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Relationships between alterations in glutathione metabolism and the disposition of inorganic mercury in rats: effects of biliary ligation and chemically induced modulation of glutathione status

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Abstract

Influences of biliary ligation and systemic depletion of glutathione (GSH) or modulation of GSH status on the disposition of a low, non-nephrotoxic i.v. dose of inorganic mercury were evaluated in rats in the present study. Renal and hepatic disposition, and the urinary and fecal excretion, of inorganic mercury were assessed 24 h after the injection of a 0.5-μmol/kg dose of mercuric chloride in control rats and rats pretreated with acivicin (two 10-mg/kg i.p. doses in 2 ml/kg normal saline, 90 min apart, 60 min before mercuric chloride), buthionine sulfoximine (BSO; 2 mmol/kg i.v. in 4 ml/kg normal saline, 2 h before mercuric chloride) or diethylmaleate (DEM; 3.37 mmol/kg i.p. in 2 ml/kg corn oil, 2 h before mercuric chloride) that either underwent or did not undergo acute biliary ligation prior to the injection of mercury. Among the groups that did not undergo biliary ligation, the pretreatments used to alter GSH status systemically had varying effects on the disposition of inorganic mercury in the kidneys, liver, and blood. Biliary ligation caused the net renal accumulation of mercury to decrease under all pretreatment conditions. By contrast, biliary ligation caused significant increases in the hepatic burden of mercury in all pretreatment groups except in the

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acivicin-pretreated group. Blood levels of mercury also increased as a result of biliary ligation, regardless of the type of pretreatment used. The present findings indicate that biliary ligation combined with methods used to modulate GSH status systemically have additive effects with respect to causing reductions in the net renal accumulation of mercury. Additionally, the findings indicate that at least some fraction of the renal accumulation of inorganic mercury is linked mechanistically to the hepato-biliary system.

Keywords: Glutathione depletion; Biliary ligation; Accumulation and excretion of mercury; Kidneys; Liver; Rats

1. Introduction

We recently investigated the effects of biliary ligation and/or three different pretreatments used to alter systemic glutathione (GSH) status on the disposition of inorganic mercury in rats 1 h after the administration of a non-nephrotoxic dose of mercuric chloride [1]. These pretreatments included administration of buthionine sulfoximine (BSO) to inhibit the γ-glutamylcysteine synthetase, administration of diethylmaleate (DEM) to deplete intracellular GSH by conjugation, and administration of acivicin to inhibit the enzyme γ-glutamyltransferase. All three pretreatments have been shown to cause significant, acute reductions in renal and hepatic content of GSH in our laboratories [2]. In animals that had not undergone biliary ligation, pretreatment with DEM and acivicin, but not BSO, caused the renal accumulation of mercury (during the initial hour after the injection of mercuric chloride) to be diminished significantly, relative to that in untreated control animals. Similar findings had also been obtained previously in our laboratory [2]. From these findings, we concluded that acute depletion of renal GSH by pretreatment with DEM or acivicin, but not with BSO, leads to reduced renal accumulation of inorganic mercury during the initial hour after exposure to, or administration of, inorganic mercury, although the mechanisms for the decreased accumulation of mercury were not yet known.

Acute biliary ligation was also shown to cause the renal accumulation of mercury to be decreased under all pretreatment conditions during the initial hour after the injection of inorganic mercury [1]. By contrast, it was shown that biliary ligation caused hepatic accumulation of mercury to increase under all pretreatment conditions except with acivicin pretreatment. As a result of all of these findings, we postulated that the decreased renal accumulation of mercury induced by biliary ligation was related to alterations in systemic thiol status (in particular GSH status) induced by preventing bile from entering into the small intestine.

To extend our findings on the effects of biliary ligation and chemical modulation of thiol status, we examine in this study the disposition of inorganic mercury 24 h after the administration of a non-nephrotoxic dose of mercuric chloride. In a separate set of experiments, we also evaluated renal and hepatic GSH status 24 h after the administration of mercuric chloride or chemical-pretreatment alone. The 24-h time-point was studied because a near maximal burden of inorganic mercury

is present in the total renal mass at this time after exposure. Studying the disposition of inorganic mercury at this time after the injection of mercuric chloride also permitted us to evaluate the effects of biliary ligation and/or chemical modulation of GSH status on both the urinary and fecal excretion of inorganic mercury.

Furthermore, we provide data to support the hypothesis that the reductions in the renal accumulation of inorganic mercury induced by chemical depletion of GSH and by acute biliary ligation are mediated by more that one mechanism. The overall data presented in this manuscript provide additional new insights into the relationships between the cellular concentrations of GSH and the accumulation of mercury in both the kidneys and liver.

2. Materials and methods

2.1. Animals and groups

Male Sprague–Dawley rats were used in the present study. The animals were purchased from Harlan Sprague-Dawley (Indianapolis, IN) at a weight of 175–200 g. After 3–4 days of acclimation, the animals were separated into groups and subgroups.

There were two principal surgical groups. One of the surgical groups underwent acute biliary ligation and the other served as a control. Each of the two surgical groups was subdivided into four pretreatment subgroups, each consisting of four to five animals. One subgroup was pretreated with normal saline (and served as a control), acivicin (to inhibit the enzyme γ-glutamyltransferase), BSO to deplete renal and hepatic GSH, or DEM as a second method to deplete renal and hepatic GSH.

During all stages of the present study, the animals were allowed water and a commercial laboratory diet for rats ad libitum and were kept in a room at 20–22°C, 50% relative humidity, on a 12-h light-dark cycle.

2.2. Surgical procedures

The surgical procedures used in the present study were the same as those outlined previously in Zalups et al. [1].

2.3. Pretreatments

As mentioned above, acivicin was administered to inhibit the activity of the enzyme γ-glutamyltransferase in the kidneys and liver. The injection protocol used in the animals pretreated with acivicin is a slight modification of the one established by Scott and Curthoys [3], which results in the inhibition of $\sim 97\%$ of the activity of γ-glutamyltransferase in the kidneys. First, the animals received a 10-mg/kg i.p. dose of acivicin in 2 ml/kg normal saline (0.9%, w/v, aqueous sodium chloride).

Then 90 min later, the animals received a second 10-mg/kg dose of acivicin that was administered into the left femoral vein (while they were anesthetized lightly with ether). And 60 min after the second dose, the animals received a non-nephrotoxic 0.5-μmol/kg i.v. dose of mercuric chloride.

BSO and DEM were chosen to deplete GSH in the kidneys and liver because depletion could be attained by different mechanisms. Depletion of GSH following pretreatment with BSO is accomplished by inhibition of γ-glutamylcysteine synthetase [4], which is the rate-limiting enzyme involved in the intracellular synthesis of GSH. Pretreatment with DEM results in depletion of GSH by formation of DEM-GSH conjugates. The injection protocols used to pretreat rats with BSO or DEM were slight modifications of ones we used previously [2]. In brief, rats pretreated with BSO were given a 2-mmol/kg dose i.v. in 4 ml/kg normal saline. Rats that were pretreated with DEM received a 3.37-mmol/kg dose i.p. in 2 ml/kg corn oil. Baggett and Berndt [5] have shown that this type of pretreatment with DEM causes the non-protein thiol content in the kidneys and liver to decrease by ~ 40 and 68%, respectively. The animals received the non-nephrotoxic 0.5-μmol/kg i.v. dose of mercuric chloride 2 h after pretreatment with either BSO or DEM.

2.4. Injection of inorganic mercury

All groups of control, acivicin-pretreated, BSO-pretreated and DEM-pretreated rats were administered a 0.5-μmol/kg dose of mercuric chloride into the femoral vein while under light anesthesia induced by ether (which lasted no more than 30 s). By the time each animal was injected with inorganic mercury, it had fully recovered from surgical anesthesia induced by ketamine and xylazine. Radioactive inorganic mercury in the form of mercuric chloride ($^{203}HgCl_2$, specific activity = 30 mCi/mg; Buffalo Materials, Buffalo, NY) was added to the injection solution containing non-radioactive mercury. The injection solution was designed to deliver 0.5 μmol Hg^{2+}/kg and 4 μCi $^{203}Hg^{2+}$/kg in 2.0 ml normal saline (0.2-ml injection volume/100 g body weight).

2.5. Collection of urine and feces

After inorganic mercury had been injected into each animal, the animal was placed individually into a plastic metabolic cage. Urine and feces were collected from each animal during the entire 24-h period of the experiment.

2.6. Acquisition of tissues and determination of the content of mercury

Then, 24 h after the injection of the 0.5-μmol/kg dose of mercuric chloride, animals were anesthetized with a 100-mg/kg dose of sodium pentobarbital (i.p.). Once the animals were anesthetized, two 1-ml samples of whole blood were obtained from the inferior vena cava. One 1-ml sample of whole blood was placed and sealed in a pre-weighed 12 × 75 mm, round-bottom, gamma-counting tube. The other 1.0 ml of whole blood was centrifuged at $10\,000 \times g$ to separate the cellular

fraction of blood from the plasma. Both plasma and cellular fractions were placed individually, and sealed, in gamma-counting tubes. After the blood had been obtained, the kidneys, liver, small intestine and large intestine were removed, cleared of fat and connective tissue and weighed quickly. Each of the two kidneys was cut along the transverse plane. One half of each kidney was placed and sealed in a pre-weighed gamma-counting tube. A 3-mm section of kidney was sliced away from the mid-region of the remaining half of the left kidney and samples of cortex, outer and inner stripes of the outer medulla and inner medulla were obtained. A 1-g sample of liver was also obtained.

Urine and fecal samples collected were removed from the cages at the end of the 24-h collection period. The amount of urine and feces excreted was determined by weighing the sample. From each sample of urine, 1 ml was removed, placed, and sealed in a 12×75-mm gamma-counting tube. In contrast, the entire amount of feces excreted was placed and sealed in multiple 16×90-mm polypropylene tubes.

The radioactivity of the samples of tissues, urine, feces and injection solution (standards) was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer that is equipped with a 3-inch sodium iodide crystal (Wallac, Gaithersburg, MD) and operates at a counting efficiency of $\sim 50\%$ for $^{203}Hg^{2+}$. The content of mercury in the samples was calculated by dividing the activity of $^{203}Hg^{2+}$ (dpm) in the sample by the specific activity of $^{203}Hg^{2+}$ (dpm/nmol) in the injection solution. Concentrations of mercury in the tissues are expressed as percent of the administered dose per gram of tissue and the content of mercury in organs is expressed simply as a percent of the administered dose. The total volume of blood in rats was estimated to be $\sim 6\%$ of body weight.

2.7. Assessment of GSH status in the kidneys and liver

The content of GSH in the kidneys and liver was assessed in 16 groups of four to five animals. A total of eight groups of rats were treated in the same manner as the eight groups of rats in which the disposition of mercury was assessed. The kidneys and liver from these animals were removed and processed for the evaluation of GSH status 24 h after the administration of the 0.5-μmol/kg dose of mercuric chloride. The other eight groups of rats were not treated with mercuric chloride. Their kidneys and liver were removed for study following the same length of time post-pretreatment with BSO, DEM or acivicin. After the kidneys and liver had been removed from each animal, the organs were weighed quickly and then samples of one half of a kidney and liver were homogenized in 10 ml of 1.0 mM bathophenanthroline disulfonate (used as an antioxidant) and 10% (v/v) perchloric acid. Acid extracts of the homogenates were derivatized with iodoacetic acid and 1-fluoro-2,4-dinitrobenzene for analysis of GSH by high-performance liquid chromatography (HPLC), according to the method of Fariss and Reed [6]. The *S*-carboxymethyl-*N*-dinitrophenyl derivative of GSH was separated on a 10-μm μBondapak amine column with gradient elusion (methanol-acetate solvent system) and was detected by absorbance at 365 nm. Quantitation of GSH in samples was performed by integration and comparison to a standard curve. The actual amount of GSH in the combined total renal mass and liver is expressed in μmol/organ(s).

2.8. Statistical analyses

Statistical differences between means, of any parameter measured in the mercury disposition experiments, were assessed using a two-way analysis of variance followed by Tukey's multiple comparison procedure, where surgery and mode of GSH modulation were the variables assessed. In the experiments designed to assess renal and hepatic GSH, however, statistical differences between means of each parameter measured were assessed using a three-way analysis of variance followed by Tukey's multiple comparison procedure. The additional variable assessed with the three-way analysis of variance was treatment with inorganic mercury. Because data expressed as a percent of total do not fit a Gaussian distribution, the percentage data were first normalized using the arcsine transformation before performing any parametric statistical procedure. The arcsine transformation takes the arcsine of the square root of the decimal fraction of the percent score. The level of significance for any of the statistical procedures used was chosen a priori to be $P < 0.05$.

3. Results

3.1. Findings pertaining to the disposition of mercury

3.1.1. Renal concentration and content of mercury

3.1.1.1. Bile duct not ligated. At the end of the initial 24 h after the i.v. injection of the 0.5-μmol/kg dose of mercuric chloride, both the renal concentration and content of mercury in BSO-pretreated rats (that did not undergo biliary ligation) were significantly less than those in the corresponding control rats (Fig. 1A and B). In fact, the mean content of mercury in the total renal mass of the rats that were pretreated with BSO was $\sim 52\%$ less than that in the corresponding control rats. Moreover, the concentration and content of mercury in the kidneys of the BSO-pretreated rats (that did not undergo biliary ligation) were significantly less than those in the corresponding groups of rats that were pretreated with DEM or acivicin, which were also significantly less than those in the corresponding control rats.

Interestingly, the kidneys in the BSO-pretreated rats had a pale appearance and were covered in a clear, gelatin-like material. No obvious gross pathological changes were detected in the kidneys of any of the other groups of rats that did not undergo biliary ligation.

3.1.1.2. Bile duct ligated. The only significant difference in the overall renal concentration or content of mercury among the four groups of rats that underwent an acute biliary ligation prior to pretreatment was detected between the group pretreated with acivicin and the group that served as a control (Fig. 1A and B). However, significant differences in both the renal concentration and content of mercury were detected between corresponding groups that underwent biliary ligation and those that did not. With the exception of the groups pretreated with BSO,

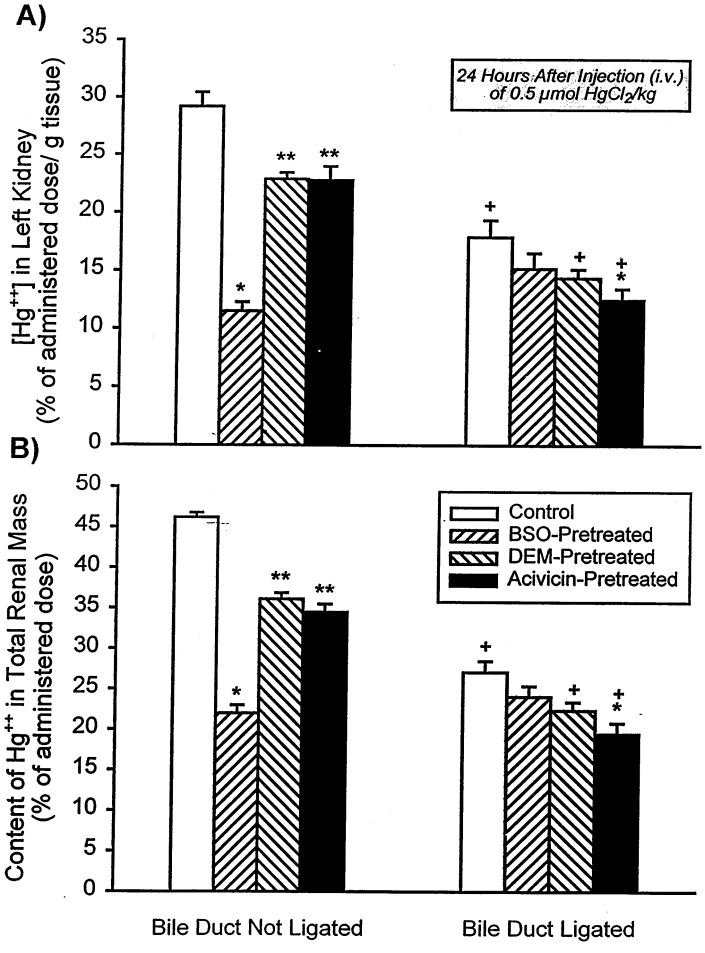

Fig. 1. Effect of acute biliary obstruction (ligation) on the renal concentration (% of administered dose/g tissue) and content of mercury (% of administered dose) in control rats and rats pretreated with buthionine sulfoximine (BSO), diethylmaleate (DEM), or acivicin 24 h after the i.v. injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of modulating glutathione metabolism systemically. Refer to Section 2 for details on pretreatments and handling of tissues. Each value represents the mean ± S.E. for four to five animals per group. *Significantly different ($P < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($P < 0.05$) from the means for the other three corresponding pretreatment groups that were treated in the same manner. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner.

the content of mercury in the total renal mass between the paired groups that were pretreated in the same manner was ~36–40% less in the group that underwent biliary ligation than in the corresponding group that did not undergo biliary ligation. There was no significant difference in either the renal concentration of mercury or content of mercury in the total renal mass between the two corresponding groups of BSO-pretreated rats. However, unlike in the BSO-pretreated rats that did not undergo biliary ligation, the kidneys in the BSO-pretreated rats had a normal appearance.

3.1.2. Intrarenal distribution of mercury

3.1.2.1. Bile duct not ligated. Concentrations of mercury in the renal cortex and outer stripe of the outer medulla were significantly less in the BSO-pretreated (that did not undergo biliary ligation) than in the other three corresponding groups of rats (Fig. 2A and B). In addition, relative to the values obtained in the corresponding control rats, the concentrations of mercury in the renal cortex and outer stripe of the outer medulla were significantly less in the DEM-pretreated and acivicin-pretreated rats.

3.1.2.2. Bile duct ligated. Among the groups that underwent biliary ligation, the only significant effects in the concentrations of mercury in the renal cortex and outer stripe of the outer medulla were detected in the group pretreated with acivicin. In this group, the concentration of mercury in the renal cortex and in the outer stripe of the outer medulla was significantly less and significantly greater, respectively, than in any of the other three groups of rats that underwent biliary ligation.

With the exception of the groups pretreated with BSO, the concentration of mercury in the renal cortex was significantly less in each group of rats that underwent biliary ligation than in the corresponding group that did not undergo biliary ligation (but that was pretreated in the same manner). On the other hand, the concentration of mercury in the outer stripe of the outer medulla was significantly less in each and every group of rats that underwent biliary ligation, relative to the corresponding paired group that did not undergo biliary ligation.

3.1.3. Content of mercury in the liver

3.1.3.1. Bile duct not ligated. In the BSO-pretreated rats that did not undergo biliary ligation, the hepatic content of mercury (24 h after the injection of mercuric chloride) was nearly threefold greater than that in the corresponding group of control rats (Fig. 3). In addition, the hepatic content of mercury in the DEM-pretreated rats was significantly greater than that in the corresponding control rats. However, no significant difference was detected in the hepatic content of mercury between the rats pretreated with acivicin and the control rats.

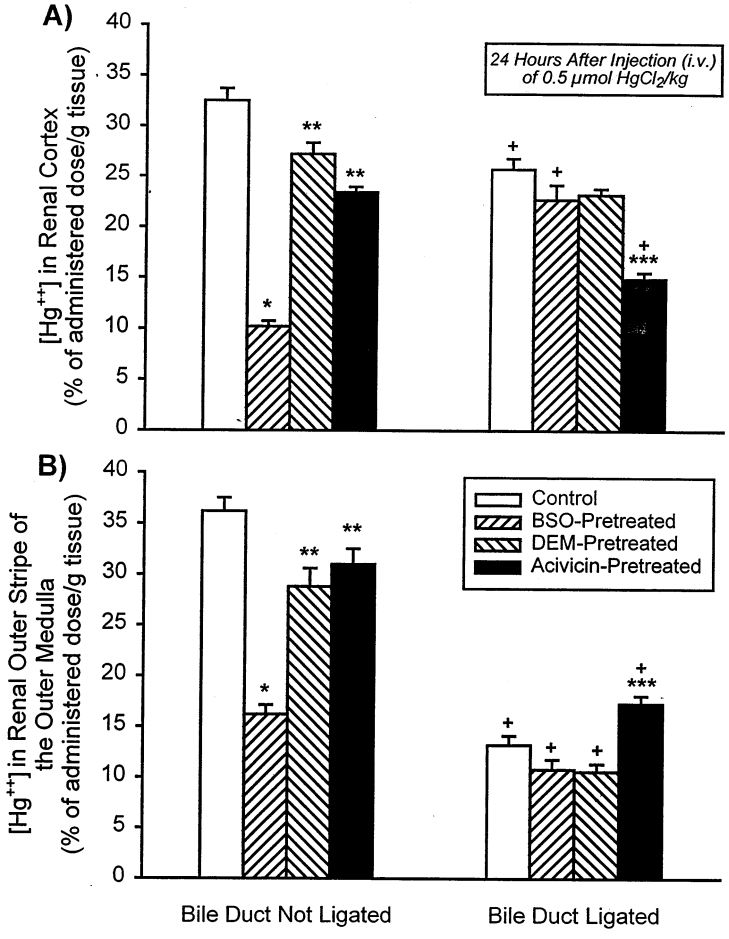

Fig. 2. Effect of acute biliary obstruction (ligation) on the concentration of mercury (% of administered dose/g tissue) in the renal cortex (A) and outer stripe of the outer medulla (B) of control rats and rats pretreated with buthionine sulfoximine (BSO), diethylmaleate (DEM), or acivicin 24 h after the i.v. injection of a 0.5-µmol/kg dose of mercuric chloride. The pretreatments used represent three different means of modulating glutathione metabolism systemically. Refer to Section 2 for details on pretreatments and handling of tissues. Each value represents the mean ± S.E. for four to five animals per group. *Significantly different ($P < 0.05$) from the means for the corresponding group of control rats that were treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($P < 0.05$) from the means for the other three corresponding pretreatment groups that were treated in the same manner. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner.

3.1.3.2. Bile duct ligated. Significant differences in the hepatic content of mercury were detected among the four groups of rats that underwent biliary ligation. The hepatic content of mercury in the groups of rats pretreated with either DEM or acivicin was significantly less than that in the group pretreated with BSO or the control group (Fig. 3). Between the paired groups of rats pretreated in the same manner, the content of mercury in the liver was significantly greater in the group that underwent biliary ligation than in the corresponding group that did not undergo biliary ligation.

3.1.4. Disposition of mercury in the blood

Among the groups that did not undergo biliary ligation, the amount of mercury in the total volume of blood of the BSO-pretreated and acivicin-pretreated rats was

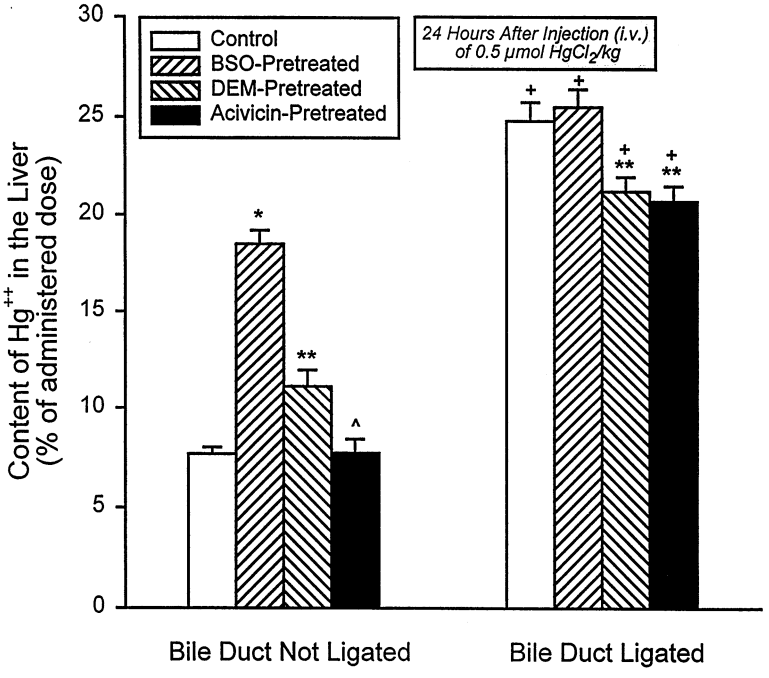

Fig. 3. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered) in the liver of control rats and rats pretreated with buthionine sulfoximine (BSO), diethylmaleate (DEM), or acivicin 24 h after the i.v. injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of modulating glutathione metabolism systemically. Refer to Section 2 for details on pretreatments and handling of tissues. Each value represents the mean ± S.E. for four to five animals per group. *Significantly different ($P < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ^Significantly different ($P < 0.05$) from means for the corresponding groups of BSO-pretreated and DEM-pretreated rats. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner.

significantly greater and significantly less, respectively, than that in any of the other three groups (Fig. 4A). No significant differences in the content of mercury in blood were detected among the four groups of rats that had undergone biliary ligation. However, the amount of mercury in blood, between the paired groups of rats pretreated in the same manner was significantly greater in the corresponding group that had undergone biliary ligation (Fig. 4A).

Significantly more mercury was present in the plasma of BSO-pretreated and acivicin-pretreated rats that had not undergone biliary ligation than in the corresponding control group. There was no significant difference in the amount of mercury in the plasma among the four groups of rats that had undergone an acute biliary ligation. Additionally, a significant difference in the amount of mercury in the plasma was found only between the paired groups of rats pretreated with BSO, which was significantly less in the group that had undergone biliary ligation.

3.1.5. Urinary excretion of mercury

3.1.5.1. Bile duct not ligated. Control rats that did not undergo biliary ligation excreted $\sim 6\%$ of the dose of mercury in the urine in 24 h (Fig. 5A). Corresponding rats pretreated with BSO excreted significantly less mercury in the urine (slightly greater than 2% of the dose), while the corresponding rats pretreated with DEM or acivicin excreted significantly more mercury in the urine. In fact, the rats pretreated with acivicin excreted more than 18% of the administered dose of inorganic mercury in 24 h, which is slightly more than threefold the amount excreted by the control rats.

3.1.5.2. Bile duct ligated. Acivicin-pretreated rats that had undergone biliary ligation also excreted significantly more mercury in the urine in 24 h than any of the other three corresponding groups of rats (Fig. 5A). No significant differences in the urinary excretion of mercury were detected among the control, BSO-pretreated and DEM-pretreated rats that had undergone biliary ligation. Between corresponding paired groups of pretreated animals that did and did not undergo biliary ligation, the amount of mercury excreted in the urine was significantly greater only in the group of control and DEM-pretreated rats that had not undergone biliary ligation.

3.1.6. Fecal excretion of mercury

3.1.6.1. Bile duct not ligated. In 24 h $\sim 5.5\%$ of the administered dose of inorganic mercury was excreted in the feces by the control animals that had not undergone biliary ligation. In the corresponding groups pretreated with DEM or acivicin, the amount of mercury excreted in the feces was significantly less. No other significant differences in the fecal excretion of mercury were detected among the four groups of rats that had not undergone biliary ligation (Fig. 5B).

3.1.6.2. Bile duct ligated. Significantly less mercury was excreted in the feces by the group of rats pretreated with acivicin and that had undergone biliary ligation than

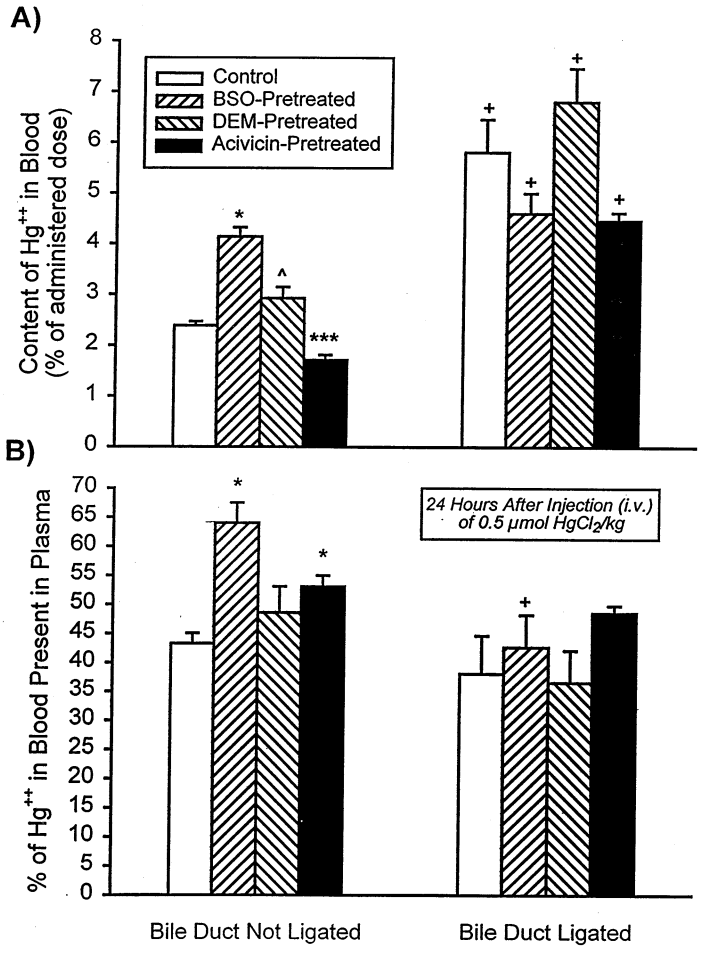

Fig. 4. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered dose) in the blood (A) and percent of mercury in blood present in plasma (B) of control rats and rats pretreated with buthionine sulfoximine (BSO), diethylmaleate (DEM), or acivicin 24 h after the i.v. injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of modulating glutathione metabolism systemically. Refer to Section 2 for details on pretreatments and handling of tissues. Each value represents the mean ± S.E. for four to five animals per group. *Significantly different ($P < 0.05$) from the means for the corresponding group of control rats that were treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($P < 0.05$) from the means for the other three corresponding pretreatment groups that were treated in the same manner. ^Significantly different ($P < 0.05$) from the mean for the corresponding group pretreated with BSO that was treated surgically in the same manner. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner.

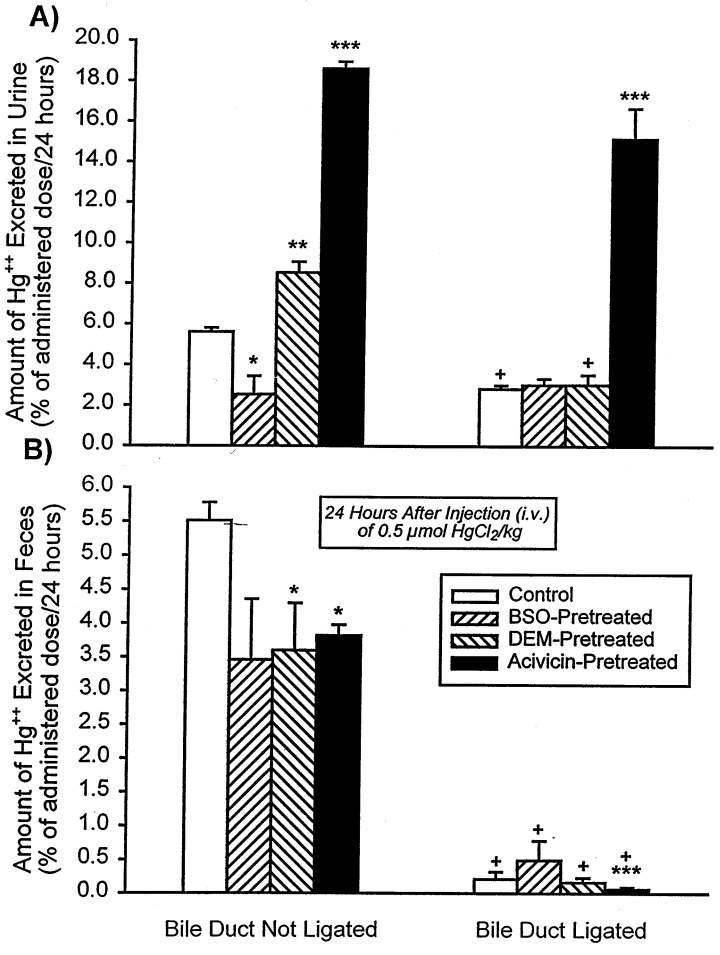

Fig. 5. Effect of acute biliary obstruction (ligation) on the urinary (A) and fecal (B) excretion of mercury (% of administered dose/24 h) in control rats and rats pretreated with buthionine sulfoximine (BSO), diethylmaleate (DEM), or acivicin 24 h after the i.v. injection of a 0.5-μmol/kg dose of mercuric chloride The pretreatments used represent three different means of modulating glutathione metabolism systemically. Refer to Section 2 for details on pretreatments and handling of tissues. Each value represents the mean ± S.E. for four to five animals per group. *Significantly different ($P < 0.05$) from the means for the corresponding group of control rats that was treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($P < 0.05$) from the means for the other three corresponding pretreatment groups that were treated in the same manner. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner.

in any of the other three corresponding groups of rats (Fig. 5B). Between all paired groups of rats pretreated in the same manner, the amount of mercury excreted in the feces was significantly less in the group that had undergone biliary ligation. It

should be mentioned that excretion of feces was greatly diminished in the animals that had undergone biliary ligation, which likely contributed to the significant differences in the fecal excretion of mercury between the paired groups of rats that did, and did not, undergo biliary ligation.

3.2. Findings pertaining to GSH status

3.2.1. GSH status in the kidney

3.2.1.1. Bile duct not ligated and not treated with inorganic mercury. Among the normal rats that did not undergo biliary ligation and that did not receive an injection of mercuric chloride, the renal concentration of GSH in the groups pretreated with BSO, DEM or acivicin was significantly less than that in the corresponding control rats (Fig. 6A). The actual concentration of GSH in the control rats averaged slightly less than 4.0 μmol/g tissue, while in the other three corresponding groups, it averaged between 1.5 and 2.0 μmol/g tissue.

3.2.1.2. Bile duct ligated and not treated with inorganic mercury. Between each of the paired groups of rats not treated with mercuric chloride, but that were pretreated chemically in the same manner, the renal concentration of GSH was significantly greater in the group that underwent biliary ligation (Fig. 6A). The smallest difference between these paired groups was detected between the two BSO-pretreated groups. Among the rats that underwent biliary ligation, those pretreated with BSO had markedly and significantly lower renal contents of GSH.

3.2.1.3. Bile duct not ligated and treated with inorganic mercury. In the group of control rats that did not undergo biliary ligation, but were treated with the 0.5-μmol/kg dose of mercuric chloride, the renal concentration of GSH averaged slightly less than 3.0 μmol/g tissue (Fig. 6B). The renal concentration of GSH in the other three corresponding groups of rats was significantly less than that in these control rats. The greatest difference in the renal concentration of GSH among these four groups of rats was detected between the control rats and the rats pretreated with BSO. In fact, the renal concentration of GSH in the BSO-pretreated group averaged at less than 0.5 μmol/g tissue, which was significantly lower than that in any of the other three treatment groups. This mean value was also statistically less than that for the BSO-pretreated group that was not treated with mercuric chloride.

3.2.1.4. Bile duct ligated and treated with inorganic mercury. Much like among the groups of rats not treated with mercuric chloride, the renal concentration of GSH between the corresponding paired groups of rats pretreated in the same manner and that were injected with inorganic mercury, was significantly greater in the corresponding group that underwent biliary ligation (Fig. 6B). Between the corresponding paired groups of rats that underwent biliary ligation and that were pretreated in the same manner, there were no significant differences between the corresponding group of rats that did or did not receive an injection of mercuric chloride. As with

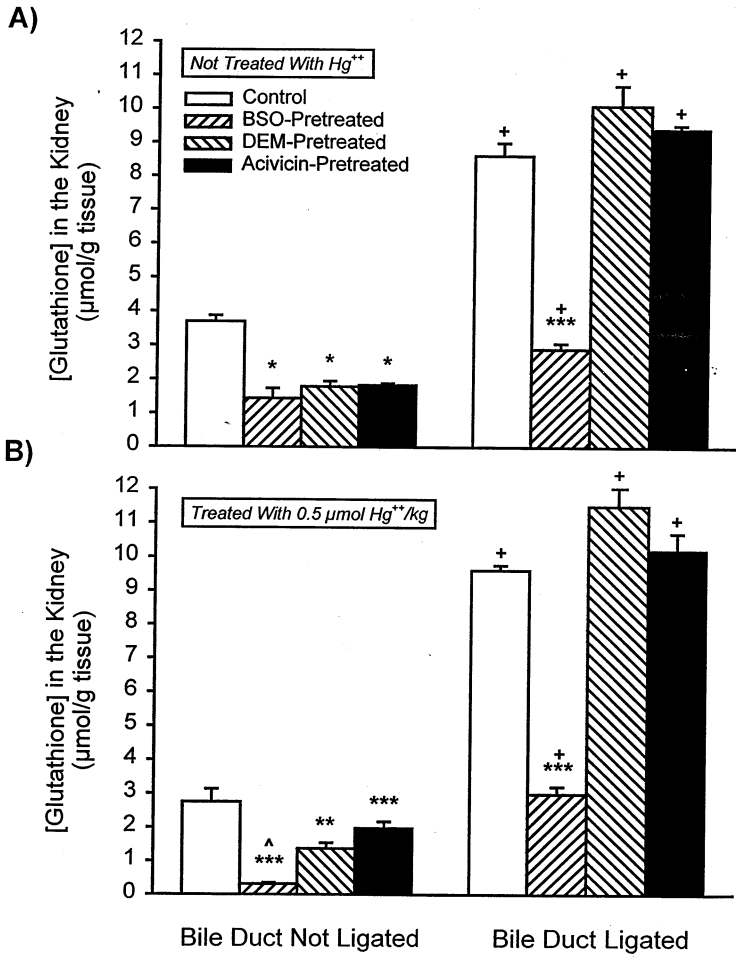

Fig. 6. Effect of acute biliary obstruction (ligation); pretreatment with saline, acivicin, buthionine sulfoximine (BSO) or diethylmaleate (DEM); and/or the i.v. injection of a 0.5-µmol/kg dose of mercuric chloride on the renal concentration of glutathione (GSH) in rats. Refer to Section 2 for details on pretreatments and handling of renal tissues. Each value represents the mean ± S.E. for four animals. *Significantly different ($P < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($P < 0.05$) from the means for the other three corresponding pretreatment groups that were treated in the same manner. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner. ^Significantly different ($P < 0.05$) from the mean for the corresponding group that was treated surgically and pretreated chemically in the same manner, but that did not receive the 0.5-µmol/kg dose of mercuric chloride.

the rats that underwent biliary ligation and were not treated with inorganic mercury, renal GSH concentration for rats that underwent biliary ligation and that were treated with inorganic mercury was significantly lower in the subgroup pretreated with BSO than in any of the three other treatment groups.

3.2.2. GSH status in the liver

3.2.2.1. Bile duct not ligated and not treated with inorganic mercury. The only significant differences in the hepatic concentration of GSH detected among the normal rats that did not undergo biliary ligation and that did not receive an injection of mercuric chloride was between the BSO-pretreated and the DEM- and acivicin-pretreated rats (Fig. 7A): the hepatic concentration of GSH was significantly less in the BSO-pretreated rats.

3.2.2.2. Bile duct ligated and not treated with inorganic mercury. Relative to the corresponding paired group pretreated in the same manner (and that was not treated with mercuric chloride), the hepatic concentration of GSH was significantly greater in the group that had undergone biliary ligation, regardless of the pretreatment (Fig. 7A). The only significant difference in the hepatic concentration of GSH among the four groups of rats that had undergone biliary ligation, but that did not receive an i.v. dose of mercuric chloride, was that the hepatic concentration of GSH in DEM-pretreated rats was significantly lower than that in the control rats.

3.2.2.3. Bile duct not ligated and treated with inorganic mercury. In the BSO-pretreated of rats that did not undergo biliary ligation, but that were treated with the 0.5-μmol/kg dose of mercuric chloride, the hepatic concentration of GSH was significantly less than that in any of the other three corresponding groups of rats (Fig. 7B).

3.2.2.4. Bile duct ligated and treated with inorganic mercury. The concentration of GSH in the liver of the BSO-pretreated rats that underwent biliary ligation and that were treated with mercuric chloride was significantly less than that in the corresponding control group (Fig. 7B). Moreover, the hepatic concentration of GSH in this BSO-pretreated group was also significantly less than that in the corresponding BSO-pretreated group that underwent biliary ligation, but was not treated with inorganic mercury. The concentration of GSH in the liver of each of the four pretreatment groups from rats that were bile duct ligated was significantly higher than that in the liver of correspondingly treated rats that were not bile duct ligated.

4. Discussion

GSH is the most abundant low molecular weight thiol present in all cells of the body. It has numerous functions, not least of which involves the formation of GSH-S-conjugates with various molecules. In recent years, interest has arisen

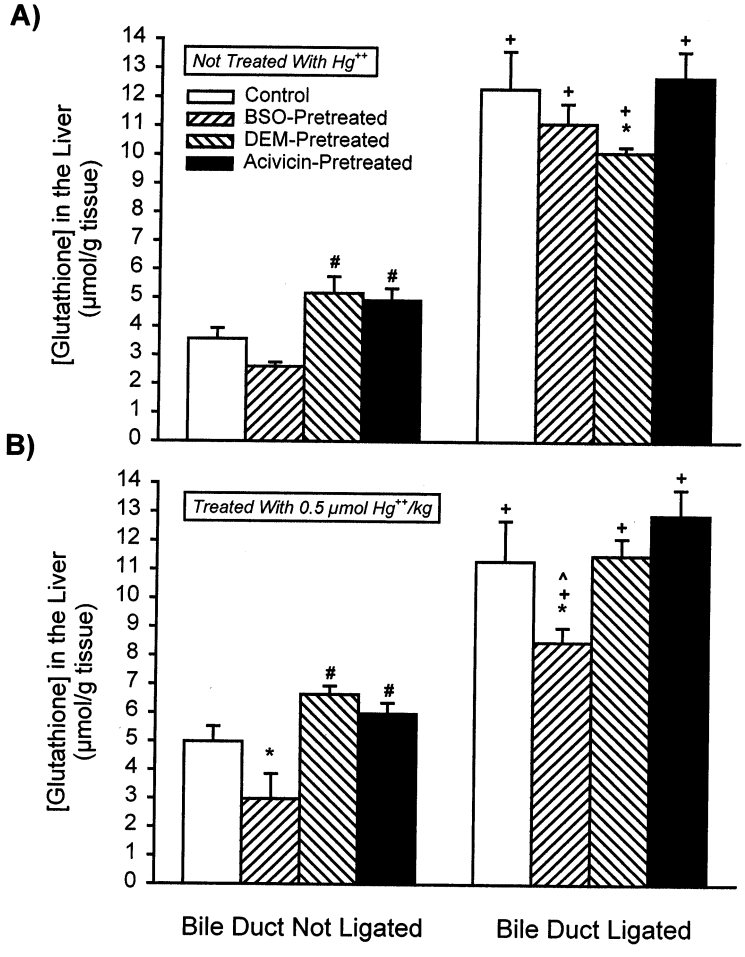

Fig. 7. Effect of acute biliary obstruction (ligation); pretreatment with saline, acivicin, buthionine sulfoximine (BSO) or diethylmaleate (DEM); and/or the i.v. injection of a 0.5-µmol/kg dose of mercuric chloride on the hepatic concentration of glutathione (GSH) in rats. Refer to Section 2 for details on pretreatments and handling of renal tissues. Each value represents the mean ± S.E. for four animals. *Significantly different ($P < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. **Significantly different ($P < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($P < 0.05$) from the means for the other three corresponding pretreatment groups that were treated in the same manner. + Significantly different ($P < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation, but was pretreated in the same manner. ^Significantly different ($P < 0.05$) from the mean for the corresponding group that was treated surgically and pretreated chemically in the same manner, but that did not receive the 0.5-µmol/kg dose of mercuric chloride. # Significantly different ($P < 0.05$) from the mean for the corresponding group, treated surgically in the same manner, that was pretreated with BSO.

regarding the formation of conjugates between GSH and the cationic forms of heavy metals, such as mercury. Chemical evidence reveals that when GSH is present, in at least a 2:1 mole ratio, with inorganic mercuric ions in aqueous solutions, they form linear II coordinate covalent, thermodynamically stable, complexes (conjugates) involving two molecules of GSH with each mercuric ion [7].

In the present study, renal and hepatic accumulation of inorganic mercury and content of GSH were measured in rats that had or had not undergone acute biliary ligation 24 h after administration of a low, relatively non-toxic dose of inorganic mercury. This study provides new and important findings that were not attainable in our previous 1-h study [1]. Moreover, several important quantitative and qualitative differences in the results obtained at the two times (after the injection of inorganic mercury) were discovered, and these are highlighted below. Furthermore, significant new insights into the inter-relationships between the renal and hepatic disposition of both inorganic mercury and GSH are provided.

4.1. Inhibition of γ-glutamyltransferase and the renal disposition of inorganic mercury and GSH

The formation and presence of mercuric conjugates of GSH in various organs and compartments in the body have been implicated in the uptake, transport, accumulation and excretion of mercury in the kidneys and liver [7]. For instance, there is a large body of evidence implicating the involvement of mercuric conjugates of GSH in the luminal (and possibly basolateral) uptake of inorganic mercury by renal (proximal) tubular epithelial cells [5,8–17]. The data from the present study support this hypothesis. We demonstrate that when the activity of γ-glutamyltransferase is inhibited before the exposure to inorganic mercury, there is a significant reduction in the renal uptake of mercury and a significant increase in the urinary excretion of mercury (and GSH) [1,5,9–12]. Our findings also indicate that inhibition of γ-glutamyltransferase (with acivicin) causes the renal concentration of GSH to decrease significantly. This is most likely due to diminished rates of recycling of secreted GSH (or its constituent amino acids) back into proximal tubular epithelial cells.

4.2. Temporal effects of inhibiting γ-glutamyltransferase on the hepatic disposition of inorganic mercury and GSH

Besides being present in abundance on the brush-border plasma membrane of proximal tubular epithelial cells, γ-glutamyltransferase is also localized on the canalicular membrane of hepatocytes. When we inhibited this enzyme systemically with acivicin, hepatic accumulation of mercury was decreased significantly only at 1 h [1], but not at 24 h, after the injection of a non-nephrotoxic dose of mercuric chloride. Intracellular concentrations of GSH did not appear to be affected in hepatocytes at 24 h after pretreatment with acivicin or the pretreatment with acivicin combined with the injection of mercuric chloride. Thus, changes in GSH status and the hepatic disposition of injected inorganic mercury induced by

pretreatment with acivicin appear to be very short-lived within the liver. This short-lived effect may be related to the rates of turnover of γ-glutamyltransferase in hepatocytes.

Hepatic GSH likely plays a key role in the disposition of inorganic mercury because the metal is secreted into the bile primarily in the form of a GSH conjugate [18,19]. Other hepatic and or biliary thiols, such as lipoid acid [20] or metallothionein [21] may also be important in the hepatic disposition of inorganic mercury. In support of a role for hepatic GSH status in determining the disposition of metals such as inorganic mercury, Klaassen and colleagues [22] showed that inhibition of hepatic, biliary, and renal γ-glutamyltransferase with acivicin (as was done in the present study) markedly stimulated the biliary excretion of GSH and the urinary excretion of methylmercury, without affecting biliary excretion of methylmercury or renal and hepatic levels of GSH. This last study illustrates the complex interrelationships between GSH status and heavy metal disposition. Although the status of other thiols besides GSH, such as those derived from GSH, or metallothionein, may be important determinants of metal disposition, the relationships are not always clear. For example, Wong and Klaassen [21] showed that renal and hepatic levels of metallothionein and GSH are not always interrelated. Stein et al. [23] showed that there is a marked species-dependent variation in the rate of biliary excretion of GSH and related thiols and disulfides in mice, rats, and hamsters but no species variation in the rate of biliary excretion of methylmercury.

4.3. Temporal effects of BSO pretreatment on the renal disposition of inorganic mercury and GSH

Pretreatment with BSO (in normal rats) was shown in the present study to cause ~50% reduction in the net renal accumulation of mercury 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride. This finding is in direct contrast to our previous findings obtained from normal animals pretreated with BSO that were studied 1 h after the administration of mercuric chloride [1,2]. We showed that BSO pretreatment does not have a significant effect on the renal burden of inorganic mercury at 1 h after the injection of mercuric chloride, despite the fact that it causes a significant reduction in the renal concentration of GSH. Thus, factors other than the levels of GSH in renal tubular epithelial cells affect the short-term (and perhaps the long-term) renal disposition of inorganic mercury when the inorganic mercury is administered soon after initiating the reduction (or depletion) of renal GSH with BSO.

4.4. Changes in renal morphology induced by the combined effects of BSO and inorganic mercury

The combination of pretreatment with BSO and treatment with inorganic mercury in surgical control animals (that did not undergo biliary ligation) caused significant morphological changes in their kidneys. The renal parenchyma in these animals became homogeneously pale and off-white in appearance. Moreover, a

clear gelatinous substance formed on the outside capsular surface of the kidneys. By contrast, no signs of gross anatomical/pathological changes were detected in the surgical control, BSO-pretreated rats that were not treated with inorganic mercury (and that were used for the assessment of renal GSH status). Because treatment with inorganic mercury caused additional depletion of GSH in the surgical control, BSO-pretreated rats, the renal pathological effects detected in these animals are likely related to severe depletion of GSH, which most likely occurred via a conjugation reaction between mercuric ions and the remaining GSH in the renal tubular epithelial cells. This renal pathology was not detected in BSO-pretreated animals that underwent biliary ligation. Because biliary ligation caused renal levels of GSH to increase, even in BSO-pretreated animals, it seems plausible that sufficient amounts of GSH were present in the kidneys of these animals to prevent the induction of the pathological changes detected in the surgical control animals pretreated with BSO.

4.5. Temporal effects of DEM pretreatment on the renal disposition of inorganic mercury and GSH

Pretreatment with DEM causes reductions in the renal content of GSH and the net renal accumulation of inorganic mercury [1,2,5]. The findings from the present study indicate that the renal content of GSH and the net renal accumulation of mercury are reduced significantly throughout the initial 24 h after pretreatment and injection of inorganic mercury. However, the reduction in the net renal accumulation of inorganic mercury is not as great as that detected in DEM-pretreated animals studied 1 h after the injection of mercuric chloride [1]. Thus, it appears that some fraction of mercury redistributes back into the kidneys between the 1st and 24th hour after the injection of mercury when GSH is depleted with DEM.

4.6. Alterations in renal and hepatic disposition of GSH and inorganic mercury following biliary ligation

In the present study we demonstrate that biliary ligation also causes a net reduction in the renal burden of inorganic mercury 24 h after the injection of mercuric chloride in control rats and rats pretreated with DEM and acivicin, but not BSO. We also demonstrated, in a previous study, that biliary ligation causes reductions in the 1-h accumulation of mercury under all pretreatment conditions used in the present study [1]. A surprising finding, however, is that while biliary ligation causes a general reduction in the renal accumulation of inorganic mercury, it occurs under conditions where the intracellular concentrations and contents of GSH in the kidneys are increased. This apparent inverse relationship between the renal content of GSH and the net renal accumulation of mercury under these conditions seems to contradict the generally accepted notion that the renal accumulation of mercury should parallel (in a direct relationship) changes in the renal cellular concentrations of GSH.

In contrast to the inverse relationship between the contents of GSH and inorganic mercury in the kidneys after biliary ligation, hepatic contents of GSH and inorganic mercury both increase after biliary ligation. A reasonable explanation for this is that increased hepatic content of GSH may increase hepatic retention of inorganic mercury under conditions where bile flow is decreased, due to the prominent role of GSH in biliary secretion of inorganic mercury [18,19]. Changes in plasma thiol status may also be important. In preliminary studies, we found that the concentration of total acid-soluble GSH equivalents ($=GSH+2\ GSSG+CySSG$, where GSSG is glutathione disulfide and CySSG is the mixed-disulfide of GSH and cysteine) in plasma from rats that had undergone acute biliary ligation was significantly increased by 80% as compared with that in plasma from control rats. Total acid-soluble GSH equivalents (in nmol/ml plasma, means $\pm$ S.E., $n=4$) in plasma of control rats and in plasma of rats 1 h after biliary duct ligation were 13.4 ± 2.1 and 24.1 ± 1.3, respectively. One might have expected that the increased hepatic retention of inorganic mercury would have led to a decrease in blood levels of inorganic mercury. However, blood levels of inorganic mercury were increased after acute biliary ligation (cf. Fig. 4A). This may be explained by both the decreased renal content of inorganic mercury (cf. Figs. 1 and 2) and the increased plasma content of total GSH equivalents.

4.7. Relationships between the concentration of GSH and the accumulation of inorganic mercury in the kidneys and liver of rats treated chemically to deplete GSH

One factor that has been thought to play a role in the renal cellular disposition of mercury is the intracellular concentration of GSH. This is due mainly to the fact that GSH (as well as some other thiols) binds mercuric ions with great affinity and that it is present in millimolar concentrations in renal epithelial cells. Data from the present study indicate that there are far more complex relationships between the status of GSH and the accumulation of mercury in the liver and kidneys than has been assumed previously. In an attempt to understand some of these factors, we assessed the relationships between the concentration of GSH and the concentration of inorganic mercury in the kidneys and liver among the groups of animals that did, and did not, undergo biliary duct ligation and that were subjected to the four pretreatment conditions. The renal concentration of mercury among the four groups of control rats showed some dependence on the renal concentration of GSH. On the surface, this apparent dependence appears to be consistent with the generally accepted notion that as the levels of GSH are decreased in cells (particularly in renal epithelial cells), there should be corresponding decreases in the accumulation of mercury. However, in the liver under conditions where production and flow of bile are unimpeded, this did not appear to be the case. Our findings lead one to believe that as hepatic content of GSH decreases, more inorganic mercury, rather than less, is sequestered within hepatocytes, although much more research is needed to confirm this notion.

Other investigators have also postulated that hepatically synthesized GSH is linked in part to some aspects of the renal accumulation of inorganic mercury [11]. These investigators have demonstrated in mice that the renal accumulation of inorganic mercury is diminished significantly when 1,2-dichloro-4-nitrobenzene, which purportedly depletes GSH specifically in the liver, is administered prior to the administration of mercuric chloride. If a fraction of hepatically synthesized GSH is involved in some aspect of the renal uptake of mercury, then it would seem logical to postulate that perhaps some of the diminution in the renal burden of mercury detected following GSH depletion (induced by DEM and acivicin) is linked mechanistically to the depletion of GSH in the liver. If this proves to be the case, then the direct relationship between the renal levels of GSH and inorganic mercury that we detected may be misleading and may only be fortuitous. It may be that the decreased renal accumulation of mercury in animals depleted of GSH is due to both an effect of the diminished pool of GSH in the proximal tubular epithelial cells and an indirect relationship with depleted levels of GSH in the liver.

4.8. Relationships between the concentration of GSH and the accumulation of inorganic mercury in the kidneys and liver of rats that underwent acute biliary ligation

Data obtained from animals whose bile duct had been ligated provide evidence implicating a complex interplay between GSH status and accumulation of inorganic mercury in the liver with the status of GSH and the accumulation of inorganic mercury in the kidneys. First of all, biliary ligation caused the hepatic and renal contents of GSH to increase. A major mechanism by which hepatically synthesized GSH is recycled involves its secretion into the bile with subsequent intestinal degradation and absorption of its constituent amino acids [24–26]. Efflux of GSH from the liver into the bile is the principal source of GSH that is translocated through both entero-hepatic and renal-hepatic circulation to other tissues [26]. By preventing the biliary secretion of GSH, the hepatocellular concentration of GSH increases and GSH is released into the blood, moving down an electrochemical gradient. It is likely that the renal content of GSH increased following biliary ligation as a result of increased uptake of GSH from the blood [26].

In association with the increased hepatic contents of GSH induced by biliary ligation, there was increased hepatic accumulation of mercury. By contrast, biliary ligation caused a significant decrease in the renal accumulation of mercury. What is surprising, is that the reduced renal accumulation of mercury occurred under conditions where the intracellular concentration and content of GSH in the kidneys were increased. The apparent lack of a direct relationship between the renal content of GSH and the net renal accumulation of mercury under these conditions seems to contradict the notion that the renal accumulation of mercury should parallel (in a direct relationship) changes in the renal cellular concentrations of GSH. It is possible that the reduced renal accumulation of mercury detected in the rats that underwent biliary ligation is linked to the increased content of GSH and accumulation of mercury in the liver, which were increased under all pretreatment condi-

tions. We suggest that the hepatic data implicate the following: (1) that a fraction of the mercury taken up in the kidneys is derived from the liver or some process in the liver, and (2) that as a consequence of increased hepatocellular concentrations of GSH, some pool of inorganic mercury that would normally be taken up by the kidneys becomes sequestered in hepatocytes, and thus prevents this pool of mercury from ending up in the kidneys.

4.9. Relationship between the hepatic and renal concentrations of inorganic mercury

In normal animals, there appears to have been an inverse relationship between the hepatic and renal accumulation of mercury. Biliary ligation altered this relationship markedly, indicating that a critical interplay exists between the hepatic and renal handling of inorganic mercury.

Much like we discovered in our previous study [1], biliary ligation had an additive effect on reducing the net renal accumulation of mercury when it is combined with chemical pretreatments used to deplete GSH systemically. This additive effect of biliary ligation may reflect that biliary ligation reduces the renal accumulation of mercury by a mechanism different from the one involved in chemical depletion of GSH.

4.10. Conclusion

In summary, we demonstrate that chemical modulation of GSH status and biliary ligation, either individually or in combination, alter significantly the accumulation of inorganic mercury in the kidneys and liver during the initial 24 h following the injection of a non-nephrotoxic dose of mercuric chloride. Biliary ligation causes increases in both the renal and hepatic content of GSH, but causes a decrease in the renal accumulation of inorganic mercury while the hepatic accumulation of inorganic mercury is increased. Based on these associations, we postulate that the decreased renal accumulation of mercury induced by biliary ligation is linked to increased hepatic retention of mercury. We also conclude that chemical depletion of renal GSH is associated with a net decrease in the renal accumulation of mercury, although there are temporal components and different mechanisms associated with the various means by which renal GSH is depleted. Some aspects of the reduction in the renal accumulation of inorganic mercury induced by chemical depletion of GSH may also be linked to hepatic processing of GSH and/or inorganic mercury.

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