Sodium selenosulfate at an innocuous dose markedly prevents cisplatin-induced gastrointestinal toxicity

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A B S T R A C T

Our previous studies in mice revealed that two weeks short-term toxicity of sodium selenosulfate was significantly lower than that of sodium selenite, but selenium repletion efficacy of both compounds was equivalent. In addition, we showed that sodium selenosulfate reduced nephrotoxicity of cisplatin (CDDP) without compromising its anticancer activity, thus leading to a dramatic increase of cancer cure rate from 25% to 75%. Hydration has been used in clinical practice to reduce CDDP-induced nephrotoxicity, but it cannot mitigate CDDP-induced gastrointestinal toxicity. The present work investigated whether sodium selenosulfate is a potential preventive agent for the gastrointestinal toxicity. In tumor-bearing mice, sodium selenosulfate was administered at a dose of 9.5 μmol/kg daily for 11 days, CDDP alone resulted in diarrhea by 88% on day 12, whereas the co-administration of CDDP and sodium selenosulfate dramatically reduced diarrhea to 6% (p<0.0001). Such a prominent protective effect promoted us to evaluate the safety potential of long-term sodium selenosulfate application. Mice were administered with sodium selenosulfate or sodium selenite for 55 days at the doses of 12.7 and 19 μmol/kg. The low-dose sodium selenite caused growth suppression and hepatotoxicity which were aggravated by the high-dose, leading to 40% mortality rate, but no toxic symptoms were observed in the two sodium selenosulfate groups. Altogether these results clearly show that sodium selenosulfate at an innocuous dose can markedly prevent CDDP-induced gastrointestinal toxicity.

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Introduction

Cisplatin (CDDP) is one of the most widely used chemotherapeutic agents for cancer treatment. The therapeutical efficacy of CDDP is largely limited by its severe toxic side effects, such as nephrotoxicity, gastrointestinal toxicity, ototoxicity and neurotoxicity (Mckeage, 1995). It has been known that sodium selenite is able to attenuate gastrointestinal toxicity, ototoxicity and neurotoxicity (Mckeage, 1995; Satoh et al., 1989, 1992), but the potential toxicity of sodium selenite largely limited by its severe toxic side effects, such as nephrotoxicity, gastrointestinal toxicity, ototoxicity and neurotoxicity (Mckeage, 1995).

Abbreviations: ALT, alanine aminotransferase; BWL, body weight loss; CAT, catalase; CDDP, cisplatin; H22 cells, ascitic hepatoma 22 cells; NPPT, nonprotein free thiol; GpX, glutathione peroxidase; GR, glutathione reductase; H&E, hematoxylin and eosin; i.p., intraperitoneally; s.c., subcutaneously; SOD, superoxide dismutase; TrxR, thioredoxin reductase.

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Materials and methods

Chemicals and drugs. Nicotinamide-adenine dinucleotide phosphate, 5,5'-dithiobis (2-nitrobenzoic acid), oxidized glutathione, reduced glutathione, glutathione reductase (GR) (Escherichia coli), CDDP and sodium selenite were purchased from Sigma (St. Louis, MO, USA). Auranofin was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, California, USA). Other chemicals were of the highest grade available. Sodium selenosulfate was freshly prepared daily before administration as described previously (Peng et al., 2007; Zhang et al., 2008a, 2008b).

Animals. Se-sufficient male Kunming mice (21–22 g) and their diet were purchased from Shanghai SLAC Laboratory Animal Co. Ltd., China. The mice were housed in plastic cages in a room with controlled temperature (22 ± 1 °C) and humidity (50 ± 10%) and 12 h light/dark cycle. The mice were allowed to obtain food and water ad libitum.

Cancer cells. Ascitic hepatoma 22 (H22) cells of murine carcinoma were maintained in our laboratory. In brief, ascitic fluid of 0.2 ml that contained 100 × 10^6 viable cells was intraperitoneally (i.p.) injected to mice for ascitic cell growth. The transplantation procedure was carried out once weekly.

Animal treatments. All experiments involving mice were performed in compliance with the ethical guidelines issued by Anhui Agriculture University.

In the first experiment designed to investigate the preventive effect of sodium selenosulfate on CDDP-induced gastrointestinal toxicity, 34 mice were inoculated with H22 cells (4 million/mouse) at their right flank on day 0, and then they were randomly divided into two groups (17 mice/group). Each mouse was marked with trinitrophenol for paired body weight record day by day. Mice in the two groups were i.p. injected with 9.5 μmol/kg sodium selenosulfate or equivalent volume of saline as control daily for 11 days. On day 5 and day 7, mice in both groups were i.p. injected with 8 mg/kg CDDP 1 h later after sodium selenosulfate or saline treatment. Body weight loss (BWL) was calculated according to the formula: \( \frac{B.W.\text{day} 5 - B.W.\text{day} x}{B.W.\text{day} 5} \times 100\% \). Tumor volume was calculated according to the formula: length × width × width/2. Gastrointestinal toxicity indicated by diarrhea was carefully inspected daily since the last CDDP administration on day 7.

In the second set of experiment designed to observe the influence of sodium selenosulfate on intestinal antioxidant parameters, 16 mice were inoculated with H22 cells (4 million/mouse) at their right flank on day 0, and then they were randomly divided into two groups (8 mice/group). Mice were i.p. injected with saline or 9.5 μmol/kg sodium selenosulfate daily for 11 days. On day 12, all mice were terminated by cervical dislocation; intestine tissues were excised, rinsed in ice cold saline, and stored at −30 °C before assay.

In the third set of experiments designed to observe the influence of sodium selenosulfate on intestinal histological alterations. Twenty > mice were randomly divided into four groups (5 mice/group) as control group, selenosulfate group, CDDP group, and CDDP plus selenosulfate group. Mice were i.p. injected with saline or 9.5 μmol/kg sodium selenosulfate daily for 11 days. On day 5 and day 7, mice in the groups with CDDP and without CDDP were i.p. injected with 8 mg/kg CDDP and saline, respectively. On day 12, all mice were terminated by cervical dislocation; intestine tissues were excised, and then fixed in formaldehyde (10% in phosphate-buffered saline) for more than 8 h before histopathological observation.

In the fourth set of experiments designed to compare long-term toxicity of sodium selenosulfate and sodium selenite. Fifty mice were randomly divided into five groups (10 mice/group). In the control group, mice were daily i.p. injected with saline; mice in other four groups were daily i.p. injected with sodium selenite or sodium selenosulfate at the doses of 12.7 and 19 μmol/kg. After 8 weeks, the mice were terminated by cervical dislocation. Blood was obtained from ophthalmic veins to obtain serum after centrifugation; liver tissues were excised, rinsed in ice cold saline, and stored at −30 °C before assay. Liver tissues were excised, and then fixed in the formaldehyde for more than 8 h before histopathological observation.

Biochemical assessments. Serum alanine aminotransferase (ALT) activity was determined by spectrophotometry using a commercially available kit. Liver and intestine tissues were homogenized in ice-cold 150 mM and pH7.2 PBS containing 1 mM EDTA-Na2 (1:9, v/v), and then the homogenate was centrifuged at 15,000 g for 15 min. The resulting supernatants were used for determinations of soluble protein levels with bovine serum albumin as standard (Bradford, 1976), nonprotein free thiol (NPFT) (Wang et al., 2008), and antioxidant enzymes including thioredoxin reductase (TrxR) (Smith et al., 2001), glutathione peroxidase (Gpx) (Smith et al., 2001), GR (Carlberg and Mannervik, 1985), catalase (CAT) (Claiborne, 1985), and superoxide dismutase (SOD) (Sun et al., 1988). Tissue selenium levels were determined using fluorescent detection protocol (Olson et al., 1975).

Histopathological observation. The fixed tissues were dehydrated in graded ethanol and embedded in paraffin. Four-micrometer tissue sections were stained with the hematoxylin and eosin (H&E) and then observed under a light microscope by a pathologic expert in a blind fashion.

Statistical analysis. Data are presented as mean ± SEM. The differences between groups were examined by two-way ANOVA, one-way ANOVA, student’s tests, chi-square test, and log-rank test as indicated in results or illustrations using GraphPad software (Prism version 5, San Diego, California, USA). A p value of less than 0.05 was considered statistically significant.

Results

Effect of sodium selenosulfate on CDDP-induced diarrhea

CDDP-induced gastrointestinal toxicity can be evaluated by inspecting diarrhea incidence. Given that sodium selenosulfate is able to prevent CDDP-induced diarrhea, a simultaneous demonstration that this selenium compound does not disturb the therapeutic efficacy of CDDP would be more appreciated, thus we used tumor-bearing mice. Since the diarrhea incidence was examined through chi-square testing for statistical significance, to make the conclusion more convincing we included a large cohort of mice in each group (n = 17). On day 0, mice were inoculated with H22 cells. During day 1 to day 11 mice were i.p. administered with saline as control group or sodium selenosulfate as preventive group. On day 5 and day 7, CDDP was i.p. injected into the mice in both groups. On day 10, we witnessed the outbreak of diarrhea events in the control group, body contamination by excrement occurred in 94% mice, the half was mild; the rest was of moderate to severe degree (Fig. 1A). On the same day sodium selenosulfate reduced the incidence to 24% in which there was no moderate or severe diarrhea (p < 0.0001, compared with the control in view of the incidence irrespective of degree by Fisher’s exact test). On days 11 and 12, diarrhea in both groups appeared to alleviate but the contras of the two groups became more prominent (88% vs. 12% on day 11; 88% vs. 6% on day 12, p all < 0.0001, in view of the incidence irrespective of degree by Fisher’s exact test). BWL is a well known marker indicative of CDDP toxicity in mice, and BWL over 25% is an extremely dangerous toxicity event in general. On day 12, we found that the incidence rates of BWL over 25% in the control and preventive groups were 88% and 6%, respectively (p < 0.0001, Fisher’s exact test) (Fig. 1B). It
is worth noting that BWL over 25% well mirrors diarrhea incidence on day 12 (Figs. 1A, B). On the other hand, tumor volumes in both groups were identical throughout the whole experimental period (Fig. 1C).

Influence of sodium selenosulfate on intestinal antioxidant parameters

To inspect whether selenium treatment alters intestinal antioxidant parameters, 9.5 μmol/kg sodium selenosulfate was i.p. injected into tumor-bearing mice daily for 11 days. As shown in Table 1, NPFT and the test antioxidant enzymes in the intestines did not respond to the selenium treatment as compared with the control tumor-bearing mice (p all >0.05).

Influence of sodium selenosulfate on histological structure of intestine

To observe whether selenium treatment alters intestinal histological structure of CDDP-untreated and CDDP-treated mice, 9.5 μmol/kg sodium selenosulfate was i.p. injected into mice daily for 11 days. As shown in Fig. 2, sodium selenosulfate itself did not alter histological structure compared to the control, CDDP resulted in large amounts of inflammatory cells and disorder of gland arrangement, sodium selenosulfate effectively prevented CDDP-induced pathological alterations.

Long-term toxicity

Since daily i.p. administration of sodium selenosulfate for 11 days at the dose of 9.5 μmol/kg could generate prominent protective effect on CDDP-induced gastrointestinal toxicity, we were interested in knowing whether the mice are tolerant to long-term intervention of sodium selenosulfate. Mice were i.p. injected with either sodium selenosulfate or sodium selenite at the doses of 12.7 and 19 μmol/kg for total 55 days, which is five-fold time used for the prevention of CDDP-induced gastrointestinal toxicity. As compared with the saline control, from day 14 to day 56, growth was suppressed by sodium selenite (p<0.05 or 0.01 for 12.7 μmol/kg group, p all<0.001 for 19 μmol/kg group, by two way ANOVA analysis post hoc Bonferroni test, Fig. 3A). In contrast, sodium selenosulfate at 12.7 and 19 μmol/kg did not suppress growth (Fig. 3A). The significant growth suppression of sodium selenite starting on day 14 was associated with the reduction of daily consumed-diet by roughly 30% from day 9 to day 13 (Fig. 3B). Sodium selenite at 19 μmol/kg resulted in 40% mortality (p<0.05, as compared with other groups by log-rank test, Fig. 3C), whereas mice in other groups could survive through the whole experimental period. Abnormal tissues such as rigid belly, pale liver, and intestine with bad smell were found in sodium selenite-treated mice in a dose-dependent manner, but not in the mice treated with the two doses of sodium selenosulfate (Figs. 3D, E, F). Histological observation of liver indicated that sodium selenosulfate did not cause pathological alterations, whereas sodium selenite caused dose-dependent hepatocellular necrosis (Fig. 4). Biochemical analysis of blood and liver corroborated that sodium selenite, but not sodium selenosulfate, generated liver toxicity (Fig. 5). Sodium selenite but not sodium selenosulfate decreased liver protein levels and increased liver TrxR activity significantly (Figs. 5A, B). Noteworthy, increased hepatic TrxR activity is a biomarker indicating liver injury in selenium-sufficient subjects as revealed by two recent papers (Zhang et al., 2007, 2008c). Compared to the other groups, the high-dose of sodium selenite significantly increased serum ALT activity although it

Table 1

<table>
<thead>
<tr>
<th>Antioxidant parameters</th>
<th>Control</th>
<th>Selenosulfate</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPFT (nmol/mg protein)</td>
<td>38.6±2.2</td>
<td>35.3±2.3</td>
<td>0.327</td>
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<tr>
<td>TrxR (U/mg protein)</td>
<td>2.23±0.11</td>
<td>2.04±0.06</td>
<td>0.153</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>0.66±0.05</td>
<td>0.69±0.06</td>
<td>0.730</td>
</tr>
<tr>
<td>GR (U/mg protein)</td>
<td>1.57±0.12</td>
<td>1.30±0.08</td>
<td>0.072</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>6.04±0.79</td>
<td>4.14±0.59</td>
<td>0.074</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>1.11±0.08</td>
<td>1.26±0.10</td>
<td>0.253</td>
</tr>
</tbody>
</table>

a Student’s t test.
remained in normal values (Fig. 5C). Consistent with our previous reports (Zhang et al., 2008b), liver selenium accumulation in sodium selenosulfate-treated mice was not lower than that in sodium selenite-treated mice (Fig. 5D).

Discussion

Originally, renal and gastrointestinal toxicities limited the clinical development of CDDP. Since the finding that hydration substantially mitigated the nephrotoxicity, CDDP was approved as an anti-tumor drug. However, under vigorous hydration treatment, gastrointestinal toxicity becomes a dose limiting side effect, thus highlighting the need for additional forms of protection (Cvitkovic, 1998).

Sodium selenite can reduce side effects of CDDP without affecting its antitumor activity (Baldew et al., 1989; Satoh et al., 1989, 1992). In tumor-bearing mice, CDDP alone failed to completely suppress the growth of malignant cells, and the mice died of either progressive disease or CDDP-induced toxicity, whereas subcutaneously (s.c.) administration of 6.3–12.7 μmol/kg sodium selenite to the mice five times weekly for 7 weeks reduced lethal toxicity of CDDP, as well as its renal and intestinal toxicities, resulting in a 100% cure rate (Satoh et al., 1992), provided that the toxicities of i.p. and s.c. injection of sodium selenite are equivalent. The present study showed 12.7 μmol/kg sodium selenite was toxic to mice (Figs. 3, 4, 5), suggesting the pharmacological and toxic doses of sodium selenite are merged. Therefore, sodium selenite appears to have little potential as a preventive agent against CDDP toxicities, including intestinal toxicity. On the contrary, the present study found long-term i.p. administration of 12.7 μmol/kg sodium selenite appeared to increase the risk of intestinal toxicity (Fig. 3F), and this risk became prominent while escalating the dose to 19 μmol/kg (Fig. 3F) since bad smell occurred in all mice of the higher dose of sodium selenite during the dissection. Sodium selenite can be metabolized into hydrogen selenide which is a highly toxic selenium compound (Tarze et al., 2007). The bad smell may come from hydrogen selenide with offensive odor.

We previously demonstrated that sodium selenosulfate attenuated CDDP nephrotoxicity without compromising its antitumor activity. Moreover, in a highly malignant ascitic hepatoma model, CDDP itself achieved at most 25% cure rate, whereas the co-administration of CDDP and sodium selenosulfate (i.p. injection of 7.6 and 9.5 μmol/kg) for 4 weeks raised the cure rate to 87.5% and 75%, respectively (Zhang et al., 2008b). The present study demonstrated i.p. injection of 9.5 μmol/kg sodium selenosulfate for 11 days was highly effective in preventing CDDP-induced diarrhea, but sodium selenosulfate at two-fold pharmacological dose (19 μmol/kg) for five-fold pharmacological intervention time (55 days) was innocuous (Figs. 3, 4, 5), suggesting sodium selenosulfate has tremendous advantage over sodium selenite for ameliorating CDDP toxicities, clinic combination of hydration and sodium selenosulfate has potential to prevent a wider spectrum of CDDP toxicity.

There exists a 20-fold difference between mice and humans in selenium requirements (Zhang et al., 2008b). Selenium administrations by i.p. injection in the present study was 9.5 μmol/kg, in view of the 20-fold difference, the recommended selenium dose for cancer patients treated with CDDP would be i.p. injection of 0.475 μmol/kg sodium selenosulfate, i.e. 2.6 mg selenium per day for a 70 kg adult. Clinical trial showed that daily oral administration of 4 mg selenium in the form of seleno-cappacarrageenan 4 days before and 4 days after CDDP chemotherapy reduced patient's nephrotoxicity, blood transfusions, and the consumption of granulocyte colony-stimulating factor (Hu et al., 1997). In addition, it has been reported that cancer patients are well tolerant of daily oral administration of selenomethionine at doses as high as 7.2 mg selenium for 28 days (Fakih et al., 2008).

Our laboratory has investigated selenium toxicity for a long time (Zhang et al., 2008b). In vitro, sodium selenosulfate at pharmacological dose (9.5 μmol/kg) did not affect cell proliferation (Zhang et al., 2008b). In vivo, low dose of sodium selenosulfate (7.6 μmol/kg) did not affect cell proliferation (Zhang et al., 2008b). In high dose of sodium selenosulfate (9.5 μmol/kg), the tumor growth was suppressed in a dose-dependent manner (Zhang et al., 2008b). In addition, sodium selenosulfate at pharmacological dose (9.5 μmol/kg) did not affect cell proliferation (Zhang et al., 2008b). In high dose of sodium selenosulfate (9.5 μmol/kg), the tumor growth was suppressed in a dose-dependent manner (Zhang et al., 2008b).
et al., 2001, 2005, 2008d; Wang et al., 2007), based on our experience, selenium toxicity dose by i.p. injection is roughly commensurate with half of the oral selenium toxicity dose, accordingly cancer patients may be tolerant to i.p. injection of 2 mg selenium in the form of seleno-cappacarrageean and 3.6 mg selenium in the form of seleno-methionine. Therefore, the inferred pharmacological doses of 2.6 mg selenium as sodium selenosulfate fall into the scope of safe selenium doses used in clinical trials.

The present study showed that the mechanism of action of selenium at a pharmacological level did not involve its impact on antioxidant parameters of selenium-adequate mice (Table 1). A recent animal experiment study has revealed that the selenium protection on CDDP-induced nephrotoxicity is associated with significantly reduced accumulation of platinum-DNA adduct in kidney tissue (García et al., 2011). This impressive preventative effect of selenium on CDDP-induced toxicities without disturbing antitumor activity of CDDP as seen in the present and previous studies (Satoh et al., 1992; Zhang et al., 2008b) altogether demonstrates normal tissues but not tumors are protected by selenium, thereby suggesting selenium at pharmacological levels reduces platinum-DNA accumulation in normal tissues but not in tumors. Two lines of evidence support selenium can selectively modulate platinum-DNA accumulation.

Firstly, in vitro experiments have demonstrated that selenium pretreatment can cause a DNA repair response in gut epithelium and bone marrow with wild-type p53, which protects DNA from subsequent challenges with DNA-damaging agents. It is further observed that such DNA repair response and subsequent DNA damage protection are p53-dependent as neither is observed in p53-null cells (Seo et al., 2002a, 2002b; Fischer et al., 2007). Tumor suppressor p53 is mutated in the vast majority of cancers but it maintains wild-type...
in normal tissues (Buganim and Rotter, 2009), thus normal p53 is an important genetic determinant for the selective protections of selenium on normal cells (Seo et al., 2002a, 2002b; Fischer et al., 2007).

Secondly, it is known that pharmacological dose of selenium can normalize tumor vasculature and microenvironment, promotes tumor vessel maturation leading to enhanced accumulation of intratumor concentration of anticancer drugs, such as doxorubicin by 4-fold (Bhattacharya et al., 2008) and the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) of irinotecan by 2-fold (Azrak et al., 2011), whereas normal tissue vasculature and drug concentrations are not altered by the selenium treatment (Bhattacharya et al., 2008; Azrak et al., 2011). As to the tumors with normal p53 functions, pharmacological selenium intervention not only increases tumor DNA repair but also enhance tumor drug accumulation which counteracts selenium-promoted DNA repair, thereby maintaining the same therapy efficacy as compared with non-selenium treatment.

In summary, sodium selenosulfate, with significantly lower toxicity as compared with sodium selenite, at innocuous doses is able to prevent CDDP-induced gastrointestinal toxicity without disturbing its therapeutic effect on tumors. In clinical practices, sodium selenosulfate in combination with hydration may prevent a wider spectrum of CDDP toxicity.

Conflict of interest disclosure statement
The authors have no financial conflict of interest.

Acknowledgments
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Fig. 5. Biochemical parameters at the end of the long-term toxicity experiment. (A) Liver soluble protein levels. (B) Liver TrxR activity. (C) Serum ALT activity. (D) Liver selenium level. Data are presented as mean ± SEM (n = 10 except selenium/19 group, in which n = 6 due to 40% mortality). One way ANOVA analysis post hoc Tukey test.

References


