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Effects of diallyl sulfide and diallyl disulfide on cisplatin-induced changes in glutathione and glutathione-S-transferase activity

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The effects of diallyl sulfide (DAS) and diallyl disulfide (DADS) on cisplatin-induced changes in glutathione (GSH) and glutathione-S-transferase (GST) activity in rat liver and kidney was investigated. Cisplatin treatment significantly ($p < 0.05$) decreased GSH and GST activity in both liver and kidney. DADS treatment significantly ($p < 0.05$) enhanced GSH and GST activity in rat liver and kidney. Furthermore, DADS treatment reversed the effect of cisplatin on GSH and GST activity both in liver and kidney. Administration of DADS with cisplatin could enhance GSH and GST activity and lower cisplatin-induced nephrotoxicity.

Key words: Cisplatin, diallyl disulfide, diallyl sulfide, glutathione, glutathione-S-transferase, nephrotoxicity.

Introduction

Cisplatin is one of the most effective drugs currently available for the treatment of testicular, ovarian and other cancers.[1–3] Its clinical usefulness is compromised due to its renal toxicity.[4] The nephrotoxicity induced by cisplatin is due to covalent binding of platinum to protein thiol groups.[5] A decrease in glutathione (GSH) levels and glutathione-S-transferase (GST) activity, and depression of macromolecule synthesis in the kidney may play a role in cisplatin nephrotoxicity.[6] Diallyl sulfide (DAS) and diallyl disulfide (DADS), oil-soluble organosulfur compounds present in garlic, have been shown to protect against doxorubicin-induced cardiac toxicity.[7] The purpose of the present investigation is to study the effects of DAS and DADS on cisplatin-induced changes in GSH and GST activity in rat liver and kidney.

Materials and methods

Cisplatin, glutathione (reduced form), ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), and 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (St Louis, MO). DAS and DADS were purchased from Aldrich (Milwaukee, WI). 1-Chloro-2,4-dinitrobenzene was obtained from Eastman Kodak (Rochester, NY). Other routine chemicals were purchased from Curtin Matheson Scientific (Eden Prairie, MN).

Male Sprague-Dawley rats (200–225 g) were purchased from SASCO (Omaha, NE). Rats were housed in environmentally controlled (22 ± 1°C, humidity 40–60%, light 6:00 a.m. to 6:00 p.m.) facilities. All rats had access to food and water *ad libitum*. Rats were divided in six groups having five rats in each group. Group assignments and treatments are given in Table 1. Rats were sacrificed one week after cisplatin treatment. GSH and GST activity were assayed in liver and kidney.

GSH assay

Liver and kidney from rats were homogenized (1:4) in ice-cold 5% TCA containing 1 mM EDTA. Homogenates were centrifuged at 10 000 *g* in a refrigerated centrifuge for 10 min and supernatant was used for GSH assay using a modified spectrophotmetric method.[8,9] In brief, 0.4 ml of supernatant was mixed with 4.55 ml phosphate buffer (0.1 M, pH 8.1) and 0.05 ml of 0.01 M DTNB in phosphate buffer (0.1 M, pH 7.0). The absorbance of the resulting yellow color was measured at 412 nm in a Beckman DU-64 spectrophotometer. GSH values were obtained from a standard curve.

GST assay

Liver and kidney from rats were homogenized (1:4) in ice-cold 1.15% KCl. Homogenates were centrifuged at 105 000 *g* for 60 min in a refrigerated ultracentrifuge. The supernatant obtained was used

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Table 1. Effects of cisplatin, DAS and DADS on GSH and GST activity

Group	Treatments	GSH[a] (mg/g tissue)		GST[a] (μmol/mg/min)	
		Liver	Kidney	Liver	Kidney
1	control	11.6 ± 1.6	4.6 ± 1.1	1.9 ± 0.5	1.5 ± 0.2
2	cisplatin (5 mg/kg, i.p.) one injection	8.9 ± 1.6[b] (76.5)	2.8 ± 0.9[b] (61.9)	1.1 ± 0.2[b] (59.1)	1.2 ± 0.3[b] (80.2)
3	DAS (200 mg/kg, p.o.) daily for 1 week	12.3 ± 1.2 (105.5)	3.4 ± 0.8[b] (75.1)	1.0 ± 0.7[b] (57.1)	1.3 ± 0.4 (89.8)
4	DADS (200 mg/kg, p.o.) daily for 1 week	14.6 ± 0.9[c] (125.3)	10.3 ± 0.4[c] (223.9)	3.0 ± 0.1[c] (156)	2.5 ± 0.5[c] (172.1)
5	cisplatin (5 mg/kg, i.p.) one injection and DAS (200 mg/kg, p.o.) daily for 1 week	11.3 ± 0.6 (96.9)	2.3 ± 0.2 (49.4)	1.6 ± 0.6 (82.1)	1.6 ± 0.8 (106.8)
6	cisplatin (5 mg/kg, i.p.) one injection and DADS (200 mg/kg, p.o.) daily for 1 week	18.1 ± 1.4[d] (155.8)	10.0 ± 2.4[d] (218.7)	2.2 ± 0.1[d] (116.2)	2.4 ± 0.8[d] (165.3)

Cisplatin was dissolved in normal saline. DAS and DADS were dissolved in sesame oil. Rats in the control group received an equivalent volume of appropriate vehicle.
[a]Values are mean ± SD derived from five rats. Each assay was run in triplicate. Values in parentheses indicate percent control.
[b]Significantly lower than control group ($p < 0.05$).
[c]Significantly higher than control group ($p < 0.05$).
[d]Significantly higher than cisplatin group ($p < 0.05$).

for GST assay using a spectrophotometric method.[9,10] In brief, 100 μl of supernatant was incubated with 100 μl of phosphate buffer (1 mM, pH 6.5), 100 μl of glutathione (reduced form 1 mM), 40 μl of 1-chloro-2,4-dinitro benzene (1 mM) and 660 μl of H_2O for 5 min. Absorbance was read at 340 nm using a Beckman DU-64 spectrophotometer. GST activity was calculated using extinction coefficient (ΔE) 9.6 mM^{-1} cm^{-1}.

Protein assay

Protein was assayed in samples using a Bio-Rad (Richmond, CA) kit using albumin as standard.

Statistical analysis

The software package INSTAT (GraphPad, San Diego, CA) was used to analyze the data for Student's *t*-test. Significance was considered at $p < 0.05$.

Results and discussion

The effects of cisplatin, DAS and DADS on GSH and GST activity in rat liver and kidney are presented in Table 1. Cisplatin treatment (5 mg/kg, i.p.) significantly ($p < 0.05$) decreased the GSH and GST activity in both rat liver and kidney when compared with the control group. Administration of DAS (200 mg/kg, p.o.) for 1 week did not have any significant effect on liver GSH and kidney GST activity but significantly lowered the GSH level in kidney and GST activity in the kidney. DADS treatment (200 mg/kg, p.o.) significantly ($p < 0.05$) increased GSH levels and GST activity in both liver and kidney. Daily administration of DAS to cisplatin injected rats did not significantly ($p < 0.05$) influence the GSH level and GST activity in the liver and kidney. However, daily treatment with DADS to cisplatin injected rats significantly increased the GSH and GST activity in rat liver and kidney.

Studies from our laboratory have indicated the antiperoxidant and protective effects against doxorubicin-induced cardiac toxicity by DAS and DADS, organosulfur compounds present in garlic.[7] DAS and DADS are also chemopreventive against 7,12-dimethyl benz[*a*]anthracene (DMBA)-initiated and 12-*O*-tetradecanoylphorbol acetate (TPA)-promoted skin cancer in mice.[11] Sumiyoshi and Wargovich[12] have reported the chemopreventive effects of organosulfur compounds present in onion and garlic against chemically-induced colon cancer.

The results from this investigation demonstrate that DADS significantly increases GSH and GST activity in liver and kidney. DADS also reverses the

effect of cisplatin on GSH and GST activity in liver and kidney. DADS, an organosulfur compound present in garlic, could be used to decrease the nephrotoxicity caused by cisplatin. Thus, DADS administration with cisplatin may enhance the clinical effectiveness of cisplatin. However, further studies on the effects of DADS treatment on cisplatin-induced renal toxicity are required to establish the protective effects of DADS.

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