein expression and associated drug resistance. *Mol. Pharmacol.* **39,** 461–467.
20. Mannervik, B., Ålin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M., and Jörnvall, H. (1985) Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. *Proc. Natl. Acad. Sci. USA* **82,** 7202–7206.
21. Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M., and Ketterer, B. (1991) Theta, a new class of glutathione transferases purified from rat and man. *Biochem. J.* **274,** 409–414.
22. Ahmad, H., Wilson, D. E., Fritz, R. R., Singh, S. V., Medh, R. D., Nagle, G. T., Awasthi, Y. C., and Kurosky, A. (1990) Primary and secondary structural analyses of glutathione *S*-transferase π from human placenta. *Arch. Biochem. Biophys.* **278,** 398–408.
23. Board, P. G., Webb, G. C., and Coggan, M. (1989) Isolation of a cDNA clone and localization of the human glutathione S-transferase 3 genes to chromosome bands 11q13 and 12q13-14. *Ann. Hum. Genet.* **53,** 205–213.
24. Ali-Osman, F., Akande, O., Antoun, G., Mao, J-X., and Buolamwini, J. (1997) Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNAs of three human glutathione S-transferase Pi gene variants: Evidence for differential catalytic activity of the encoded proteins. *J. Biol. Chem.* **272,** 10004–10012.
25. Harries, L. W., Stubbins, M. J., Forman, D., Howard, G. C. W., and Wolf, C. R. (1997) Identification of genetic polymorphisms at

the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* **18,** 641–644.

26. Watson, M. A., Stewart, R. K., Smith, G. B. J., Massey, T. E., and Bell, D. A. (1998) Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* **19,** 275–280.
27. Helzlsouer, K. J., Selmin, O., Huang, H-Y., Strickland, P. T., Hoffman, S., Alberg, A. J., Watson, M., Comstock, G. W., and Bell, D. (1998) Association between glutathione *S*-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. *J. Natl. Cancer Inst.* **90,** 512–518.
28. Hu, X., Xia, H., Srivastava, S. K., Herzog, C., Awasthi, Y. C., Ji, X., Zimniak, P., and Singh, S. V. (1997) Activity of four allelic forms of glutathione S-transferase hGSTP1-1 for diol epoxides of polycyclic aromatic hydrocarbons. *Biochem. Biophys. Res. Commun.* **238,** 397–402.
29. Simons, P. C., and Vander Jagt, D. L. (1977) Purification of glutathione S-transferases from human liver by glutathione-affinity chromatography. *Anal. Biochem.* **82,** 334–341.
30. Singh, S. V., Leal, T., Ansari, G. A. S., and Awasthi, Y. C. (1987) Purification and characterization of glutathione S-transferases of human kidney. *Biochem. J.* **246,** 179–186.
31. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.
32. Horton, J. K., Roy, G., Piper, J. T., van Houten, B., Awasthi, Y. C., Mitra, S., Moulay, A., Alaoui-Jamali, M. A., Boldogh, I., and Singhal, S. S. (1999) Characterization of a chlorambucil-resistant human ovarian carcinoma cell line overexpressing glutathione *S*-transferase μ. *Biochem. Pharmacol.* **58,** 693–702.
33. Srivastava, S. K., Singhal, S. S., Hu, X., Awasthi, Y. C., Zimniak, P., and Singh, S. V. (1999) Differential catalytic efficiency of allelic variants of human glutathione *S*-transferase Pi in catalyzing the glutathione conjugation of thiotepa. *Arch. Biochem. Biophys.* **366,** 89–94.
34. Hu, X., Ji, X., Srivastava, S. K., Xia, H., Awasthi, S., Nanduri, B., Awasthi, Y. C., Zimniak, P., and Singh, S. V. (1997) Mechanism of differential catalytic efficiency of two polymorphic forms of human glutathione *S*-transferase P1-1 in the glutathione conjugation of carcinogenic diol epoxide of chrysene. *Arch. Biochem. Biophys.* **345,** 32–38.
35. Hu, X., Pal, A., Krzeminski, J., Amin, S., Awasthi, Y. C., Zimniak, P., and Singh, S. V. (1998) Specificities of human glutathione *S*-transferase isozymes toward *anti*-diol epoxides of methylchrysenes. *Carcinogenesis* **19,** 1685–1689.
36. Hu, X., Xia, H., Srivastava, S. K., Pal, A., Awasthi, Y. C., Zimniak, P., and Singh, S. V. (1998) Catalytic efficiencies of allelic variants of human glutathione *S*-transferase P1-1 toward carcinogenic *anti*-diol epoxides of benzo[c]phenanthrene and benzo[g]chrysene. *Cancer Res.* **58,** 5340–5343.
37. Buening, M. K., Wislocki, P. G., Levin, W., Yagi, H., Thakker, D. R., Akagi, H., Koreeda, M., Jerina, D. M., and Conney, A. H. (1978) Tumorigenicity of the optical enantiomers of the diastereomeric benzo[a]pyrene 7,8-diol-9,10-epoxides in newborn mice: Exceptional activity of (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. *Proc. Natl. Acad. Sci. USA* **75,** 5358–5361.
38. Ji, X., Blaszczyk, J., Xiao, B., O'Donnell, R., Hu, X., Herzog, C., Singh, S. V., and Zimniak, P. (1999) Structure and function of residue 104 and water molecules in the xenobiotic substrate-binding site in human glutathione *S*-transferase P1-1. *Biochemistry* **38,** 10231–10238.