Selective Reduction of cis-Diaminedichloroplatinum(II) Nephrotoxicity by Ebselen

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INTRODUCTION

Cisplatin is the most widely used cytostatic drug that is effective against various tumors (1). However, clinical application of cisplatin is often accompanied with severe side effects, notably toxicity to the kidneys, the gastrointestinal tract, the peripheral nerves, and the bone marrow (1, 2). Cisplatin-induced functional and histopathological changes in the proximal tubular area of the kidney have been described (2) as well as various methods to reduce cisplatin-induced nephrotoxicity, a major dose-limiting factor in clinical studies (3). Various platinum analogues have been developed, which are less nephrotoxic than cisplatin. Carboplatin is less nephrotoxic than cisplatin, but bone marrow depression and a narrower antitumor spectrum limit its usefulness (4). Hydration and induction of chloruresis provide some protection against cisplatin nephrotoxicity (5). Several agents have been tested for their ability to protect against cisplatin toxicity in animals (6, 7), but until now none of them has led to an improved therapeutic index of cisplatin in patients. The major problems encountered in this field are the intrinsic toxicity of the chemoprotectors (8) and reduction of the antitumor activity of cisplatin (6). Little is known about the molecular mechanism of cisplatin-induced nephrotoxicity, and chemoprotection against cisplatin-induced nephrotoxicity has not been achieved via a mechanistic approach. This is in contrast to, for example, the mechanism-based protection against the hepatotoxicity induced by an overdose of the analgesic drug paracetamol (9) or other compounds (10).

An interesting chemoprotector is the essential trace element selenium, which has been reported to reduce cisplatin-induced nephrotoxicity in mice, without reducing its antitumor activity against several tumors (11–17). Selenium is effective in rodents at nonhepatotoxic doses when administered as sodium selenite. Unfortunately, the clinical use of inorganic selenium compounds might be limited by their potential toxicity. The mechanism of the protective effect of selenite against cisplatin-induced nephrotoxicity is still unknown. Most probably, however, selenite is selectively activated in the kidneys to selenols (RSeH) (18, 19) which might be capable of scavenging either cisplatin or its hydrolysis products, thereby preventing the nephrotoxicity induced by cisplatin. The relatively new seleno-organic compound ebselen (20) is relatively nontoxic, probably because of its strongly bound selenium moiety (21). Ebselen is now in Phase I clinical trials as an antiinflammatory drug.

The present results indicate that ebselen may provide protection against cisplatin-induced nephrotoxicity, when it is given before or after cisplatin. This might open new perspectives in cancer chemotherapy.

MATERIALS AND METHODS

Chemicals. Ebselen was a gift of Rhône-Poulenc Nattermann (Cologne, Federal Republic of Germany). Cisplatin (Platinol) was a gift of Bristol Myers, Weesp, The Netherlands. BUN and serum creatinine were measured spectrophotometrically using the Merckatest urea reagent kit and the Merccktest creatinine reagent kit from Merck, Darmstadt, Federal Republic of Germany. sGPT and sGOT were measured with reagent kits from J. T. Baker, Deventer, The Netherlands.
Tumors. MPC 11 tumor cells were obtained from the Institute of Pathology, University of Utrecht, The Netherlands. The MPC 11 tumor originated as a plasmacytoma and has originally been obtained from Dr. D. Catty, Birmingham, UK. The tumor cells were maintained by weekly passage in BALB/c mice. Freshly harvested ascitic cells were used in the experiments. Cells were counted with a hemocytometer. Transplantable Prima breast tumor cells were obtained from the Radio-biological Institute TNO-Rijswijk, The Netherlands. The Prima tumor originated as a breast carcinoma, induced by forced breeding in BALB/c mice bearing murine mammary tumor virus. The Prima tumor cell-line was cultured in vitro in standard Dulbecco's modification of minimal essential medium (Gibco, Paisley, UK), supplemented with L-glutamine (500 mg/liter), 2-mercaptoethanol (60 μmol/liter), and 10% fetal calf serum (Sera-Lab, Ltd, Sussex, United Kingdom).

Laboratory Animals. Female BALB/c mice were obtained from the Central Institute for the Breeding of Laboratory Animals-Harlan Sprague Dawley (CPB/HSD), Zeist, The Netherlands. The mice were 8 wk of age and weighed 18 to 20 g at the start of the experiment. All animals were provided with standard laboratory food (SRMA chow; Hope Farms, Woerden, The Netherlands) and water ad libitum.

Animal Treatment. Animals were divided at random into groups of 8 animals. Cisplatin was administered in 1.0 ml of physiological saline. Ebselen was dissolved in a mixture of dimethyl sulfoxide/polyethylene-glycol/physiological saline (1/4/20) and administered i.p. in a volume of 0.6 ml.

Kidney Function. The influence of ebselen on cisplatin-induced nephrotoxicity was studied by injection of ebselen i.p. 1 h prior to or 1 h after cisplatin administration. Control groups were treated with ebselen or the vehicle. Blood samples were obtained from the retroorbital venous plexus in the mice. Serum creatinine and BUN were measured daily in pilot studies (data not shown) and in more extensive studies at the time of maximally observed toxicity, Day 4.

Liver Function. sGPT and sGOT were determined on Day 1 and Day 4 after treatment with cisplatin or ebselen and cisplatin. Control groups were treated with ebselen or the vehicle.

Histology. Mice were sacrificed 4 days after treatment with cisplatin or ebselen and cisplatin. Control groups were treated with ebselen or the vehicle. Kidneys and livers were removed and processed for light microscopy. Sections of 6 μm thickness were cut and stained in hematoxylin and eosin. All slides were examined without prior knowledge of the treatment given to the animal from which the specimen under investigation was taken.

Evaluation of Antitumor Activity. The influence of ebselen on the antitumor activity of cisplatin against an ascitic tumor was examined in BALB/c mice, i.p. inoculated with 10^6 MPC 11 tumor cells (Day 0). After 24 h, the mice were treated with a single i.p. dose of cisplatin. The influence of ebselen was assessed by injecting ebselen i.p. 1 h prior to cisplatin. Control groups were treated with ebselen or the vehicle. Mice were examined daily for occurrence of tumors. The experiments were terminated on Day 42, and MSTs were calculated.

BALB/c mice, inoculated with 0.5 × 10^6 Prima breast tumor cells s.c. in the left thigh (Day 0), were used to investigate the effect of ebselen on the antitumor activity of cisplatin against solid tumors. One group of mice was treated with a single i.p. dose of cisplatin 24 h after inoculation of tumor cells. Another group was treated with a single i.p. injection of ebselen 1 h before cisplatin. Control groups were treated with ebselen or the vehicle. The occurrence of tumors was examined daily by palpation. The experiments were terminated on Day 15, when the tumors were excised and weighed.

Statistics. Student's t test, unpaired, was used to evaluate the significance of differences between experimental groups. The level of significance was set at P < 0.05.

RESULTS

Toxicity of Ebselen

Ebselen, 10 mg/kg, did not cause changes in sGPT or sGOT levels in BALB/c mice at Day 1 or Day 4 posttreatment, nor did ebselen followed by cisplatin injection 1 h later. Treatment of the animals with ebselen, 10 mg/kg, alone did not cause changes in BUN or serum creatinine levels. Similar results were obtained with the other dose regimens tested (data not shown). These experiments demonstrate that ebselen, 10.0 mg/kg, did not cause functional liver or kidney damage in BALB/c mice. In a further search for the toxic effects of ebselen, kidneys and livers of BALB/c mice treated with ebselen, 10 mg/kg, with or without various cisplatin doses were examined by routine histology. No kidney damage was observed 4 days after treatment (light micrographs not shown). Liver damage was absent or very moderate and focal, occurring mainly at the edge of liver lobules.

Influence of Ebselen on the Nephrotoxicity of Cisplatin

The data in Table 1 demonstrate a dose-dependent protective effect of ebselen against cisplatin-induced nephrotoxicity in mice. Maximal protection was obtained with an ebselen dose of 10.0 mg/kg. The results, summarized in Table 2, demonstrate a protective effect of ebselen, 10.0 mg/kg, against nephrotoxicity induced by various cisplatin doses in mice. Administration of cisplatin in a dose range of 11.5 to 19.0 mg/kg increased BUN and serum creatinine levels at Day 4 posttreatment. Administration of ebselen 1 h before cisplatin diminished the increase of BUN and creatinine levels at all cisplatin doses tested with the exception of the highest dose. When ebselen was administered 1 h after cisplatin, a protective effect against cisplatin-induced elevations of BUN and serum creatinine was also observed, but not so marked. The protective effect of ebselen against cisplatin-induced kidney damage, as observed

| Table 1 Influence of various ebselen doses on the nephrotoxicity of cisplatin in BALB/c mice |
|-----------------------------|-----------------|--------|----------------|-----------------|
| Cisplatin (mg/kg)           | Ebselen (mg/kg) | BUN (mg/100 ml) | Creatinine (mg/100 ml) |
| 0                           | 0               | 22 ± 2    | 0.54 ± 0.03    |
| 14.5                        | 0               | 18 ± 3    | 0.53 ± 0.03    |
| 14.5                        | 2.5             | 154 ± 59  | 5.6 ± 0.7      |
| 14.5                        | 5.0             | 105 ± 20  | 3.0 ± 1.0^d    |
| 14.5                        | 7.5             | 72 ± 17^d | 1.7 ± 0.4^d    |
| 14.5                        | 10.0            | 37 ± 10^d | 0.73 ± 0.0^d   |
| 14.5                        | 12.5            | 44 ± 18^d | 0.69 ± 0.08^d  |

| Table 2 Influence of ebselen on the nephrotoxicity of various cisplatin doses in BALB/c mice |
|-----------------------------|-----------------|--------|----------------|-----------------|
| Cisplatin (mg/kg)           | Ebselen (mg/kg) | BUN (mg/100 ml) | Creatinine (mg/100 ml) |
| 0                           | 0               | 20 ± 3^a  | 0.53 ± 0.03    |
| 0                           | 10.0^a          | 19 ± 3    | 0.53 ± 0.02    |
| 11.5                        | 0               | 70 ± 42   | 1.6 ± 0.6      |
| 11.5                        | 10.0^a          | 22 ± 5^a  | 0.54 ± 0.04^a  |
| 13.0                        | 0               | 130 ± 38  | 4.5 ± 1.0      |
| 13.0                        | 10.0^a          | 40 ± 27^a | 0.83 ± 0.05^d  |
| 13.0                        | 10.0^a          | 67 ± 23^a | 1.5 ± 0.3^a    |
| 14.5                        | 0               | 175 ± 52  | 5.8 ± 0.7      |
| 14.5                        | 10.0^a          | 57 ± 16^a | 1.2 ± 0.3^d    |
| 14.5                        | 10.0^a          | 102 ± 22^a| 2.8 ± 0.3^d    |
| 16.0                        | 0               | 232 ± 40  | 6.4 ± 1.0      |
| 16.0                        | 10.0^a          | 108 ± 55^a| 3.2 ± 0.6      |
| 19.0                        | 0               | 275 ± 26  | 6.9 ± 0.2      |
| 19.0                        | 10.0^a          | 133 ± 70^a| 4.5 ± 0.8^d    |

^d Control animals were given injections of the vehicle.
^a Ebselen was administered i.p. 1 h before cisplatin.
^b Control animals were given injections of the vehicle.
^c Mean ± SD (n = 8).
^d P < 0.05 compared with the groups treated with cisplatin alone.
^e Ebselen was given 1 h after cisplatin.
by BUN and creatinine measurements, was confirmed by routine histology. Fig 1 demonstrates that tubules of the kidneys of mice treated with ebselen, 10 mg/kg, and 1 h thereafter with cisplatin, 13.0 mg/kg, show considerably less degeneration and less cell loss of the tubular epithelium at Day 4 posttreatment than those of mice treated with cisplatin, 13.0 mg/kg, alone.

Retroperitoneal ganglionic tissue, attached to the kidneys, was also examined histologically. Damage to retroperitoneal ganglionic cells was more pronounced in mice treated with cisplatin, 13.0 mg/kg, and showed considerably less degeneration and less cell loss of the tubular epithelium at Day 4 posttreatment than those of mice treated with cisplatin, 13.0 mg/kg, alone.

Influence of Ebselen on the Antitumor Activity of Cisplatin

MPC 11 Plasmacytoma. The antitumor activities of various cisplatin/ebselen combinations in BALB/c mice, inoculated with MPC 11 tumor cells, are shown in Table 3. Ebselen, 10 mg/kg, did not reduce the antitumor activity of cisplatin. Down to cisplatin doses as low as 6.5 mg/kg, there were no significant differences in MST of mice treated with cisplatin compared with mice treated with the corresponding cisplatin dose and ebselen. Treatment with ebselen, 10 mg/kg, alone, resulted in a MST identical to that of the control group, treated with the vehicle.

The data in Table 3 also show that ebselen protects BALB/c mice against cisplatin nephrotoxicity without reducing its antitumor activity against the MPC 11 tumor. The mean BUN level of mice inoculated with MPC 11 tumor cells and treated with cisplatin, 13.0 mg/kg, was 162 ± 56 mg/100 ml on Day 5. Four animals of this group (n = 8) were dead on Day 7. The surviving animals did not develop tumors (MST >42 days).

The MST of mice inoculated with 10⁶ MPC 11 cells on Day 0 was 16 days. All mice (n = 8) treated with ebselen, 10 mg/kg, and cisplatin, 13.0 mg/kg, survived: MST >48 days. These mice did not develop tumors. The mean BUN level of these animals was 29 ± 10 mg/100 ml 4 days after cisplatin treatment, which is much lower than that of the group treated with cisplatin alone.

Prima Breast Tumor. As shown in Table 4, cisplatin in doses of 9.0 and 11.5 mg/kg was effective against the Prima breast tumor in BALB/c mice. Ebselen, 10 mg/kg, was not effective against the Prima tumor and did not reduce the antitumor activity of cisplatin.

The data in Table 4 also show that ebselen reduced the nephrotoxicity, but not the antitumor activity of cisplatin in Prima tumor-bearing mice. Mice (n = 8) treated with cisplatin alone, 13.0 mg/kg, showed highly elevated BUN levels (mean, 148 ± 85 mg/100 ml) on Day 5, but the animals survived. None of the mice of this group had tumors as assessed by palpation on Day 7. On the other hand, none of the mice treated with ebselen, 10 mg/kg, and cisplatin, 13.0 mg/kg, showed kidney damage. All mice survived, and none of them had tumors. Neither BUN levels on Day 5 nor mean tumor weight on Day 15 of mice treated with ebselen, 10 mg/kg, alone, was significantly different from those of the control group, treated with the vehicle.

DISCUSSION

The data presented in this paper demonstrate that physiologically nontoxic doses of ebselen provide protection against cisplatin-induced nephrotoxicity without reducing its antitumor activity in BALB/c mice inoculated with MPC 11 plasmacytoma cells or Prima breast tumor cells. It is important to note that ebselen also did not reduce the antitumor activity of much lower, nonnephrotoxic cisplatin doses. We have previously shown that sodium selenite provides protection against cisplatin-induced nephrotoxicity, without reducing the antitumor activity of the drug in these tumor models (12). These results were in agreement with results of similar studies with a variety of other tumor models (11, 14–16). A number of important aspects concerning the chemoprotection against cisplatin-induced nephrotoxicity will be discussed.

Toxicity of Ebselen. A major problem in the clinical application of chemoprotectors is their intrinsic toxicity. DDTC, for example, has been successfully applied to reduce the nephrotoxicity induced by cisplatin in rodents, without loss of antitumor activity. Clinical tests, however, have failed due to the severe toxicity of DDTC (8). In our experiments, ebselen doses effective in protecting against cisplatin-induced nephrotoxicity in BALB/c mice were not toxic to the liver or kidney. These observations are in agreement with the reported low toxicity of ebselen (21). In contrast to other selenium compounds such as sodium selenite (Na₂SeO₃) and selenocysteine, ebselen is classified as relatively nontoxic (21). The toxicity of selenium compounds is supposed to be caused by the bioavailability of their selenium moiety and by their bioactivation to hydrogen selenide (H₂Se) (21). In contrast to most selenium compounds, the selenium moiety of ebselen is not available for incorporation into proteins and, moreover, ebselen is not converted into hydrogen selenide (21, 24). In the present study ebselen doses of 10.0 mg/kg (36 μmol/kg) were administered to BALB/c mice without significant toxicities. Ebselen doses of 5.0 mg/kg and 7.5 mg/kg (27 μmol/kg) protected against severe nephrotoxicity induced by cisplatin (Table 1). The maximal tolerable dose of sodium selenite in BALB/c mice is 25 μmol/kg (12, 13).

Dosage Schedules. In the present study a single dose of ebselen, 10 mg/kg, provided effective protection against cisplatin-induced nephrotoxicity in mice when administered 1 h prior to a single dose of cisplatin and also when given 1 h thereafter (Tables 1 and 2). This indicates that the time of administration is less critical for ebselen than for other chemoprotectors. Sodium selenite and sodium thiosulfate only protect against cisplatin-induced nephrotoxicity when administered before or simultaneously with cisplatin (6, 12, 14). DDTC exerts its protective effect only when administered 1 to 4 h after, but not before, cisplatin treatment (7).

Mechanism of Protection. Chemoprotection against cisplatin-induced toxicity can be achieved through nucleophilic agents, which are capable of alkylating cisplatin and its hydrolys products (7, 25). The protection of 5'-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) against cisplatin-induced nephrotoxicity, for example, is probably based on chelation of platinum complexes in the kidney by a nucleophilic metabolite of WR-2721 (26). Cisplatin is supposed to exert its nephrotoxic activity by inactivation of thiol-containing enzymes in the kidneys (7), while its antitumor activity is based on formation of bifunctional adducts with DNA (27). Lempers et al. (28) have studied the interactions between platinum and thiols (RSH). With [PtCl₂(dien)]Cl as the model compound for cisplatin, they have shown that nucleophiles are capable of abstracting Pt(dien) from thiols. Based on these observations they have concluded that nucleophilic agents should be able to protect against cisplatin-induced nephrotoxicity by abstracting.
Fig. 1. Light micrographs of the kidneys from BALB/c mice 4 days after treatment. In A, mice were treated with cisplatin, 13.0 mg/kg. The arrow indicates severe degeneration of tubules in the cortex. In B, mice were treated with ebselen, 10.0 mg/kg, and 1 h thereafter with cisplatin, 13.0 mg/kg. No degeneration of tubules or other histological damage was observed. H & E, × 500.
platinum from platinum-protein bonds. In that way the original structure and function of the protein are probably restored. Bodenner et al. (7) have demonstrated that DDTC protects against cisplatin-induced nephrotoxicity by abstracting platinum from platinum-protein bonds. In that way the original structure and function of the protein are probably restored.

The mechanisms of the protective effect of ebselen are unknown. Ebselen can react with thiols, generating a selenol intermediate (Fig. 2). This selenol-intermediate may play an important role in the protective effect of ebselen. The selective protection of ebselen may be explained by assuming that the formation of the selenol intermediate is more predominant in kidney cells than in tumor cells. Presently it is unknown whether selenols are capable of abstracting platinum from platinum-protein bonds. Recently, it has been suggested that cisplatin-induced nephrotoxicity is related to a decreased activity of GSH peroxidase (32). Whether the well-established GSH peroxidase activity of ebselen (20) contributes to the protective effect of ebselen against cisplatin-induced nephrotoxicity is also unknown.

Clinical Perspectives. Insight into the molecular mechanism of the interactions between ebselen and cisplatin is required for designing optimal clinical dosage schedules and modes of administration for cisplatin/ebselen combinations. Attempts to enhance selenol levels in kidney cells by selective targeting of ebselen to the kidney or by coadministration of ebselen and suitable thiols (33) should be made. To further improve the therapeutic index of cisplatin, investigations of the effect of coadministration of BSO and ebselen would be interesting. It has been demonstrated that BSO is able to potentiate the antitumor activity of cisplatin (34) and other chemotherapeutic agents, such as cyclophosphamide (35). BSO achieves these effects by depletion of GSH levels in tumors. In the design of a rational treatment strategy, a more severe GSH depletion in tumor cells compared with kidney cells might be a goal worth pursuing.

Ebselen exerts a thiol-dependent peroxidase-like activity. The combination of this pharmacological activity with the protective effect against cisplatin-induced nephrotoxicity may have important implications for cancer chemotherapy. Whether ebselen also provides protection against the toxicities of other antitumor drugs, such as doxorubicin, is an especially intriguing question.

In conclusion, it has been demonstrated that ebselen protects against cisplatin-induced nephrotoxicity without reducing the antitumor activity of the drug. Preliminary results indicate that ebselen might also be capable of protecting against cisplatin-induced nephrotoxicity by abstracting platinum from platinum-protein bonds.
induced neurotoxicity. This might have important implications for cancer chemotherapy.

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