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DIQUAT- AND ACETAMINOPHEN-INDUCED ALTERATIONS OF BILIARY EFFLUX OF IRON IN RATS

ARTHUR E. BENZICK, SUDHESHNA L. REDDY, SANJIV GUPTA, LYNETTE K. ROGERS and CHARLES V. SMITH*

Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, U.S.A.

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Abstract—The effects of diquat on the biliary efflux of nonheme iron in rats were studied as a means of examining the possible effects of diquat metabolism on hepatocellular iron metabolism and the association of altered iron metabolism with the initiation of acute hepatic necrosis. Administration of hepatotoxic doses (0.1 mmol/kg) of diquat to male Fischer-344 rats increased biliary iron concentrations from 6 μM to more than 15 μM. However, increases in biliary efflux of iron were not observed during the first 60 min following exposure to diquat, despite the rapid increases in biliary glutathione disulfide concentrations, which increased maximally within 40 min. Biliary efflux of iron was not altered by diquat in Sprague–Dawley rats, which are resistant to hepatic necrosis in response to diquat, despite the marked oxidant stress responses observed in these animals. Conversely, hepatotoxic doses of acetaminophen (1500 mg/kg) caused significant *decreases* in biliary iron efflux. The rapid decreases in biliary iron caused by acetaminophen and the delay in diquat-induced iron efflux suggested the possibility that some fraction of the biliary iron was being excreted as reversibly formed GS-Fe^{2+} chelates, with inhibition of export by glutathione disulfide (GSSG) in the case of diquat, or by 3-(glutathion-*S*-yl)-acetaminophen (GS-AAP) in the case of the acetaminophen-treated animals. However, 50–200 mg/kg doses of acetaminophen showed little effect on biliary iron excretion despite producing biliary GS-AAP conjugate concentrations almost 1000 times the 6 μM concentrations of iron, which would not appear to support the hypothesis of excretion of GS-Fe^{2+} chelates. The data demonstrate a significant effect of diquat on hepatic iron metabolism in Fischer-344 rats, and the possible importance of this iron redistribution to reactive oxygen-mediated cell damage *in vivo* is indicated by the absence of similar responses in diquat-treated Sprague–Dawley rats.

Key words: diquat; acetaminophen; iron; biliary excretion; glutathione; glutathione disulfide

Reactive oxygen species are thought to contribute significantly to many pathologies, presumably through the cellular dysfunction caused by chemical alterations of critical biological molecules [1, 2]. For this reason, identification of the alterations caused by exposure of biological systems to reactive oxygen species and understanding the pathophysiological consequences of the respective transformations are goals of biomedical research that have broad implications for the study of many human diseases. The hepatic necrosis that is produced in Fischer-344 rats by administration of diquat appears to be mediated by reactive oxygen species generated by cyclic reduction and oxidation of the bipyridylium [3]. Diquat-treated Fischer-344 rats thus offer a useful experimental model for the study of reactive oxygen-mediated acute tissue damage *in vivo* [4, 5].

Increased production of (GSSG)† is probably the most frequently employed index of exposure of cells to reactive oxygen species, but cells can withstand truly remarkable increases in disulfide production without loss of viability, particularly *in vivo* [3]. In contrast, rather modest amounts of reactive oxygen can become lethal if the normally tight control over redox-active iron chelates is defeated [6–8]. Cellular iron in excess of immediate requirements is stored primarily in the form of ferritin, and this iron can be liberated for other cellular functions through reduction of the ferritin-bound Fe^{3+} form [9].

Thomas and Aust [10] showed that diquat stimulates the release of iron from ferritin *in vitro* through the transfer of electrons from NADPH-cytochrome P450 reductase. Subsequently, Reif *et al.* [11] showed that administration of diquat to rats causes a net loss of ferritin-bound hepatic iron, without a decrease in ferritin protein [11]. However, the latter study examined effects observed 36 hr after drug exposure, whereas many of the toxicologically important interactions occur much earlier [5, 7]. Release of ferritin-bound iron to intracellular pools could provide a source of chemically reactive iron chelates if the rates of release exceed the capacities for controlled disposition or protected binding of the iron. Gregus and Klaassen [12] proposed the existence of a similar pool of hepatocellular iron based upon their observation that the fraction of an i.v. dose of iron that was excreted into the bile increased with increasing dose. The latter study further suggests that intracellular iron in excess of normal binding capacities, which might reasonably be expected to be less protected from relatively unregulated redox activities, might also be preferentially excreted into the bile.

* Corresponding author: Charles V. Smith, Ph.D., Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Tel: (713) 798-3556; FAX: (713) 798 5691.

† Abbreviations: GSSG, glutathione disulfide; GS-AAP, 3-(glutathion-*S*-yl)-acetaminophen; and GSH, glutathione.

We therefore investigated the effects of diquat on the biliary excretion of iron in Fischer-344 and Sprague–Dawley rats as a means of assessing the short-term effects of the redox cycling metabolism of diquat on intrahepatic iron homeostasis and the possible toxicological relevance of any such effects.

MATERIALS AND METHODS

Male Fischer 344 and Sprague–Dawley rats (200–350 g) were purchased from Harlan-Sprague Dawley and maintained in an air-conditioned room on a 12 hr light/dark cycle. Food and water were available *ad lib*. Diquat was provided by Dr. Ian Wyatt of Imperial Chemical Industries (Macclesfield, Cheshire, England). Other reagents were obtained from either the Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Houston, TX). Polyethylene tubing was purchased from Clay Adams.

Animal studies were initiated between 8:00 and 10:00 a.m. to minimize possible effects of diurnal variations. Animals were anesthetized by administration of sodium pentobarbital (50 mg/kg, i.p.). Bile ducts were cannulated with polyethylene tubing for timed collections in tared tubes that contained 100 μL of 0.1 M phosphoric acid. Diquat and acetaminophen were dissolved in saline and administered i.p. after two collections of 20 min each, with the time of administration defined as 0 min. Biliary GSH and GSSG were measured enzymatically using an HP 8452A diode array spectrophotometer by Tietze-derived methodology as previously described [7].

Biliary iron was measured using a modification of the method of Artiss *et al.* [13]. For these determinations, 200 μL of bile was added to 200 μL of the trichloroacetic acid-ascorbate-thiourea reagent described by Artiss. The samples were vortexed and centrifuged for 15 min in a Fisher model 235C Micro Centrifuge, and 350 μL of the resulting supernatant was removed and mixed with 100 μL of ammonium acetate and 250 μL of deionized water. The absorbance of this mixture was measured at 594 nm before and after addition of 20 μL of the ferene S reagent, prepared as described by Artiss. A time period of 6 min was allowed for complete color development. The concentration of iron in the biological sample was calculated by comparison with $FeCl_3$ standards prepared in 0.1 N HCl. The correction of the final measured absorbance for the absorbance at 594 nm in the absence of ferene-S permits correction for possible interfering substances in the biological samples and improves the selectivity and, therefore, the accuracy of the assay, particularly at low concentrations.

Concentrations of GS-AAP conjugate were measured by reverse phase HPLC with detection of absorbance at 254 nm [14]. Peak areas of conjugate were compared with standard curves obtained by injection of known amounts of parent drug.

Data are given as means ± SEM. Statistical comparisons were performed using ANOVA followed by Newman–Keuls, with significance assigned at $P < 0.05$ [15].

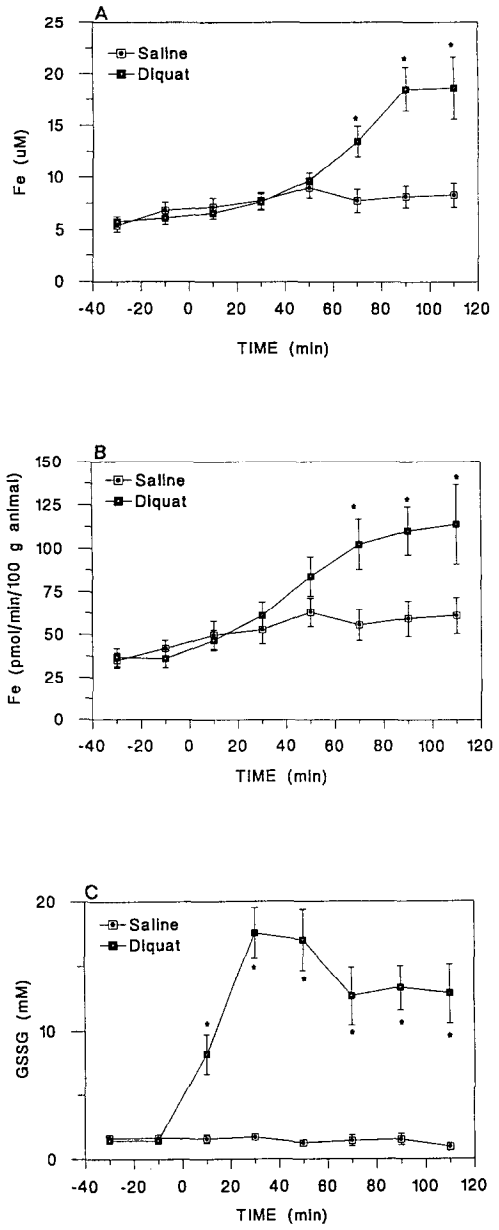

Fig. 1. Effect of diquat on biliary efflux of iron and GSSG. Male Fischer-344 rats were anesthetized with pentobarbital, and bile was collected. After two baseline collections, animals were given 0.1 mmol/kg of diquat or equal volumes of saline, i.p., at 0 min. Biliary nonheme iron contents were measured with ferene-S, as described in Materials and Methods. Data in this and in subsequent figures are plotted at the midpoints of the 20-min periods of collection of the bile samples, but it should be recognized that the data represent average rates and concentrations over the collection periods. The biliary iron concentrations (A) and efflux rates (concentrations times bile flow rates) (B) in the diquat-treated animals were different from saline-treated controls, $P < 0.05$, after 60 min (*). Biliary concentrations of GSSG, measured by a modification [7] of the method of Tietze, as described in Materials and Methods and shown in C, were significantly different from control ($P < 0.05$) in all samples collected after administration of diquat (*). Data are given as means ± SEM of 15 animals per group, and the data were evaluated statistically by ANOVA Newman–Keuls.

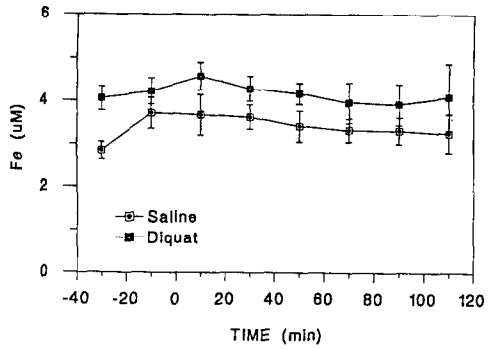

Fig. 2. Effect of diquat on biliary iron concentrations in Sprague–Dawley rats. Biliary iron concentrations were measured in samples from male Sprague–Dawley rats before and after administration of 0.1 mmol/kg of diquat or equal volumes of saline, i.p., at 0 min. Data are means ± SEM of 3 control and 4 diquat-treated rats. Increased concentrations of GSSG in these bile samples verified significant diquat-induced oxidant stress responses in these animals (data not shown), but biliary iron concentrations were not statistically different (at $P < 0.05$) in the animals given diquat.

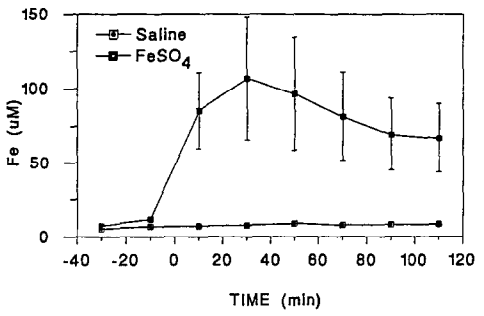

Fig. 3. Effect of i.p. administration of ferrous sulfate on biliary iron concentrations. Biliary iron contents were measured in Fischer-344 rats before and after i.p. administration of 0.36 mmol/kg of $FeSO_4$ or equal volumes of saline, i.p., at 0 min. Data are given as means ± SEM for 3 control rats and 4 rats given $FeSO_4$. The effects of ferrous sulfate were statistically significant ($P < 0.05$) at all time points following administration.

RESULTS

Biliary iron concentrations in Fischer-344 rats averaged 6 μM and administration of 0.1 mmol/kg of diquat to Fischer-344 rats increased biliary iron concentrations (Fig. 1A) and rates of efflux (Fig. 1B, concentrations times rates of bile flow), but the observed increases were noticeably delayed relative to the rapid increases in biliary GSSG concentrations (Fig. 1C). The biliary iron concentrations of diquat-treated rats increased to around three times the control values by 90 min, whereas biliary GSSG concentrations rose during the collection of the first sample following diquat administration and reached the respective maximum levels within the first 40 min. A trend toward somewhat higher bile flow rates after diquat resulted in the minor differences in the time profile of iron efflux when viewed as rates. Administration of 0.1 mmol/kg of diquat to male Sprague–Dawley rats did not alter biliary export of iron (Fig. 2), despite the clear demonstration of drug-stimulated redox activity and marked oxidant stress that we have reported previously [3] and which was documented in these specific animals by observed increases in biliary GSSG contents (data not shown).

The administration of ferrous sulfate i.p. to Fischer-344 rats resulted in rapid increases in biliary iron concentrations (Fig. 3). Similar increases were observed in Sprague–Dawley rats given ferrous sulfate (data not shown). Diquat enhances expiration of ethane and pentane in Fischer-344 rats [7], which suggests drug-induced release of bound iron to a pool of more chemically reactive iron chelates with subsequent stimulation of β-scission of the corresponding lipid hydroperoxides [16]. The diquat-induced increases in alkane expiration are significant by 30 min and are maximal within 60 min, and the responses are potentiated in animals pretreated acutely with ferrous sulfate [7]. However, the rapid response of the biochemical markers suggestive of redox-active iron (ethane and pentane) contrast with the lag observed in biliary iron excretion (Fig. 1). One possibility for this difference in the time courses was that the mechanisms for excretion of iron from the pool of chemically reactive chelates function more slowly than do the mechanisms for biliary export of GSSG. The rapid increases in biliary iron content of the ferrous sulfate-treated rats (Fig. 3) suggest that the delays in the increases in biliary iron observed in the rats treated with diquat (Fig. 1, A and B) are not readily attributable to slower rates of excretion of iron from the redox-active intracellular pool. However, the involvement of multiple pools and routes of transfer of iron to bile in the ferrous sulfate-treated animals is certainly possible, and additional studies will be needed.

The diquat-induced increases in biliary iron efflux also could have been secondary to tissue injury. With the initial intent of testing this hypothesis, we examined the effects of a second hepatotoxin, acetaminophen, on biliary release of iron in Fischer-344 rats. Administration of a dose (1.5 g/kg) of acetaminophen that causes hepatic injury comparable in magnitude to that caused by 0.1 mmol/kg of diquat [3, 17] produced a rapid decline in biliary iron concentrations (Fig. 4A). Addition of known amounts of iron to bile samples from acetaminophen-treated rats showed the expected increases in measured iron content, indicating that the effects we observed were not due to inhibition of our assay method by metabolites of acetaminophen. Biliary iron excretion rates also decreased in these animals (Fig. 4B), indicating that the decreases in concentration were not simply due to dilution by iron-poor bile during acetaminophen-induced choleresis.

Dose–response studies on the effects of acetaminophen revealed that doses as low as 200 mg/kg could produce statistically significant decreases in biliary iron concentrations (Fig. 5A). Moderate effects were

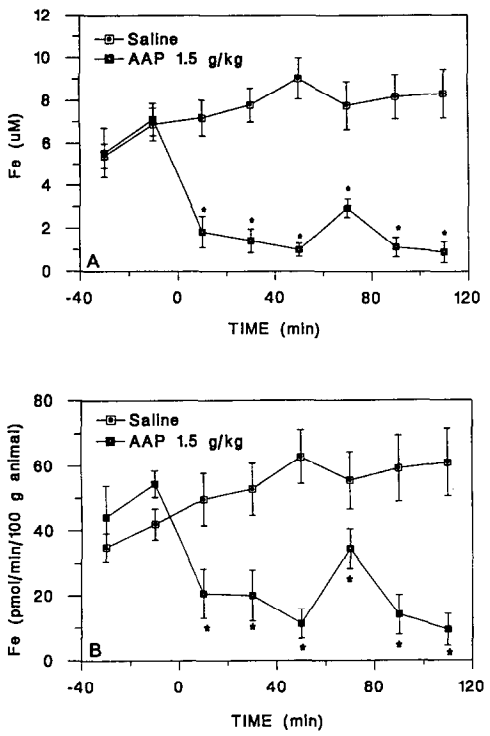

Fig. 4. Effects of hepatotoxic doses of acetaminophen on biliary iron concentrations and rates of efflux. Biliary iron concentrations (A) and rates of efflux (B) are shown for Fischer-344 rats given 1.5 g/kg of acetaminophen or saline, i.p., as indicated. Data are means ± SEM for 15 control and 4 acetaminophen-treated animals, respectively. By ANOVA Newman–Keuls, the effects of acetaminophen were statistically significant ($P < 0.05$) at all time points following administration of drug (*).

observed following 50 or 100 mg/kg doses, but the effects were not uniformly different from the saline-treated animals. The administration of acetaminophen at 50, 100, or 200 mg/kg did produce significant increases in bile flow in these animals (data not shown), and the biliary iron efflux rates were not statistically different in the animals given acetaminophen (Fig. 5B). The effects of the 200 mg/kg dose approached statistical significance (at $P < 0.05$) at some time points, but the major effect of the lower doses of acetaminophen on biliary iron concentrations appeared to be one of dilution. Biliary GSH concentrations were not depleted substantially in these animals (Fig. 5C), indicating that decreased biliary iron concentrations were not likely to be due to depletion of intrahepatic GSH to levels low enough to compromise the formation of a hypothetical GS-Fe chelate with intracellular iron. Biliary GSSG concentrations were significantly lower in animals given the highest dose of acetaminophen than in the saline-treated controls (Fig. 5D).

The biliary concentrations of the *S*-glutathionyl conjugate of acetaminophen, measured in these animals, are presented in Fig. 6. The data show that even the lowest dose (50 mg/kg) of acetaminophen results in millimolar concentrations of GS-AAP in bile. The lack of a change in the iron export processes that lead to concentrations in the low micromolar range, during the export of GS-AAP conjugate at concentrations that are almost three orders of magnitude greater, would not appear to support the idea that the effects of acetaminophen reflect competitive inhibition of the excretion of a GS-Fe^{2+} chelate [18].

DISCUSSION

The diquat-induced increases in biliary iron concentrations and rates of efflux (Fig. 1) demonstrated a drug-induced change in hepatic iron homeostasis that is consistent with a stimulation of release of iron from bound stores and subsequent biliary excretion of some part of this iron. However, the delay in increased biliary efflux of iron relative to the rapid increase in diquat-induced redox stresses indicated by efflux of GSSG (Fig. 1C) suggests the involvement of other factors or mechanisms.

One possible reason for the delay in iron excretion relative to increased redox activity is that the biliary excretion of iron from the putative intracellular pool of redox-active iron is a measurably slower process. In Fischer-344 rats, ethane and pentane expiration are elevated significantly within 30 min following administration of diquat [7]. Inasmuch as the most probable mechanism for the production of ethane and pentane *in vivo* is through the iron-catalyzed β-scission of the corresponding lipid hydroperoxides, the rapid rise in alkane expiration indicates a similarly rapid increase in the intracellular availability of reactive iron chelates following administration of diquat. Intraperitoneal administration of ferrous sulfate was found to potentiate the diquat-induced increases in ethane and pentane expiration [7], suggesting that at least some of the administered iron accessed the pool of reactive chelates. In the present studies, similar i.p. doses of ferrous sulfate were found to increase biliary iron efflux (Fig. 3), but without the delay seen with diquat-induced effects on endogenous iron (Fig. 1). These data do not support the idea that the time-course of increased biliary iron efflux after diquat is the result of an inherent slowness of the process of excretion of iron from the redox-active pool. However, other pools or routes of transport [19] of the exogenous iron to the bile may obscure the relevant time-course response through functional intracellular pools.

The possibility that the diquat-induced increase in biliary iron efflux was a result of cell damage rather than a marker of a mechanism contributing to initiation of injury also was considered. Therefore, with the initial intent of testing this hypothesis, we examined the effects of comparably damaging doses [3, 17] of a mechanistically different hepatotoxin. The rapid decrease in biliary iron excretion caused by acetaminophen (Fig. 4) did not resolve the initial hypothesis, but did suggest the possibility of the involvement of GS-dependent mechanisms in the cellular export of the iron. The involvement of the GS-conjugate export pump would explain the effects of the hepatotoxic doses of acetaminophen, because the formation and excretion of the GS–acetamino-

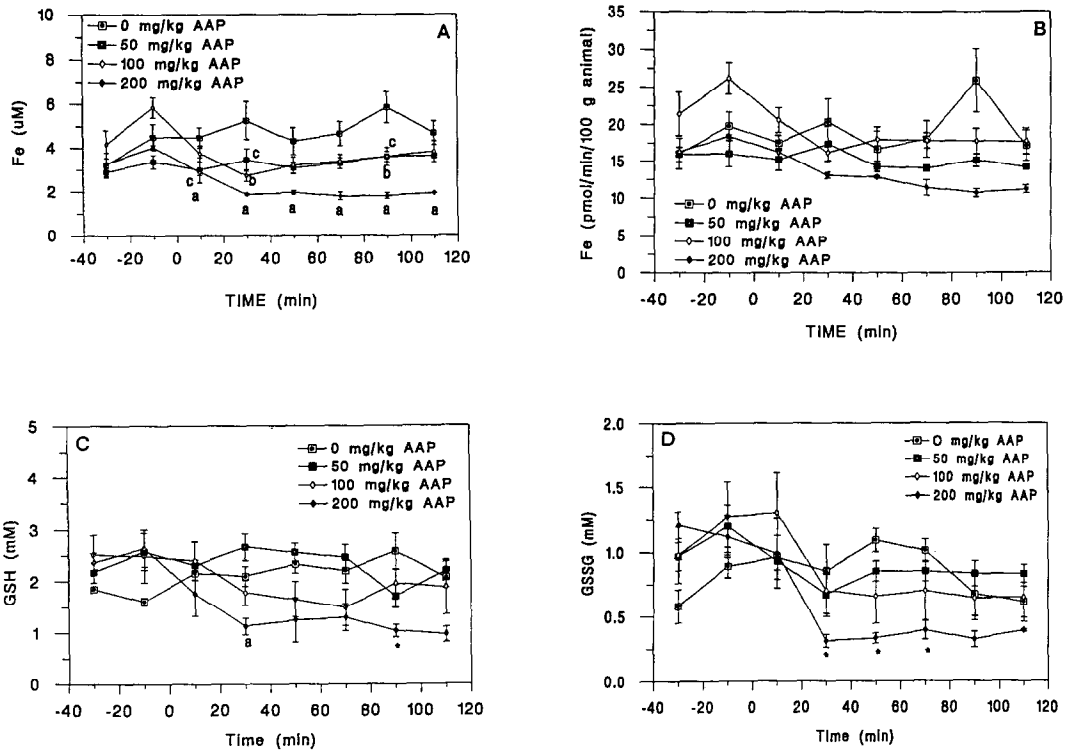

Fig. 5. Effects of moderate doses of acetaminophen on biliary excretion of iron and glutathione. Biliary iron concentrations (A) and rates of efflux (B) are shown for male Fischer-344 rats given the indicated doses of acetaminophen (0, 50, 100, or 200 mg/kg), i.p., at 0 min. Iron concentrations were significantly different ($P < 0.05$) in the animals given 200 mg/kg of acetaminophen (**a**) than in controls. The effects of 100 mg/kg (**b**) and 50 mg/kg (**c**) of acetaminophen on biliary iron concentrations were statistically significant ($P < 0.05$) in some time periods. No statistical differences in the rates of iron efflux (B) were indicated by ANOVA. Biliary GSH concentrations (C) in the animals given 200 mg/kg of acetaminophen were not statistically different ($P < 0.05$) from the animals given 50 mg/kg or saline except for points labeled (**a**) and (*) respectively. Biliary GSSG concentrations (D) differed significantly from control (*$P < 0.05$) at the 200 mg/kg dose of acetaminophen from 20 to 80 min.(*) Rates of efflux of GSH and GSSG were not altered significantly in these animals by the doses of acetaminophen employed (data not shown). Data in all panels are given as means ± SEM for 4–7 animals per group.

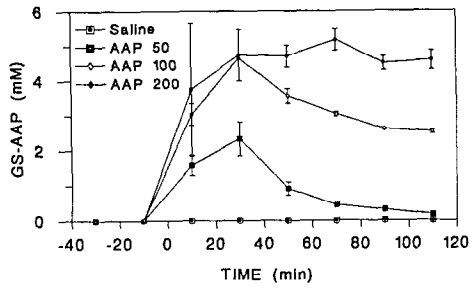

Fig. 6. Biliary concentrations of the *S*-glutathionyl conjugate of acetaminophen (GS-AAP). Biliary concentrations were measured by HPLC in samples collected from the Fischer-344 rats, described in Fig. 5, given up to 200 mg/kg of acetaminophen. Data are means ± SEM of 4–7 animals per group.

phen conjugate are rapid enough (Fig. 6) to account for the time-course of the decreased biliary iron export caused by hepatotoxic doses of acetaminophen (Fig. 4). The involvement of the conjugate export pump also would offer an explanation for the lag in biliary excretion of iron following administration of diquat (Fig. 1), because the rapid increase in hepatic formation and biliary export of GSSG [7], which has been shown to compete with other GS-conjugates for pump activity [18], could suppress biliary export of intracellular GS-Fe chelates.

The GSH-dependent biliary excretion of other transition metals has been described by a number of investigators, including the recent reports from Gyurasics *et al.* [20, 21]. These investigators showed that intravenous administration of soluble salts of antimony, arsenic, and bismuth results in biliary excretion of the metals and in the stimulation of biliary GSH efflux in parallel. They further showed that substrates for GSH conjugation and subsequent biliary excretion by the GS-conjugate export pump, bromosulfophthalein and diethyl maleate, inhibit

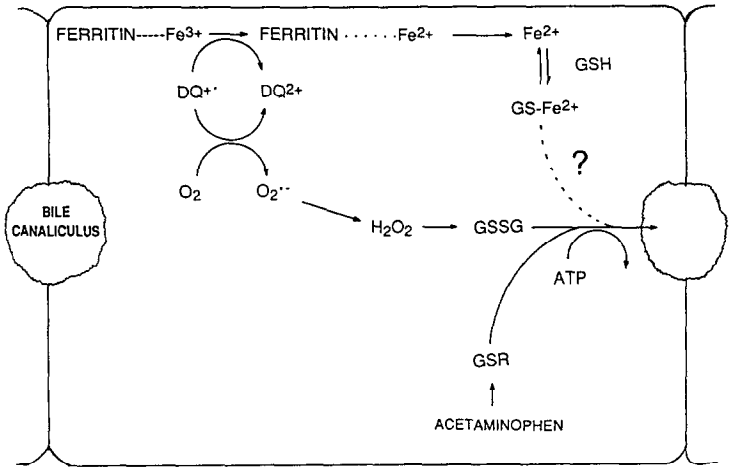

Fig. 7. Scheme indicating possible sites of drug-induced alterations in intrahepatic iron metabolism that could be reflected in altered biliary concentrations or rates of efflux of nonheme iron. The data presently available do not support the significant contributions of GS-Fe excretion to the biliary export of nonheme iron as depicted.

metal excretion and prevent the metal-induced increases in biliary GSH concentrations. Their data strongly suggest the reversible formation of GS–metal chelates that appear to be recognized by the GS-conjugate export pump. Following excretion into the bile, the thiol ligands can dissociate, particularly under conditions for measurement of GSH. Although free iron would not be expected to show a high affinity for GSH, the stability of iron complexes with thiol ligands is greater for Fe^{2+}, which is a softer electrophile than is Fe^{3+} [22], and complex stability could be enhanced further by the stereoelectronic influences of the other ligands in the presumably hexacoordinate complex.

The lack of an increase in biliary iron efflux in diquat-treated Sprague–Dawley rats (Fig. 2), despite the marked intrahepatic redox stress observed in these animals in the form of GSSG production, further suggests the release of iron to redox active intracellular pools as a significant step in initiation of lethal injury by reactive oxygen species because of the resistance of Sprague–Dawley rats to diquat-induced hepatic damage [3, 23]. Additional evidence that altered iron metabolism is important to the initiation of diquat-induced hepatic injury is provided by recent studies in which we observed hepatic injury in diquat-treated Sprague–Dawley rats if those rats were pretreated with ferrous sulfate (Gupta *et al.*, unpublished).

Additional support for a critical role for redox-active iron in diquat-induced cell injury has been published recently by Rikans and Cai [4] who reported a greater cytotoxicity of diquat to hepatocytes isolated from old Fischer-344 rats than hepatocytes isolated from younger mature animals. The difference in susceptibilities that they observed did not appear to be due to rates of GSH depletion or inherent sensitivities to iron-induced lipid peroxidation, which were the same in both groups of hepatocytes, or in the rates of diquat-induced microsomal generation of superoxide, which were significantly lower in the older animals. A greater diquat-induced formation of thiobarbituric acid reactive substances (TBARS) was observed in the hepatocytes from the older rats, and these investigators speculate that differences in the availability of iron to react with diquat-generated hydrogen peroxide may account for the increased sensitivity of the hepatocytes from the older rats.

In another study, Rikans *et al.* reported greater concentrations of nonheme iron and of a low molecular weight chelatable pool of iron in hepatocytes from older rats [24], but did not characterize the possible effects of diquat on intracellular redistribution of iron. We found no significant difference in hepatic nonheme iron in Fischer-344 and Sprague–Dawley rats [25], but we did find that administration of diquat results in measurable redistribution of nonheme iron in the Fischer rats, whereas no significant changes are observed in the Sprague–Dawley rats. In related studies, we have also found Fischer-344 rats to be more susceptible to hyperoxic lung injury than are Sprague–Dawley rats [26], which again coincides with a measurable redistribution of nonheme iron content in the lungs of the Fischer rats during hyperoxic exposure, whereas no similar change was observed in the Sprague–Dawley rats [27].

The data presently available do not enable us to distinguish between cause and effect in the relationship between altered iron metabolism and reactive oxygen-mediated hepatic injury in the diquat-treated animals, but this uncertainty is similar to that encountered in the numerous studies in which lipid peroxidation has been invoked as a causal mechanism of cell damage. The ideas of cause versus effect are not necessarily conflicting, because both iron metabolism and lipid peroxidation probably are affected by the processes associated with cell damage,

and both are capable of contributing to the expansion or progression of the injury.

In view of the apparent importance of careful control of intracellular iron homeostasis in the essential functioning of cellular antioxidant defense mechanisms [2, 6, 28, 29], and the clearly recognized difficulties in experimentally examining the flux of iron through the critical pool(s), thought to consist mainly of low molecular weight chelates in transit between cellular sites of uptake, utilization, storage, or excretion [30], the differences in the abilities of Sprague–Dawley and Fischer-344 rats to manage iron homeostasis in the presence of at least two quite distinctive redox challenges may offer important clues to the optimal functioning of this apparently critical element of cellular defense. The present data do not disprove completely the possible involvement of GS-dependent mechanisms in some aspect of iron excretion, but the absence of any appreciable effect on iron export (Fig. 5) in response to doses of acetaminophen that yield millimolar concentrations of GS conjugate in the bile (Fig. 6) do not support hypotheses of GS–Fe chelate extrusion by the GS-conjugate export pump, such as is illustrated in Fig. 7.

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REFERENCES

1. Smith CV, Free radical mechanisms of tissue injury. In: *Free Radical Mechanisms of Tissue Injury* (Eds. Moslen MT and Smith CV), pp. 1–22. CRC Press, Boca Raton, FL, 1992.
2. Kehrer JP, Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol* **23**: 21–48, 1993.
3. Smith CV, Hughes H, Lauterburg BH and Mitchell JR, Oxidant stress and hepatic necrosis in rats treated with diquat. *J Pharmacol Exp Ther* **235**: 172–177, 1985.
4. Rikans LE and Cai Y, Age-associated enhancement of diquat-induced lipid peroxidation and cytotoxicity in isolated rat hepatocytes. *J Pharmacol Exp Ther* **262**: 271–278, 1992.
5. Petry TW, Wolfgang GHI, Jolly RA, Ochoa R and Donarski WJ, Antioxidant-dependent inhibition of diquat-induced toxicity *in vivo*. *Toxicology* **74**: 33–43, 1992.
6. Minotti G, Sources and role of iron in lipid peroxidation. *Chem Res Toxicol* **6**: 134–146, 1993.
7. Smith CV, Evidence for the participation of lipid peroxidation and iron in diquat-induced hepatic necrosis *in vivo*. *Mol Pharmacol* **32**: 417–422, 1987.
8. Balla G, Vercellotti GM, Eaton JW and Jacob HS, Iron loading of endothelial cells augments oxidant damage. *J Lab Clin Med* **116**: 546–554, 1990.
9. Reif DW, Ferritin as a source of iron for oxidative damage. *Free Radic Biol Med* **12**: 417–427, 1992.
10. Thomas CE and Aust SD, Reductive release of iron from ferritin by cation free radicals of paraquat and other bipyridyls. *J Biol Chem* **261**: 13064–13070, 1986.
11. Reif DW, Beales ILP, Thomas CE and Aust SD, Effect of diquat on the distribution of iron in rat liver. *Toxicol Appl Pharmacol* **93**: 506–510, 1988.
12. Gregus Z and Klaassen CD, Disposition of metals in rats: A comparative study of fecal, urinary and biliary excretion and tissue distribution of eighteen metals. *Toxicol Appl Pharmacol* **85**: 24–38, 1986.
13. Artiss JD, Vinogradov S and Zak B, Spectrophotometric study of several sensitive reagents for serum iron. *Clin Biochem* **14**: 311–315, 1981.
14. Buckpitt AR, Rollins DE, Nelson SD, Franklin RB and Mitchell JR, Quantitative determination of the glutathione, cysteine, and *N*-acetyl cysteine conjugates of acetaminophen by high-pressure liquid chromatography. *Anal Biochem* **83**: 168–177, 1977.
15. Zar JH, *Biostatistical Analysis*, 2nd Edn. Prentice-Hall, Englewood Cliffs, NJ, 1984.
16. Smith CV and Reilly MH, Formation of pentane versus 1-pentanol in the ferrous sulfate initiated decomposition of 15-hydroperoxyeicosatetraenoic acid in hypoxic and hyperoxic conditions. *Biochem Pharmacol* **38**: 1362–1364, 1989.
17. Smith CV and Mitchell JR, Acetaminophen hepatotoxicity *in vivo* is not accompanied by oxidant stress. *Biochem Biophys Res Commun* **133**: 329–336, 1985.
18. Lauterburg BH, Smith CV, Hughes H and Mitchell JR, Biliary excretion of glutathione and glutathione disulfide in the rat: Regulation and response to oxidative stress. *J Clin Invest* **73**: 124–133, 1984.
19. Ballatori N, Mechanisms of metal transport across liver cell plasma membranes. *Drug Metab Rev* **23**: 83–132, 1991.
20. Gyurasics A, Koszorus L, Varga F and Gregus Z, Increased biliary excretion of glutathione is generated by the glutathione-dependent hepatobiliary transport of antimony and bismuth. *Biochem Pharmacol* **44**: 1275–1281, 1992.
21. Gyurasics A, Varga F and Gregus Z, Effect of arsenicals on biliary excretion of endogenous glutathione and xenobiotics with glutathione-dependent hepatobiliary transport. *Biochem Pharmacol* **41**: 937–944, 1991.
22. Pearson RG, Hard and soft acids and bases. *J Am Chem Soc* **85**: 3533–3539, 1963.
23. Smith CV, Effect of BCNU pretreatment on diquat-induced oxidant stress and hepatotoxicity. *Biochem Biophys Res Commun* **144**: 415–421, 1987.
24. Rikans LE, Cai Y, Kosanke S and Venkataraman PS, Redox cycling and hepatotoxicity of diquat in aging male Fischer 344 rats. *Drug Metab Dispos* **21**: 605–610, 1993.
25. Smith CV, Gupta S, Chang A, Benzick AE and Rogers LK, Diquat-induced oxidant stress and hepatic necrosis in rats *in vivo* and the redistribution of hepatic iron. *Toxicol Lett* **Suppl**: 219, 1992.
26. Stenzel JD, Welty SE, Benzick AE, Smith EO, Smith CV and Hansen TN, Hyperoxic lung injury in Fischer-344 and Sprague–Dawley rats *in vivo*. *Free Radic Biol Med* **14**: 531–539, 1993.
27. Stenzel JD, Welty SE, Hansen TN and Smith CV, Subcellular iron localization in lungs of Fischer-344 (F344) and Sprague–Dawley (SD) rats exposed to >95% oxygen. *Pediatr Res* **33**: 42, 1993.
28. Rothman RJ, Serroni A and Farber JL, Cellular pool of transient ferric iron, chelatable by deferoxamine and distinct from ferritin, that is involved in oxidative cell injury. *Mol Pharmacol* **42**: 703–710, 1992.
29. Ryan TP and Aust SD, The role of iron in oxygen-mediated toxicities. *Crit Rev Toxicol* **22**: 119–141, 1992.
30. Crichton RR and Ward RJ, Iron metabolism—New perspectives in view. *Biochemistry* **31**: 11255–11264, 1992.