Generation and Functional Characterization of Mice with a Disrupted Glutathione S-Transferase, Theta 1 Gene

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ABSTRACT:

Glutathione S-transferase (GST) theta 1 (GSTT1) has been regarded as one of the key enzymes involved in phase II reactions because of its unique substrate specificity. In this study, we generated mice with the disrupted Gstt1 gene (Gstt1-null mice) by gene targeting and analyzed the metabolic properties in cytotoxic and in vivo studies. The resulting Gstt1-null mice failed to express the Gstt1 mRNA and GSTT1 protein by reverse transcriptase-polymerase chain reaction analysis and two-dimensional fluorescence difference gel electrophoresis/mass spectrometry analysis, respectively. However, the Gstt1-null mice appeared to be normal and were fertile. In an enzymatic study using cytosolic samples from the liver and kidney, GST activity toward 1,2-epoxy-3-(p-nitrophenoxy)propane (EPNP), dichloro-methane (DCM), and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was markedly lower in Gstt1-null mice than in the wild-type controls, despite there being no difference in GST activity toward 1-chloro-2,4-dinitrobenzene between Gstt1-null mice and the wild-type controls. Gstt1-null mice had GST activity of only 8.7 to 42.1% of the wild-type controls to EPNP, less than 2.2% of the wild-type controls to DCM, and 13.2 to 23.9% of the wild-type controls to BCNU. Plasma BCNU concentrations after a single i.p. administration of BCNU to Gstt1-null mice were significantly higher, and there was a larger area under the curves 60 min. (male, 2.38 times; female, 2.28 times, versus the wild-type controls) based on the results. In conclusion, Gstt1-null mice would be useful as an animal model of humans with the GSTT1-null genotype.

Glutathione S-transferases (GSTs) form a superfamily that is characterized by catalysis of the conjugation of glutathione (GSH) with various electrophilic compounds. At least seven distinct classes (alpha, mu, pi, theta, zeta, omega, and sigma) of soluble GSTs have been identified so far, according to substrate specificity, chemical affinity, structure, and the kinetic behavior of the enzyme (Mannervik et al., 1985; Meyer et al., 1991; Board et al., 1997, 2000; Jowsey et al., 2001). Theta class GSTs are distinguished from other classes by their failure to bind to immobilized GSH affinity matrices and their negligible activity toward 1-chloro-2,4-dinitrobenzene (CDNB), which is the substrate of various GSTs (Meyer et al., 1984, 1991). There is a genetic polymorphism of the null genotype in the human GSTT1 gene, with about 15% of Caucasians and 60% of Asians lacking GSTT1 activity (Nelson et al., 1995; Chen et al., 1996). The relationship between the GSTT1-null genotype and the incidence of cancer has been investigated extensively to detect GSTT1-associated differential susceptibility toward carcinogens. Many epidemiologic investigations have shown that the GSTT1-null genotype is related to a slightly increased risk of cancer of the bladder and gastrointestinal tract and smoking-related cancer of the lung or oral cavity (Landi, 2000). However, there is no direct evidence yet that the GSTT1-null genotype causes carcinogenesis.

The alkylating drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) is used to treat brain tumors, multiple myeloma, Hodgkin’s disease, and non-Hodgkin’s lymphomas (Wasserman et al., 1975). BCNU is thought to exert the antitumor effect by the formation of a chloroethyl-adduct at the O6 position of guanine bases in the DNA (Bodell et al., 1984). The critical problem in BCNU treatment is the acquisition of BCNU resistance, which is implicated in many mechanisms in the tumor cells. The major resistant factors are O6-alkylguanine-DNA-alkyltransferase, which repairs DNA damage by removing the alkyl groups from the O6 position of guanine, and GSTs as a BCNU-detoxifying enzyme (Bodell et al., 1986; Smith et al., 1989). It has been reported that human GSTM2, GSTM3, and GSTT1 have BCNU denitrosation activity (Lien et al., 2002). In particular, GSTT1 displayed a much higher activity toward BCNU. The existence of a GSTT1-null genotype may influence both the sensitivity of tumors to BCNU and the severity of adverse side effects of BCNU in patients. Therefore, it is important to study the metabolic properties to BCNU in GSTT1-null individuals. However, there is no report about it yet.

ABBREVIATIONS: GST, glutathione S-transferase; GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; GSTT1, glutathione S-transferase \( \theta \) 1; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; EPNP, 1,2-epoxy-3-(p-nitrophenoxy)propane; DCM, dichloromethane; Neo', neomycin-resistant; PCR, polymerase chain reaction; RT, reverse transcriptase; DIGE, with fluorescence difference gel electrophoresis; IEF, isoelectric focusing; 2D, two-dimensional; TFA, trifluoroacetic acid; MALDI, matrix-assisted laser desorption ionization; TOF, time of flight; MS, mass spectrometry; AUC, area under the curve.
In this study, we generated mice with a disrupted Gstt1 gene (Gstt1-null mice) and analyzed the metabolic activity toward the specific substrates 1,2-epoxy-3-(p-nitrophenoxy)propene (EPNP), DCM, and BCNU. Furthermore, we measured the plasma BCNU concentrations after a single i.p. administration of BCNU to evaluate the metabolic properties of Gstt1 to the specific substrates.

Materials and Methods

Generation of Gstt1-Null Mice. To construct the targeting vector, DNA from a W9.5 ES cell was used to amplify the Gstt1 gene fragments for both the 5'- and 3'-arms. For the 5'-arm, a 4.9-kb 5'-flanking sequence of the Gstt1 gene was cloned into the HpaI and XhoI sites of the pKO Scrambler V901 plasmid. For the 3'-arm, a 2.5-kb 3'-flanking sequence of the Gstt1 gene was cloned into the SacII and SalI sites. The primers for the 5'-arm were 5'-ACACCCGGGCGGACTGCTTCTAGCAGT-3' and 5'-CCCTCGTGAAGCTTAATGACGCTTGCTACCATCTC-3'. The primers for the 3'-arm were 5'-AGACCGGCCGGAAGGAGTGAGCCTAGTGAATCTGCTC-3' and reverse, 5'-AGACCGGCCGGAAGGAGTGAGCCTAGTGAATCTGCTC-3'.

The primers for the mouse Gstt1 gene were cloned into the HpaI and XhoI sites of the pKO SelectDT V840 plasmid were used. All the plasmids were supplied by pKO SelectNeo V800 plasmid and the Diphtheria Toxin A chain gene (DT) from pKO SelectDT V840 plasmid were used. All the plasmids were supplied by Takara Bio. (Otsu, Japan). W9.5 ES cells (10^6) were electroporated (250 V, 500 F) with 20 μg of linearized targeting vector and selected with 250 μg (active form)/ml G418 for 8 to 10 days (Fujimoto et al., 2006). G418-resistant clones were primarily screened on the 3'-arm for PCR with the primer pair: NeoF3, 5'-CTCTGCTAAAGCGCAGCTGCAGCTGGTTG-3' and NeoR1, 5'-ACATTTTCCGGTCTTACCTTATGCCACAGGG-3'. For the positive negative selection, a neomycin-resistant (Neo+) cassette from the pKO SelectNeo V800 plasmid was homogenized with fluorescence difference gel electrophoresis (FDGE) and subjected to isoelectric focusing (IEF) and SDS-polyacrylamide gel electrophoresis in the above-mentioned manner. After migration, the gels were treated with SYPRO Ruby dye (Invitrogen), and the protein images were visualized. The significant spots were excised from the gels using an Etan-spot picker (GE Healthcare). The gel pieces were washed twice with 50% (v/v) methanol containing 50 mM ammonium bicarbonate for 20 min, dehydrated with 75% (v/v) acetonitrile, and dried completely in a vacuum centrifuge. The proteins were digested overnight at 37°C with sequencing grade modified trypsin solution (Promega, Madison, WI; 10 ng/μl in 20 mM ammonium bicarbonate). After digestion, the peptides in each gel piece were eluted sequentially with 50% (v/v) acetonitrile for the normalization of spot abundance. The Cy2-, Cy3-, and Cy5-labeled protein samples were mixed with 1% (v/v) trifluoroacetic acid (TFA), 50% (v/v) acetonitrile with 0.2% (v/v) TFA, and subjected to protein identification. Protein extracts (500 μg) were separated by IEF and SDS-polyacrylamide gel electrophoresis in the above-mentioned manner. After migration, the gels were treated with SYPRO Ruby dye (Invitrogen), and the protein images were visualized. The significant spots were excised from the gels using an Etan-spot picker (GE Healthcare). The gel pieces were washed twice with 50% (v/v) methanol containing 50 mM ammonium bicarbonate for 20 min, dehydrated with 75% (v/v) acetonitrile, and dried completely in a vacuum centrifuge. The proteins were digested overnight at 37°C with sequencing grade modified trypsin solution (Promega, Madison, WI; 10 ng/μl in 20 mM ammonium bicarbonate). After digestion, the peptides in each gel piece were eluted sequentially with 50% (v/v) acetonitrile with 1% (v/v) trifluoroacetic acid (TFA), 50% (v/v) acetonitrile with 0.2% (v/v) TFA, and 100% acetonitrile with sonication. The samples were concentrated with a vacuum centrifuge and dried after desalination with C18 ZipTip pipette tips (Millipore Corporation, Billerica, MA). The peptides were resuspended in 1 μl of α-cyano-4-hydroxy cinnamic acid-saturated matrix solution containing 0.5% (v/v) TFA and 50% (v/v) acetonitrile. The 0.5-μl peptide solution was transferred onto the MALDI target and dried. Mass spectrometry analysis was performed using an Ettan MALDI-TOF software version 1.11 (GE Healthcare).

For protein identification, the obtained mass information was searched against the National Center for Biotechnology Information nonredundant database using Ettan MALDI-TOF software version 1.11 (GE Healthcare).

Measurement of GST Activities toward CDNB, EPNP, DCM, and BCNU in the Cytosols of the Liver and Kidney. The liver and kidney samples of the Gstt1-null mice, the heterozygotes, and the wild-type controls were mixed with 0.154 M potassium chloride and homogenized in an ice bath. The homogenates were centrifuged at 9000 g for 20 min at 4°C, and the supernatant fraction was further centrifuged at 105,000 g for 1 h at 4°C to isolate the cytosolic fractions. The protein concentrations in the cytosolic fractions were determined by the method of Lowry et al. (1951).

The GST activity toward CDNB (GST-CDNB activity) and EPNP (GSTM-EPNP activity) was measured according to the method of Habig et al. (1974). In the measurement of GST-CDNB activity, the cytosols were diluted 30-fold with 0.154 M potassium chloride, and 0.3 ml of 20 μM GSH and 0.06 μl of the diluted cytosols were mixed in 5.34 ml of 100 mM potassium phosphate buffer, pH 6.5. After the addition of CDNB at a final concentration of 1 mM, the change in absorbance at 340 nm was measured for 1 min with a spectrophotometer. In the measurement of GST-EPNP activity, 0.3 ml of 100 mM GSH and 0.2 ml of the cytosols were mixed in 5.2 ml of 100 mM potassium phosphate buffer, pH 6.5. After the addition of EPNP solution (10 mM in
ethanol) at a final concentration of 0.5 mM, the change in absorbance at 360 nm was measured for 2 min with a spectrophotometer. The GST-CDNB and GST-EPNP activities were expressed as the amount of CDNB-GSH and EPNP-GSH conjugates formed per unit weight of protein per unit of time (nanomoles per minute per milligram of protein), respectively.

The GST activity toward DCM (GST-DCM activity) and BCNU (GST-BCNU activity) was measured according to the method of Nash (1953) and Talcott and Levin (1983), respectively. Briefly, the cytosols of the liver and kidney were diluted 5- and 2-fold with 0.154 M potassium chloride, respectively. Briefly, the cytosols of the liver and kidney were diluted 5- and 2-fold with 0.154 M potassium chloride, respectively. In the measurement of GST-DCM activity, 0.5 ml of reaction buffer (10 mM GSH, 30 mM Tris/HCl, pH 7.4) was mixed with 0.1 ml of the diluted cytosols and 2 μl of DCM. After incubation for 30 min at 37°C, this reaction was mixed with 0.2 ml of 20% trichloroacetic acid solution and centrifuged for 2 min at 16,000 x g. An amount of 0.1 ml of the supernatant was mixed with 0.1 ml of Nash reagent (15 g of ammonium acetate, 0.2 ml of acetylacetone, 0.3 ml of acetic acid brought to a volume of 100 ml with distilled water) and incubated at 37°C. After 60 min, the absorption at 412 nm was measured using a spectrophotometer. The GST-DCM activity was expressed as the amount of formaldehyde moles formed per unit weight of protein per unit of time (nanomoles per minute per milligram of protein).

Measurement of Plasma BCNU Concentration after a Single i.p. Administration of BCNU. Gstt1-null mice and the wild-type controls were treated via a single i.p. administration with 20 mg/ml BCNU dissolved in 5% ethanol/saline solution at dose levels of 20 mg/kg. Approximately 0.5 ml of blood was collected at 5, 15, 30, and 60 min postdose. The blood samples were centrifuged at 10,000 rpm for 2 min at room temperature to prepare the plasma samples. Then, 10 μl of plasma sample was placed in disposable glass tubes, and 465 μl of 0.1% acetic acid in water was added and vortexed. After adding 25 μl of ethanol, the solution was applied to 1 ml of Oasis HLB solid phase extraction cartridge (Waters, Milford, MA). The cartridge was washed previously with 1 ml of acetonitrile followed by 1 ml of water. After the sample was loaded, the cartridge was washed with 1 ml of 0.1% acetic acid in water and then eluted with 0.5 ml of 0.1% acetic acid in acetonitrile. The eluted sample was analyzed on a C18 reverse-phase column (Sunfire; 3.5 μm, 2.1 x 50 mm; Waters) using a step gradient of 95% B until 6 min followed by a step gradient of 5% B until 12 min at a flow rate of 0.2 ml/min. The column effluent was directed into the electrospray ionization source of a QTRAP mass spectrometer (MD Sciex, Toronto, ON, Canada). The electrospray ionization conditions were: assist gas flow rate, 85 l/min; evaporation gas, 85 l/min; ionization voltage, −4.5 kV; evaporation temperature, 300°C; field voltage, −0.3 V; and measurement of GST-BCNU activity, 0.65 ml of 0.1 M phosphate buffer, pH 7.4, with 5 mM GSH was mixed with 0.1 ml of the diluted cytosols and 7.5 μl of 200 mM BCNU. After the incubation for 20 min at 37°C, this reaction was mixed with 0.75 ml of chloroform. After 5 min of centrifugation at 21,500 g, 0.5 ml of the aqueous phase was extracted with 0.75 ml of chloroform again. After the second extraction, 0.2 ml of the aqueous phase was mixed with 0.4 ml of 50 mM sulfanilamide and 0.5 mM N-(1-naphthyl)ethylenediamine dihydrochloride dissolved in 3 M HCl and incubated at 55°C. After 20 min, the absorption at 540 nm was measured using a spectrophotometer. The GST-BCNU activity was expressed as the amount of nitrite moles formed per unit weight of protein per unit of time (nanomoles per minute per milligram of protein).

Fig. 1. Targeted disruption of the mouse Gstt1 gene. A, targeting vector construct (top), the wild allele of the Gstt1 gene (middle), and the predicted mutant allele (bottom) are shown. The targeting vector was constructed by replacing exon 1 (E1) and exon 2 (E2) of the Gstt1 gene with a neomycin-resistant (Neor) cassette. The Diphtheria toxin A chain gene (DT) fragment was ligated at the 5′-end of the vector for negative selection. The mutant allele was detectable by PCR using the indicated primer sets, GT43/NP3 and NeoF3/T1R1, which confirmed the homologous recombination on the 5′ and 3′ arms, respectively. B, PCR analyses of the homologous combination on the 5′ arm (left). NeoF3/T1R1 primer set was amplified by a 5.1-kb fragment contained in the 5′ arm on the mutant allele (left). NeoF3/T1R1 primer set was amplified by a 2.7-kb fragment contained in the 3′ arm on the mutant allele (right). λ/HindIII digest was used as a molecular size marker. WT-ES, intact ES cell. 9D5-ES, the homologous recombinant ES cell clone. WT-F1, F1 pup with the wild allele. Hetero-F1, F1 heterozygote with the mutant allele.

Fig. 2. PCR genotyping and RT-PCR analysis of Gstt1-null mice. A, genotype was determined by PCR using two forward primers distinctive between the wild allele and the mutant allele and one common reverse primer. The wild allele and the mutant allele indicated 196- and 154-bp fragments, respectively. B, RT-PCR was performed using the primers specific for the mouse Gstt1 gene and β-actin gene. The Gstt1 and β-actin mRNA expression indicated 270- and 323-bp fragments, respectively. The amplified fragments were confirmed by direct sequence using the ABI PRISM 3700 DNA Analyzer. M, φ X174/HindII digest as a molecular size marker; Homo, homozygote (Gstt1-null mice); Hetero, heterozygote; Wild, wild-type control.
collision energy, −6 V. The data were acquired in negative ion mode using Analyst software (MDS Sciex). BCNU was detected as ion pairs at m/z 271.9/211.9.

**Statistical Analysis.** All data were analyzed by an F test to evaluate the homogeneity of variance. If the variance was homogeneous, a Student’s t test was applied. If the variance was heterogeneous, an Aspin-Welch’s t test was performed. The value of P < 0.05 was chosen as an indication of statistical significance except hepatic protein expression analysis. Microsoft Excel (Microsoft, Redmond, WA) was used for statistical analysis.

**Results**

**Generation of Gstt1-Null Mice.** Mice with a disrupted Gstt1 gene were generated by a homologous recombination method with mouse ES cell. The targeting vector used in this study is shown in Fig. 1A. The mouse Gstt1 gene consists of five exons in a region of about 5 kb. This targeting vector was designed to achieve the deletion of exons 1 and 2 of the Gstt1 gene by replacing them with a Neo cassette. W9.5 ES cells were electroporated with the targeting vector, and 306 of the colonies with G418 resistance were selected and screened by PCR for homologous recombination. Six independent PCR-positive clones, 1A3, 2A2, 3C6, 9D5, 11D4, and 12B6, were obtained by primary PCR screening with a GT43/NP3 primer pair. However, secondary PCR screening with a NeoF3/T1R1 primer pair. Chimera mice were generated using the 9D5 ES cell clone with near-diploid karyotype. The F1 offspring were confirmed to succeed to the homologous recombinant allele (Fig. 1B). The F1 heterozygous mice were intercrossed to produce F2 mice, which were determined to be wild type, heterozygote, or homozygote (Gstt1-null mice) by PCR genotyping (Fig. 2). Furthermore, RT-PCR analysis showed Gstt1 mRNA expression was not detected in the livers of Gstt1-null mice (Fig. 2). Gstt1-null mice appeared to be normal and were as fertile as the wild-type and heterozygote mice. No obvious histological, hematological, and blood chemical differences in the basal condition were detected between Gstt1-null mice and the wild-type controls (data not shown).

**Hepatic Protein Expression Analysis by 2D-DIGE/Mass Spectrometry.** Hepatic protein expression analysis by 2D-DIGE/mass spectrometry (MS) is summarized in Table 1. The expression levels of the 3, 16, and 4 proteins were significantly different between Gstt1-null mice and the heterozygotes, the Gstt1-null mice, and the wild-type control and the heterozygotes and the wild-type controls, respectively. Among them, the expression levels of the two proteins were significantly different between all the genotypes. These proteins were identified as GSTT1 and albumin 1 by MALDI-TOF MS. In particular, GSTT1 protein was absent in Gstt1-null mice (Fig. 3). Although we succeeded in the identification of 13 proteins among the 16 proteins that showed significant differences in the expression level between Gstt1-null mice and the wild-type controls, the other GSTs were not among them.

**GST Activities toward CDNB, EPNP, DCM, and BCNU in the Cytosols of the Liver and Kidney.** We measured the GST-CDNB, EPNP, DCM, and BCNU in the cytosols of the liver and kidney.
GST-EPNP, GST-DCM, and GST-BCNU activity in cytosolic samples from the livers and kidneys of \textit{Gstt1}-null mice. The results showed significantly low activity of GST-EPNP, GST-DCM, and GST-BCNU in \textit{Gstt1}-null mice, despite the same level of GST-CDNB activity in all the genotypes (Fig. 4). The GST-EPNP activity of \textit{Gstt1}-null mice was low in the livers of the males (42.1% of the wild-type control) and markedly low in the livers of the females (8.7% of the wild-type control) and in the kidneys of both sexes (male, 27.7%; female, 18.3% of the wild-type control). The GST-DCM activity of \textit{Gstt1}-null mice was nearly absent in the livers and kidneys of both sexes. The GST-BCNU activity of \textit{Gstt1}-null mice was 13.2 to 23.9% of the wild-type control in the livers and kidneys of both sexes. The heterozygotes showed intermediate activity of GST-EPNP, GST-DCM, and GST-BCNU between that of the homozygotes and wild-type controls, except for the GST-EPNP and GST-BCNU activity of the female kidney.

**Plasma BCNU Concentration after Single i.p. Administration of BCNU.** We measured the plasma concentrations of BCNU after a single i.p. administration of BCNU (20 mg/kg) to \textit{Gstt1}-null mice and the wild-type controls by liquid chromatography-tandem mass spec-

![Liver](image1.png) ![Kidney](image2.png)
Expression analysis by RT-PCR and 2D-DIGE/MS showed that analyses were used to generate mice with a disrupted mutant allele, confirmed by genomic PCR and direct sequencing substrates. Homologous recombinant ES clones with the predicted combination to evaluate the metabolic properties of GSTT1 to the specific Gstt1-null mice that failed to express Gstt1-null mice. These results indicated success in the generation of Gstt1 protein.

Furthermore, we succeeded in the identification of 13 proteins among the 16 proteins that showed significant differences in the expression level between Gstt1-null mice and the wild-type controls. Although four proteins among them (protein disulfide isomerase-related protein, aflatoxin B1 aldehyde reductase 1, carbonic anhydrase 3, and glutathione peroxidise) were reported to cause an increase in the expression levels by oxidative stress (Kyang et al., 2003; Ellis et al., 2003; Engle et al., 2004; Yamamoto et al., 2006), we observed that the expression levels of aflatoxin B1 aldehyde reductase 1 and carbonic anhydrase 3 were decreased in Gstt1-null mice. Therefore, these results suggest that GSTT1-deficiency does not necessarily cause an increase in oxidative stress under basal conditions.

In the cytosolic study, GST-CDNB activity, a general GST activity, in the liver and kidney was not different between the Gstt1-null mice and the wild-type control. This result was consistent with the low GST-CDNB activity of mouse recombinant GSTT1 protein (Whittington et al., 1999). Furthermore, we also observed that P450 activity, assessed by measurement of 7-alkoxycoumarin O-dealkylase activity using a microsomal fraction, was not different between the Gstt1-null mice and the wild-type controls (data not shown). The GST-EPNP activity of the Gstt1-null mice was low in the liver and kidney of both sexes. However, the remaining GST-EPNP activity of the Gstt1-null mice ranged from 8.7 to 42.1% in the liver and from 18.3 to 27.7% in the kidney. This result suggests that EPNP is metabolized by not only GSTT1 but also by other xenobiotic metabolizing enzymes. Actually, it has been reported that EPNP is also metabolized by GSTA3, GSTA4, GSTM1, GSTM4, GSTP1, and GSTT3 (Jowsey et al., 2003).

The expression level of GSTP1 in the liver was observed to be higher in males than in females, whereas that in the kidney was not different between both sexes (Mitchell et al., 1997). We suppose that GSTP1 may contribute to the higher remaining GST-EPNP activity in the male liver. On the other hand, the GST-DCM activity was nearly absent in the liver and kidney of both sexes in the Gstt1-null mice. Similar to Gstt1-null mice, humans with the GST-null genotype also showed loss of GST-DCM activity in the cytosol of the liver and kidney (Thier et al., 1998). These results suggest that DCM is a highly specific substrate to GSTT1 from mice to humans.

The GST-BCNU activity of Gstt1-null mice was markedly low in the liver and kidney of both sexes. In humans, GSTT1, GSTM2, and GSTM3 have GST-BCNU activity, and GSTT1 was demonstrated to have 14-fold higher GST-BCNU activity than GSTM2 and GSTM3 (Lien et al., 2002). In this study, we also observed that GSTT1 was the most efficient catalyst in the liver and kidney cytosols of mice. The remaining GST-BCNU activity of Gstt1-null mice ranged from 13.2
to 19.9% in the liver and from 21.8 to 23.9% in the kidney. We
suppose that the other GSTs contribute to the remaining GST-BCNU
activity, which may be mouse counterparts of human GSTM2 and
GSTM3. Furthermore, we measured the plasma BCNU concentrations
after a single i.p. administration of BCNU (20 mg/kg) to Gstt1-null
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to evaluate the pharmacokinetics of BCNU in the whole body. This
result showed that plasma BCNU concentrations after a single i.p.
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than those of the wild-type controls and that there was a larger
AUC_{max} (male, 2.30 times; female, 2.28 times versus the wild-type
controls) based on these results. Considering these results, Gstt1-null
mice administered a specific substrate for GSTT1, such as BCNU, are
highly exposed and toxicologically susceptible to the substrate.
In humans, GSTT1 is expressed in the brain, a clinical target for BCNU
treatment (Juroen et al., 1996; Sherratt et al., 1997). Therefore,
the existence of a GSTT1-null genotype may influence both the sensitivity
of tumors to BCNU and the severity of the adverse side-effects of
BCNU in patients.

In conclusion, we generated knockout mice for the Gstt1 gene and
found that GST-EPNP, GST-DCM, and GST-BCNU activity in the
liver and kidney cytosols markedly decreased in Gstt1-null mice and
that a single i.p. administration of BCNU to Gstt1-null mice resulted in
larger AUC_{5–60 min} for the plasma BCNU concentration. Finally,
we concluded that Gstt1-null mice would be useful as a toxicokineti-
cally modified animal model, i.e., an animal model of a poor metabo-
lizer to the specific substrates for GSTT1, such as an individual with
a GSTT1-null genotype. Although the activity, expression level,
and distribution of GSTT1 are markedly different between humans and mice (Mainwaring et al., 1996; Thier et al., 1998), we believe that
Gstt1-null mice would offer great advantages in determining the roles
of GSTT1 in physiological homeostasis, drug metabolism, and cancer
susceptibility.

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