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Effect of bismuth nitrate given in combination with *cis*-diamminedichloroplatinum(II) on the antitumor activity and renal toxicity of the latter in nude mice inoculated with human bladder tumor

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Summary. The effects of bismuth nitrate pretreatment on the toxicity and antitumor activity of *cis*-diamminedichloroplatinum (cisplatin, CDDP) were examined in nude mice that had been inoculated with human bladder-tumor tissue. Pretreatment with bismuth nitrate depressed the renal toxicity of CDDP without compromising its activity against a transplantable human bladder tumor. Renal metallothionein (MT) and bismuth (Bi) levels in nude mice were markedly increased by Bi preadministration, but no significant MT induction was observed in inoculated human bladder-tumor tissue in which only a trace amount of Bi was incorporated. Furthermore, it was confirmed that tumor platinum (Pt) concentrations in CDDP-treated mice were not affected by Bi pretreatment. Thus, the administration of Bi compounds prior to chemotherapy with CDDP may provide an effective mode of treatment for advanced bladder tumors.

Introduction

Advanced bladder tumors is generally treated with combination chemotherapy using *cis*-diamminedichloroplatinum (CDDP) [27, 28, 34–36]. However, the adverse side effects produced by platinum-based anticancer agents prevent their effective clinical application [13, 32]. Metallothionein (MT) is a cysteine-rich protein of low molecular weight that is known to be both protective against heavy metal toxicity [12, 33, 37] and highly reactive to alkylating agents [7, 8] and free radicals [17, 21–23, 26, 30, 31]. We have recently reported that the administration of bismuth (Bi) compounds prior to the injection of CDDP depresses the lethal and renal toxicity of CDDP in mice without compromising its antitumor activity [16]. The specific protection against CDDP toxicity provided by Bi preadministration may be explained by the observation that Bi induces MT in the kidney, a major target organ of CDDP toxicity, but not in the tumor tissues. Since the inducibility of MT by Bi in human tumor tissue has not yet been clarified, nude mice that had been inoculated with human bladder-tumor tissue underwent s.c. pretreatment with bismuth nitrate followed by treatment with CDDP in a study carried out to examine the induction of MT in tumor tissues and the efficacy of CDDP in depressing tumor growth.

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Materials and methods

Animals. Male Jcl/AF nude mice aged 6 weeks were purchased from Crea Japan Co. Ltd. (Tokyo, Japan). Five mice (22–24 g) were housed in a cage under specific pathogen-free conditions and were given free access to water and food.

Chemicals. CDDP was supplied by Bristol Myers Japan Co. Ltd. (Tokyo, Japan). Bismuth nitrate (BN) was purchased from Iwaki Co. Ltd. (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). CDDP and BN were dissolved in saline and distilled water, respectively, prior to their use.

Tumor. Human bladder-tumor (NMB-1) tissue was obtained during a urinary cystectomy performed in 1978 at Nippon Medical School Department of Urology (Tokyo, Japan). The NMB-1 tumor is a poorly differentiated transitional-cell carcinoma that exhibits partial squamous metaplasia, and its successful transplantation rate is almost 100% [18]. The other human urological tumors (prostatic tumor, renal-cell carcinoma, and Wilms' tumor) were also obtained during surgery at Nippon Medical School Department of Urology. These tumor cells were maintained by s.c. transplantation onto the backs of male Jcl/AF nude mice under specific pathogen-free conditions.

Treatment with drugs. BN was injected s.c. at the abdominal site once a day for 2 days. The mice were given a single i.p. dose of CDDP (25 μmol/kg) at 24 h after the last administration of BN. These BN and CDDP treatments were performed in two courses for evaluation of the activity of CDDP against human bladder tumors. To examine the induction of MT by Bi in the kidney and tumors, the animals were inoculated with various urological tumors and BN was given to the mice once a day for 2 days after the maximal diameter of the tumors had reached 10 mm.

Evaluation of antitumor activity. The effects of BN on renal and tumor MT and Bi concentrations were examined using NMB-1-inoculated nude mice. On day 0, NMB-1 was inoculated s.c. into the backs of nude mice

(Jcl/AF) aged 7 weeks; these animals also received BN (50 μmol/kg) on days 18 and 19.

The effect of BN pretreatment on the activity of CDDP against human bladder tumors was also examined in NMB-1-inoculated nude mice. Tumor tissue was inoculated s. c. into the backs of the animals on day 0. Two s. c. dose of BN were given at 24 h intervals on days 12 and 13, and CDDP was injected i. p. on day 14. This sequence of administration was repeated at 7 days after the first CDDP injection. Blood urea nitrogen (BUN) values were determined on day 26. Antitumor activity was evaluated twice a week by tumor volume according to a Battelle Columbus Laboratories protocol [19]. For each tumor, perpendicular diameters were recorded and the tumor volumes were calculated using the formula

$v = 1/2ab^2$,

where a is the maximal diameter of the tumor and b is the diameter at right angles to a. The curves were statistically analyzed using analysis of variance.

Histology. Nude mice were killed at 5 days after the last injection of CDDP. The kidneys were removed and processed by routine histology. Sections measuring 3 μm in thickness were cut and stained in hematoxylin-eosin. All slides were examined without prior knowledge of the treatment given to the animal from which the specimen under investigation was taken.

Analysis. Concentrations of MT and Bi in the kidney and tumors were determined at 24 h after the last BN injection. The MT levels in various tissues were determined using the ^{203}Hg-binding assay as modified by us [10, 11, 20]. Tissues were digested with nitric acid for the analysis of metal species. Bi was assayed by the hydride-generation method [3] using a flame atomic absorption spectrophotometer (Shimazu model AA-640-12) equipped with a Varian VGA-76 vapor-generation accessory. Platinum (Pt) in the tissues of CDDP-treated mice was measured on day 15 by furnace atomic absorption spectrophotometry (Varian SpectrAA 300/400 System). BUN values were measured spectrophotometrically using a BUN assay kit (Urea-N-test, Wako).

Results

Tissue concentrations of MT and Bi

MT and Bi contents in the kidneys and tumors of tumor-bearing nude mice are shown in Fig. 1. Levels of MT and Bi in the kidneys were significantly increased by BN administration as compared with those measured in control mice. In tumors of Bi-treated mice, only a slight increase in Bi concentration was detected, but MT levels did not change.

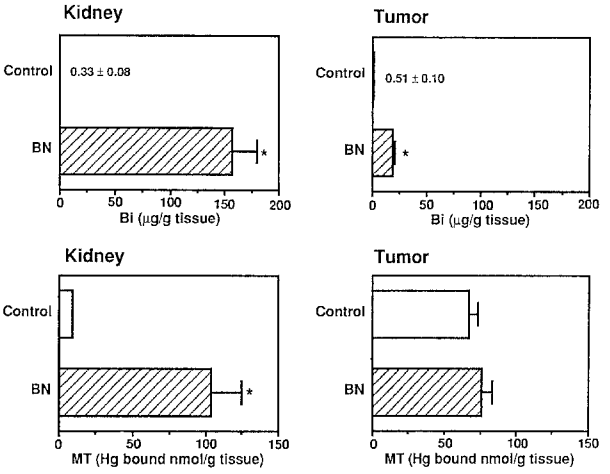

Fig. 1. Concentrations of Bi and MT in tissues of tumor-bearing mice that had been pretreated with BN. Mice (AF nude) were inoculated s. c. with human bladder-tumor tissue on day 0. Animals received s. c. injections of BN (50 μmol/kg) at 24 h intervals on days 18 and 19. Levels of Bi and MT in the kidney and tumor tissues were determined at 24 h after the last injection of BN. Each value for Bi and MT content represents the mean ± SD for 5 mice. *Significantly different from control values ($P < 0.001$)

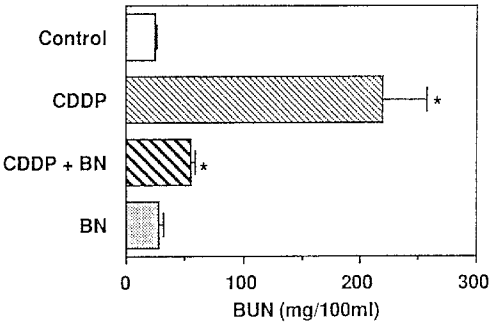

Fig. 2. Effect of BN pretreatment on CDDP renal toxicity in nude mice. Mice (AF nude) were inoculated s. c. with human bladder-tumor tissue. They were pretreated s. c. with BN (50 μmol/kg) at 24 h intervals for 2 days. At 24 h after the last BN injection, CDDP was injected i. p. (25 μmol/kg). The BN and CDDP treatments were repeated once. BUN levels were determined at 5 days after the last CDDP injection. Each value for BUN represents the mean ± SD for 5 mice. *Significantly different from control ($P < 0.001$)

Effect of pretreatment with BN on the renal toxicity of CDDP

Administration of CDDP increased BUN levels on day 26 (5 days after the last CDDP injection). Pretreatment with BN significantly reduced the BUN concentrations (Fig. 2). The protective effect of BN against CDDP-induced kidney damage as indicated by BUN values was further confirmed by routine histology. A significant correlation between BUN values and renal lesions was observed. Figure 3 A shows the histology of proximal tubules of the kidneys of mice that were treated with CDDP alone. Cloudy, swelling tubular epithelial cells and markedly dilated proximal tubules were seen. Loss or thinning of the brush border was evident in some areas. Hyaline casts filled the lumen of several longitudinal sections of a tubule. Figure 3 B shows the proximal renal tubules of BN-pretreated mice in which lesions appeared to be less extensive than those observed in mice that were treated with CDDP alone. Thus, the results demonstrate a protective effect of pretreatment with BN against CDDP-induced renal toxicity in nude mice.

Effect of BN pretreatment on the antitumor activity of CDDP

The antitumor activity of CDDP given in the presence or absence of Bi pretreatment to nude mice that had been inoculated with NMB-1 is shown in Fig. 4. Preadministration of 50 μmol/kg BN did not reduce the antitumor activity of CDDP. At CDDP doses as low as 25 μmol/kg, the

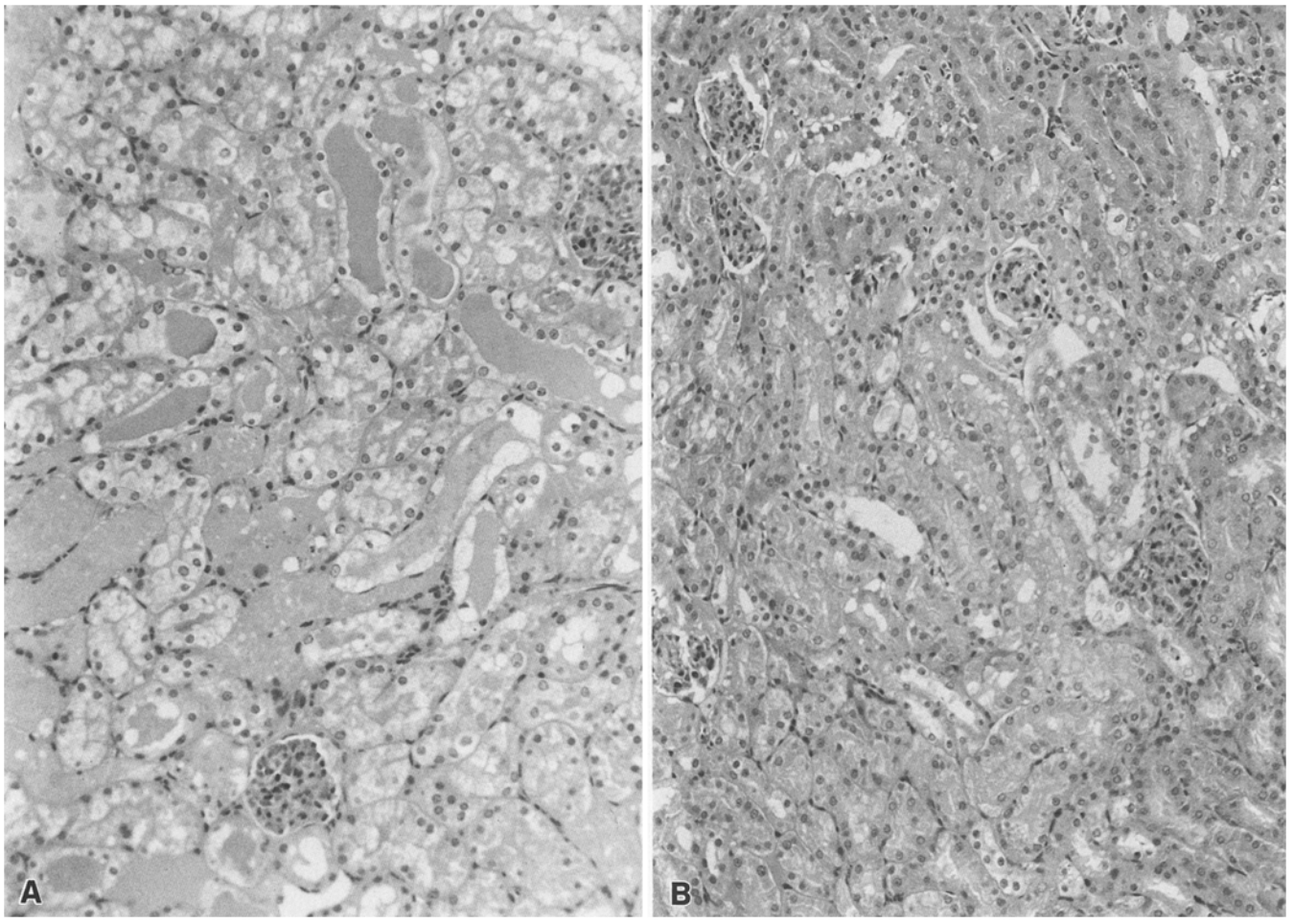

Fig. 3 A, B. Histopathological changes in renal cortices of nude mice that had been treated with **A** CDDP or **B** BN and CDDP. Hematoxylin-eosin stain, ×200

CDDP/BN combinations were as effective against the tumor as was CDDP given alone. Treatment with 50 μmol/kg BN alone produced the same results as those obtained in control animals that were given physiological saline.

Effect of BN pretreatment on tissue concentrations of Pt

Figure 5 presents Pt concentrations measured in the kidney and tumor tissues. This figure demonstrates that BN pretreatment did not influence the concentration of Pt either in the kidney or in the tumor tissues. In contrast, BN induced MT in the kidney, a major target organ of CDDP toxicity, but not in the tumor tissues (Fig. 1).

Comparison of MT concentrations in human urological tumors

Figure 6 compares the MT levels found in inoculated prostatic tumor, renal-cell carcinoma, and Wilms' tumor tissues recovered from controls and from BN-treated nude mice. Levels of MT in prostatic tumor tissue were approximately 2-fold those measured in renal-cell carcinoma and Wilms' tumor tissues. However, BN administration did not alter the concentration of MT in either tumor.

Discussion

Many efforts [1, 2, 6, 9, 14, 15, 24] have been made to reduce the renal toxicity of CDDP that limits the clinical efficacy of this drug [2, 6, 29]. We have previously reported that the administration of bismuth compounds prior to CDDP treatment efficiently reduces the renal toxicity and lethality of CDDP without diminishing its activity against transplanted murine tumors [16]. The favorable effect of Bi described above can be explained by its ability to induce MT in the kidney, a major target tissue of CDDP toxicity, but not in murine tumors. To facilitate the development of this procedure as an effective protocol in cancer chemotherapy, we inoculated nude mice with human bladder tumors to compare the effect of bismuth pretreatment on the toxicity and the antitumor activity of CDDP using the human neoplasm against which CDDP has been most effective.

The present study confirmed that BN efficiently induces MT in the kidneys but not in human bladder tumor NMB-1 in nude mice. This finding compares favorably with the

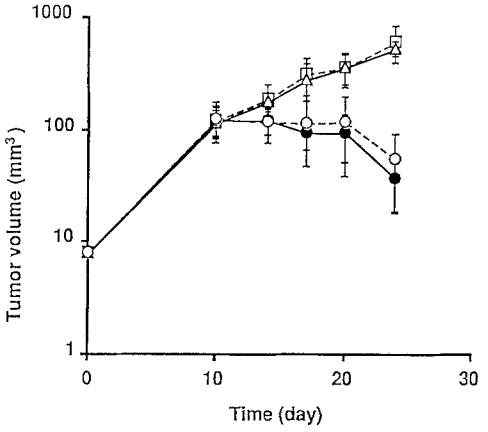

Fig. 4. Comparison of tumor size in nude mice that had been inoculated with bladder tumor and then treated with CDDP and/or BN. The mice were given two courses of CDDP chemotherapy. – – □ – – CONTROL, mice that received saline in place of BN or CDDP; —△— BN, animals that received BN and saline instead of CDDP; —●— BN+CDDP, mice that received BN and CDDP; – – ○ – – CDDP, animals that received saline instead of BN and CDDP

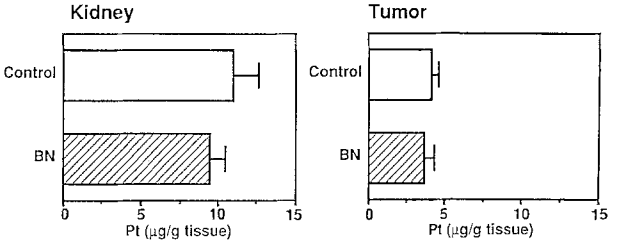

Fig. 5. Effect of pretreatment with BN on the concentration of Pt in tissues of tumor-bearing mice that had been given CDDP. Pt concentrations were determined at 24 h after the injection of CDDP. Each value for Pt content represents the mean ± SD for 5 mice

previously reported efficacy of BN [16]. These results can be explained by the observation that Bi is preferentially taken up by the kidney but is hardly transported into the human tumor tissue tested (Fig. 1). Consequently, preadministration of BN protects the host from the severe renal toxicity of CDDP without affecting its antitumor activity.

Binding of platinum to MT has been reported to occur in the liver and kidney of CDDP-treated rats [4, 25, 38]. It was suggested that this binding was associated with the replacement of zinc in zinc-bound MT by platinum [4]. Determination of Pt levels in the kidneys and inoculated human bladder tumors at 24 h after CDDP administration clearly indicated that platinum concentrations in both the kidneys and the tumors were not altered by BN preadministration, although the level of renal MT was markedly increased. The detoxication of CDDP by preinduction of renal MT may be explained by the replacement of zinc or Bi in the renal MT by platinum, which was consequently trapped firmly by the protein such that it did not exert adverse effects. Furthermore, the maintenance of tumor platinum concentrations following BN administration seems to explain at least partly the unchanged antitumor activity of CDDP.

Clinically, bismuth compounds, including bismuth subnitrate (BSN), have been given p.o. as antidiarrheal drugs.

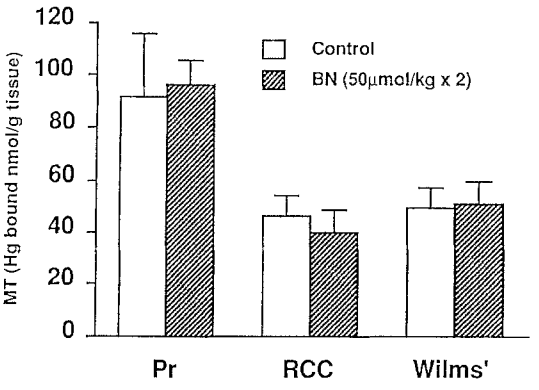

Fig. 6. Concentrations of MT in human urological tumors grown in nude mice with or without BN treatment. Each value for MT content represents the mean ± SD for 5 mice. *Pr*, Prostatic tumor; *RCC*, renal-cell carcinoma; *Wilms'*, Wilms' tumor

We have previously reported that oral BSN administration efficiently depresses the toxicity of CDDP in mice without compromising its antitumor activity [16]. Furthermore, our subsequent experiments have revealed that MT induction by BSN actually protects the host not only from the lesions caused by CDDP but also from those induced by Adriamycin or γ-ray irradiation [17, 21–23, 30, 31]. As in the case of bladder tumor, MT concentrations in human prostatic tumor, renal-cell carcinoma, and Wilms' tumor were not increased by BN treatment. This suggests that MT induction in target tissues should also be effective in preventing the adverse effects induced by Pt-containing combination chemotherapy of various neoplasms.

Although effective diuresis regimens are generally used at present to reduce CDDP toxicity [5], hydration takes a very long time and burdens the patient, especially those suffering from heart failure. The results obtained in the present study suggest that the administration of CDDP coupled with bismuth pretreatment may provide a promising protocol for chemotherapy of human bladder tumors.

References

1. Baldew GS, Hamer CJ van den, Los G, Vermeulen NP, Goeij JJ de, McVie JG (1889) Selenium-induced protection against *cis*-diamminedichloroplatinum(II) nephrotoxicity in mice and rats. Cancer Res 49: 3020
2. Borch RF, Katz JC, Lieder PH, Pleasants ME (1980) Effect of diethyldithiocarbamate resin on tumor response to *cis*-platinum in a rat model. Proc Natl Acad Sci USA 77: 5441
3. Brodie KG (1979) Analysis of arsenic and other trace elements by vapor generation. Am Lab 11: 58
4. Bühler RH, Kägi HR (1974) Human hepatic metallothioneins. FEBS Lett 39: 229
5. Corden BJ, Fine RL, Ozols RF, Collins M (1985) Clinical pharmacology of high-dose cisplatin. Cancer Chemother Pharmacol 14: 38
6. Earhart RH, Martin PA, Tutsch KD, Erturk E, Wheeler RH, Bull FE (1983) Improvement of the therapeutic index of cisplatin by pharmacologically induced chloruresis in the rat. Cancer Res 43: 1187
7. Endresen L, Bakka A, Rugstad HE (1983) Increased resistance to chlorambucil in cytoplasmic metallothionein. Cancer Res 43: 2918
8. Hiratsuka A, Kawashima K, Watabe T (1986) Detoxication of ultimate carcinogens by rat liver zinc-metallothionein (in Japanese). J Hyg Chem 32: 10

9. Howell SB, Pfeifle CE, Wung WE, Olshen RA (1983) Intraperitoneal *cis*-diamminedichloroplatinum with systemic thiosulfate protection. Cancer Res 43: 1426
10. Kotsonis FN, Klaassen CD (1978) Comparison of methods for estimating hepatic metallothionein in rats. Toxicol Appl Pharmacol 42: 583
11. Koyama Y, Nakatogawa K, Naganuma A, Imura N, Onosaka S, Tanaka K (1984) Effect of metallothionein inducing metals on toxicity of inorganic mercury. Jpn J Hyg 39: 385
12. Leber PA, Miya TS (1976) A mechanism for cadmium- and zinc-induced tolerance to cadmium toxicity: involvement of metallothionein. Toxicol Appl Pharmacol 37: 403
13. Madias NE, Harrington JT (1978) Platinum nephrotoxicity. Am J Med 65: 307
14. Naganuma A, Satoh M, Yokoyama M, Imura N (1983) Selenium efficiently depressed toxic side effects of *cis*-diamminedichloroplatinum. Res Commun Chem Pathol Pharmacol 42: 127
15. Naganuma A, Satoh M, Imura N (1984) Effect of selenite on renal toxicity and antitumor activity of *cis*-diamminedichloroplatinum in mice inoculated with Ehrlich ascites tumor cells. J Pharmacobiodyn 7: 217
16. Naganuma A, Satoh M, Imura N (1987) Prevention of lethal and renal toxicity of *cis*-diamminedichloroplatinum(II) by induction of metallothionein synthesis without compromising its antitumor activity in mice. Cancer Res 47: 983
17. Naganuma A, Satoh M, Imura N (1988) Specific reduction of toxic side-effects of Adriamycin by induction of metallothionein in mice. Jpn J Cancer Res 79: 406
18. Nakajima N (1979) Studies on heterotransplantation of human urogenital malignant neoplasms (in Japanese). Jpn J Urol 70: 849
19. Ovejera AA, Houchens DP, Barker AD (1978) Chemotherapy of human tumor xenografts in genetically athymic mice. Ann Clin Lab Sci 8: 50
20. Piotrowski JK, Bolanowska W, Sapata A (1973) Evaluation of metallothionein content in animal tissues. Acta Biochem Pol 20: 207
21. Satoh M, Naganuma A, Imura N (1988) Metallothionein induction prevents toxic side effects of cisplatin and Adriamycin used in combination. Cancer Chemother Pharmacol 21: 176
22. Satoh M, Naganuma A, Imura N (1988) Involvement of cardiac metallothionein in prevention of Adriamycin-induced lipid peroxidation in heart. Toxicology 53: 231
23. Satoh M, Miura N, Naganuma A, Matsuzaki N, Kawamura E, Imura N (1989) Prevention of adverse effects of γ-ray irradiation after metallothionein induction by bismuth subnitrate in mice. Eur J Cancer Clin Oncol 25: 1727
24. Satoh M, Naganuma A, Imura N (1989) Optimum schedule of selenium administration to reduce lethal and renal toxicities of *cis*-diamminedichloroplatinum in mice. J Pharmcobiodyn 12: 246
25. Sharma RP, Edwards IR (1983) *cis*-Platinum: subcellular distribution and binding to cytosolic ligands. Biochem Pharmacol 32: 2665
26. Shiraishi N, Utsumi K, Morimoto S, Joja I, Iida S, Takeda Y, Aono K (1982) Inhibition of nitroblue tetrazolium reduction by metallothionein. Physiol Chem Phys 14: 533
27. Sternberg CN, Yagoda A, Scher HI, Watson RC, Ahmed T, Weiselberg LR, Geller N, Hollander PS, Herr HW, Sogani PC, Morse MJ, Witmore WF (1985) Preliminary results of M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin) for transitional cell carcinoma of urothelium. J Urol 133: 403
28. Sternberg JJ, Bracken RB, Handel PB, Johnson DE (1977) Combination chemotherapy (CISCA) for advanced urinary tract carcinoma: a preliminary report. JAMA 238: 2282
29. Szymanska JA, Mogilnicka EM, Kaszper BW (1977) Binding of bismuth in the kidneys of the rat: the role of metallothionein-like proteins. Biochem Pharmacol 26: 257
30. Thomas JP, Bachowski GJ, Girotti AW (1986) Inhibition of cell membrane lipid peroxidation by cadmium- and zinc-metallothioneins. Biochim Biophys Acta 884: 448
31. Thornalley PJ, Vasak M (1985) Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydrogen radicals. Biochim Biophys Acta 827: 36
32. Von Hoff DD, Schilsky R, Reichert CM, Reddick RL, Rosencweig M, Young RC, Muggia FM (1979) Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 63: 1527
33. Webb M (1976) Protection by zinc against cadmium toxicity. Biochem Pharmacol 21: 2767
34. Williams SD, Donohue JP, Einhorn LH (1979) Advanced bladder cancer: therapy with *cis*-dichlorodiammineplatinum(II), Adriamycin, and 5-fluorouracil. Cancer Treat Rep 63: 1573
35. Yagoda A (1983) Chemotherapy for advanced urothelial cancer. Semin Urol 1: 60
36. Yagoda A, Watson RD, Gonzalez-Vitale JC, Grabstald H, Whitmore WF (1976) *cis*-Dichlorodiammineplatinum(II) in advanced bladder cancer. Cancer Treat Rep 60: 917
37. Yoshikawa H (1976) Tolerance to acute metal toxicity in mice having received a daily injection of its low dose. Ind Health 12: 175
38. Zelazowski AJ, Garvey JS, Hoeschele JD (1984) In vivo and in vitro binding of platinum to metallothionein. Arch Biochem Biophys 229: 246