Differential Combined Effect of Cadmium and Nickel on Hepatic and Renal Glutathione S-Transferases of the Guinea Pig

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When male guinea pigs were given a single dose of Cd (2.0 mg Cd^{2+}/kg, ip) 72 hr prior to sacrifice, the hepatic reduced glutathione (GSH) level did not change although glutathione S-transferase (GST) activities toward the substrates 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), ethacrynic acid (EAA), and 1,2-epoxy-3-(*p*-nitrophenoxy) propane (ENPP) increased significantly as compared to controls. Cd did not change the renal GSH level and GST activities toward CDNB and EAA. However, significant increase was observed in the GST activity for DCNB whereas GST activity for ENPP was significantly inhibited by Cd. When the animals were given a single dose of Ni (14.8 mg Ni^{2+}/kg, sc) 16 hr prior to sacrifice, significant increases were observed in hepatic GSH level and GST activities toward CDNB, DCNB, EAA and ENPP. Ni, however, depressed the renal GSH level and GST activities toward CDNB, DCNB and ENPP significantly. The renal GST activity toward EAA remained unaltered. For the combined treatment, guinea pigs received the single dose of Ni 56 hr after the single dose of Cd and then they were killed 16 hr later. In these animals, no significant alteration was observed in the hepatic GSH level. The augmentation of elevation was observed in hepatic GST activities toward CDNB and DCNB. Combined metal treatment did not potentiate the elevation of hepatic GST activities toward EAA and ENPP to any greater degree. The depression of renal GSH level was significantly ameliorated by the combined treatment. Combination treatment potentiated the depression of renal GST activity for ENPP but not for CDNB. The renal GST activities toward DCNB and EAA, however, were unaltered by the combined metal treatment. These results reveal that the hepatic and renal GSTs of the guinea pig are differentially regulated by Cd or Ni alone and in combination, and that the combination of Cd and Ni does have an additive effect on hepatic and renal GSTs depending on the substrates of GSTs.—Environ Health Perspect 102(Suppl 9):69–72 (1994)

Key words: cadmium, nickel, combined treatment, liver, kidney, reduced glutathione, glutathione S-transferases, guinea pig

Introduction

The glutathione S-transferases (GSTs, EC 2.5.1.18) comprise a family of multifunctional enzymes with broadly overlapping substrate specificities, which play important roles in the detoxification of electrophilic xenobiotics primarily through conjugation to reduce glutathione (*1,2*). Similar to other xenobiotic metabolizing enzymes, the GSTs are known to be influenced by a large number of xenobiotics such as phenobarbital, polycyclic aromatic compounds, pesticides, and metals (*3–6*). Furthermore, in the same tissue (e.g., liver or kidney), differential responses among the substrates of GSTs to a great variety of xenobiotics have been well established (*6–8*). Moreover, the responses of the substrates of GSTs to several xenobiotics have been reported to be different among the various tissues of the same animal species (*6,9*).

Concerning the capacity of the metals such as cadmium (Cd) and nickel (Ni), which are widely distributed throughout the environment, to alter the reduced glutathione (GSH) level and GST activities, various studies have been reported in different animal species (*6,8,10–14*). However, in these studies, the effects of metals on GST activities are examined mainly toward the general substrate CDNB. Furthermore, rather limited data are available for the effects of metals on the kidney GSH level and GST activities (*6,9,15*). In addition, although concomitant exposure to these metals occurs in various situations (e.g., during the production of nickel-cadmium batteries or tobacco smoking) (*16,17*), the consequences of interaction of the combination of these environmental pollutants with GSH or GSTs have not been clarified yet. We therefore studied the acute combined effects of Cd and Ni on the GSH level and GST activities of the guinea pig liver and kidney, which are the known target organs for the tested metals, and compared those effects to those of Cd or Ni alone.

Materials and Methods

Chemicals

Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (EAA), 1,2-epoxy-3-(*p*-nitrophenoxy)-propane (ENPP) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO) 3,4-Dichloronitrobenzene (DCNB) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Cadmium chloride was obtained from Riedel A.G. (Germany). Nickel chloride was obtained from Merck A.G. (Germany). Crystalline bovine serum albumin was obtained from BDH Chemicals Ltd. (Poole, UK). All the other chemicals were of analytical grade.

Animals, Treatments and Tissue Preparations

Male albino (local strain) guinea pigs (250–300 g) were used throughout the experiments. guinea pigs were fed with standard laboratory chow and water *ad libitum*. The animals were divided into four groups. The first group received only 2.0 mg Cd^{2+}/kg (3.58 mg $CdCl_2.H_2O$/kg) ip in saline (2 ml/kg) 72 hr prior to sacrifice. The second group received only 14.8

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mg Ni^{2+}/kg (59.5 mg $NiCl_2.6H_2O$/kg) sc in saline (2 ml/kg) 16 hr prior to sacrifice. The third group received Cd (2.0 mg Cd^{2+}/kg, ip) in saline (2 ml/kg) 56 hr prior to Ni (14.8 mg Ni^{2+}/kg, s.c.) which was injected in saline (2 ml/kg) 16 hr prior to sacrifice. The fourth group of animals was the control animals and they received equivalent volumes of saline. The animals were killed by decapitation. After homogenization (Potter–Elvehjem) of the tissue samples, the cytosol was prepared by ultracentrifugation (100,000*g* for 50 min). The tissue extract supernatant used in the determination of GSH was prepared as described in detail previously (*8,14*).

Analytical Procedures

GSH was assayed as a nonprotein sulfhydryl according to the spectrophotometric method of Sedlak and Lindsay (*18*). The cytosolic GST activities were assayed by the spectrophotometric method of Habig et al. (*19*) using optimized conditions determined in the preliminary experiments for the substrates CDNB (1.0 mM; 1.0 mM GSH), DCNB (1.0 mM; 5.0 mM GSH), EAA (0.25 mM; 0.3 mM GSH), and EPNN (0.5 mM, liver, 0.05 mM, kidney; 5.0 mM GSH, liver, 0.5 mM GSH, kidney). Kinetics with each substrate and GSH concentration were studied to ensure linear product formation as a function of both incubation time and protein concentration. Protein content of cytosol was determined by the method of Lowry et al. (*20*) with the use of bovine serum albumin as a standard. All reported data are the mean ± SE of five animals. The data were analyzed by the Student's *t*-test and a *p* value of 0.05 denoted significance.

Results and Discussion

The previous studies have shown that maximum inhibitions were observed in the hepatic monooxygenases, the enzyme system responsible for the activation of numerous chemicals, 72 hr after ip injection of a single dose of 2.0 mg Cd^{2+}/kg to the animals (*21,22*). Studies devoted to the effect of Ni on monooxygenases, however, demonstrated that it produced maximum inhibitions 16 hr after sc administration of a single dose of 14.8 mg Ni^{2+}/kg to the animals (*8,10*). Therefore, in our previous study (*23*), we used these doses and time points in the treatment with metals either alone or in combination to find out whether the combination of metals would elicit a synergistic effect on monooxygenases in this regimen of treatment.

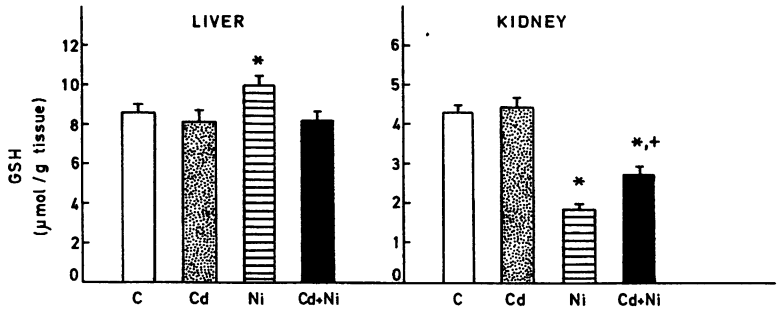

Figure 1. Effect of Cd and Ni (alone or in combination) on guinea pig hepatic and renal GSH levels. Male guinea pigs (250–300 g) were given either a single dose of Cd (3.58 mg $CdCl_2.H_2O$/kg, ip) 72 hr prior to sacrifice or a single dose of Ni (59.5 mg $NiCl_2.6H_2O$/kg, sc) 16 hr prior to sacrifice. For the combined treatment, the animals received the single dose of Ni 56 hr after the single dose of Cd and then they were killed 16 hr later. Controls received saline. The values represent the mean ± SE of five animals. *Significantly different from respective controls ($p<0.05$). +Significantly different from Cd-only treated animals and Ni-only treated animals ($p<0.05$).

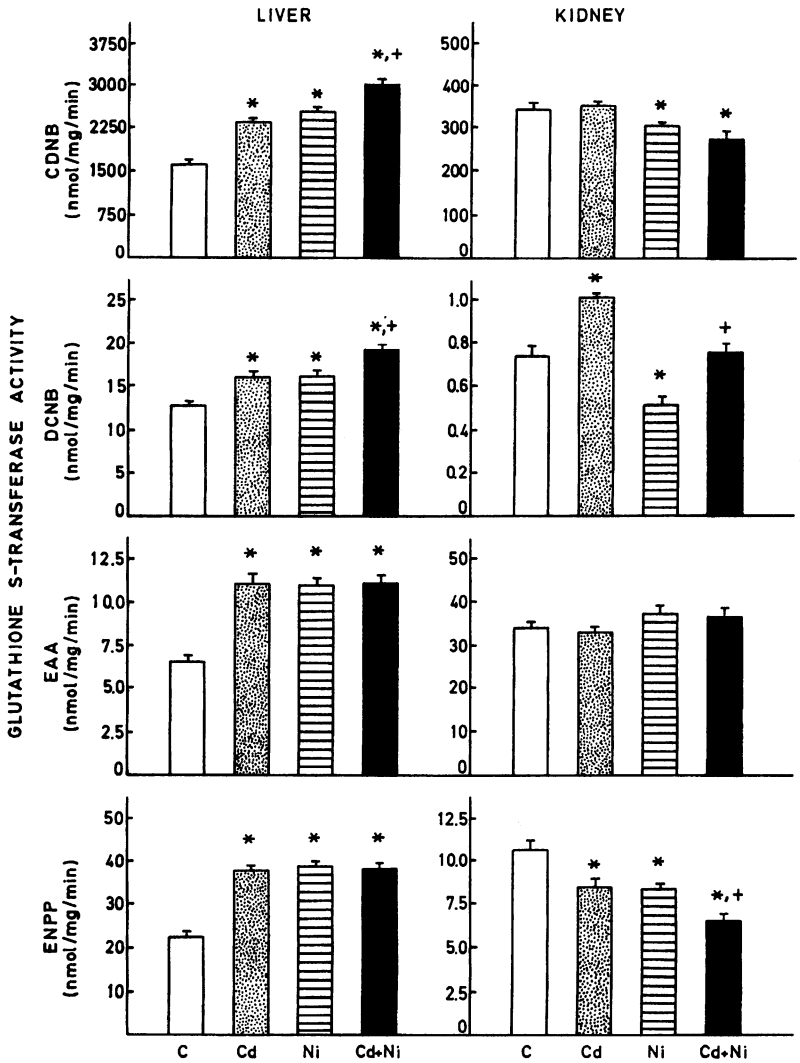

Figure 2. Effect of Cd and Ni (alone or in combination) on guinea pig hepatic and renal GST activities toward CDNB, DCNB, EAA and ENPP. Male guinea pigs (250–300g) were treated with metals (alone or in combination) as described in the legend to Figure 1. The values represent the mean ± SE of five animals. *Significantly different from respective controls ($p<0.05$). +Significantly different from only Cd-treated animals and Ni-only treated animals, respectively ($p<0.05$).

However, we observed that the combined treatment did not have synergistic effect on hepatic monoxygenases in guinea pigs (*23*). Thus, in the current study, the same regimen of treatments has been used in order to obtain the picture of the status of the hepatic and renal detoxification pathways in response to metals alone or in combination. The present study provides evidence for the differential response of hepatic and renal GSTs to metals alone or in combination.

Cd alone had no influence on liver GSH level (Figure 1). However, the GST activities toward CDNB, DCNB, EAA, and ENPP increased significantly as compared to controls (Figure 2). Similar results have been reported for other metals in other animal species (*6,8*). Cd treatment caused no alterations in the renal GSH level and GST activities toward the substrates CDNB and EAA (Figures 1,2). Significant increase, however, was observed in the GST activity for DCNB whereas GST activity for ENPP was significantly inhibited by Cd in the kidney. Although complicated by overlapping substrate specificities, the determination of several substrate activities gives insight as to the relative proportions of GST isozymes. For example, GST activities for DCNB, EAA, and ENPP are relatively specific measures of rat liver isozymes 3–3, 7–7, and 5–5, respectively (*1,2*). The CDNB, however, is the most important substrate for the demonstration of multiple forms of GST in various animal species (*1,2*). In addition, these isozymes have been found to be present in the rat kidney although the levels of expressions differed from those of liver (*2,24*). On the other hand, the detailed isozymes of GSTs have not been purified from livers and kidneys of guinea pigs yet, although few attempts of this type have been made regarding in the liver (25,26). Nevertheless, based on the evidence so far available that homologous enzymes of the animal species have similar substrate specificities (*1,2*), the examined GSTs of guinea pigs could be homologous to those of the other animal species, such as rats. Accordingly, these findings seem to indicate that the hepatic isozymes of GSTs are regulated similarly by Cd whereas renal isozymes of GSTs are differentially regulated by Cd.

Ni appeared to have rather different patterns of influence on GSH of liver and kidney than Cd. Ni significantly increased the hepatic GSH level (Figure 1). The elevation of hepatic GSH level might reflect the enhanced *de novo* synthesis of GSH as was reported for rats (*10*). Ni also significantly increased the hepatic GST activities toward all the substrates studied (Figure 2). This could be due to the activation of resting enzyme molecules or stimulation of GST synthesis. In contrast, Ni depressed the GSH level significantly in the kidney (Figure 1). The reason for the GSH depletion is not clear. However, this could result from enhanced biliary excretion of Ni-GSH complexes (*27*), interference of the metal with the GSH metabolism, and increase in the utilization of GSH in the removal of lipid peroxides, since Ni has been shown to increase lipid peroxidations in other animal species (*28*). An alternative or additional possiblity could be the increase in the rate of GSH oxidation. The significant depressions were also noted in the GST activities for CDNB, DCNB, and ENPP by Ni. The renal GST activity toward EAA remained unaltered (Figure 2). Thus, the Ni effect on GST activities in the kidney is rather different from that observed in the liver. The direct effect of the metal on the enzymes seems to be rather remote since Ni was found to be ineffective directly on GST activities (*14,15*). Nevertheless, these results seem to reveal that isozymes of GSTs of guinea pigs are similarly regulated in liver but differentially regulated in kidney by Ni.

When the metals were administered in combination, no change was observed in the hepatic GSH level (Figure 1). However, combined treatment augmented the increase in the hepatic GST activities for CDNB and DCNB. On the other hand, the combined metal treatment did not potentiate the elevation of hepatic GST activities toward EAA and ENPP to any greater degree (Figure 2). The depression of the renal GSH level observed by Ni was significantly ameliorated by the combined treatment (Figure 1). The renal GST activities for DCNB and EAA were unaltered. The combined treatment potentiated the depression of renal GST activity for ENPP to a greater degree. The inhibition of GST activity for CDNB observed for Ni was not potentiated by the combined treatment. However, since ENPP has been shown to be a good substrate for subunit 5–5, and since this enzyme has been reported to be responsible for the detoxification of almost all epoxides in rats (*1*), it appears that the detoxifying capacity of kidney against epoxides is severely damaged when exposed to the combination of Cd and Ni. These results seem to indicate that hepatic as well as renal isozymes of GSTs of guinea pigs are differentially regulated by the combination of metals.

In conclusion, the increases in GSTs suggest the ability of liver to cope against toxic insult of Cd or Ni by increasing its detoxifying capacity. Futhermore, the combined treatment, by potentiating the enhancement of detoxifying capacity to a greater degree, seems to render the liver more resistant to their toxic effects. On the other hand, Ni, rather than Cd, by decreasing the detoxifying capacity, seems to make the kidney more vulnerable to its toxic effect. In addition, combined treatment, by diminishing the cellular defense system, is also likely to render the kidney more vulnerable to the toxic effects of metals.

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