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Protection of antiproliferative effect of *cis*-diamminedichloroplatinum (II) by sodium thiosulfate

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Summary. Utilizing the phytohemagglutinin (PHA) stimulation assay of human peripheral blood mononuclear cells (PBM), the protective effect of sodium thiosulfate (STS) on the antiproliferative action of *cis*-diamminedichloroplatinum (II) (DDP) against human cells was investigated. DDP alone significantly inhibited the proliferation of PBM over the concentration range of 10^{-7} to 5×10^{-5} *M*. The antiproliferative effect of DDP was significantly blocked when STS was added to the stimulation culture at the time of exposure to DDP at molar STS/DDP ratios of more than 500. However, STS at molar ratios of less than 100 induced minimal protection. Then, STS was added at various times after DDP exposure with a molar STS/DDP ratio of 1000. The protection was effective within 10 min after exposure to DDP at a concentration of 5×10^{-5} *M*, whereas it was not effective beyond 30 min after the exposure. The results indicate that effective protection against DDP cytotoxicity in human cells can be achieved by the concurrent presence of STS with molar STS/DDP ratios of more than 500, but not when a molar STS/DDP ratio of less than 100 is used.

Introduction

cis-Diamminedichloroplatinum (II) (DDP) possesses potent activities against a large variety of human malignant tumors. Its toxicity is, however, considerable.

A number of sulfur-containing compounds have been shown to reduce the cytotoxicity of DDP [1, 2, 5, 13]. Among these compounds, sodium thiosulfate (STS) has been selected as an antagonist because of its relative safety and ready availability for use in man [5, 9]. Then, the regional use of DDP chemotherapy with concurrent protection of the systemic circulation by STS was demonstrated to improve the therapeutic efficacy in cases of peritoneally disseminated tumors, metastatic liver tumor and bladder tumor in animals [10–12]. Further, a clinical trial of this procedure in patients with intraperitoneal tumors was reported [6].

The present study was undertaken as a quantitative investigation of the dose-response relationship for antagonism by STS of DDP-induced cytotoxicity in human cells in vitro. The assay system used was phytohemagglutinin (PHA)-induced blastoid transformation of human peripheral blood mononuclear cells (PBM), since DDP has been shown to be a potent inhibitor of this reaction [3, 8]. Using the PHA stimulation assay, the protection of the antiproliferative effect of DDP by STS was examined.

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Materials and methods

Cell preparation. Peripheral blood was taken from normal donors into heparinized syringes. PBM were isolated by Ficoll-Conray density gradient centrifugation. The cells were suspended in RPMI 1640 medium supplemented with 10% pooled human AB serum, penicillin 100 units/ml, and streptomycin 100 μg/ml (complete medium).

Mitogen stimulation of PBM. PBM were cultured in complete medium in a flat-bottomed microculture plates as 0.2-ml aliquots containing 2×10^5 cells. PHA at a concentration of 5.0 μg/ml culture medium was used for stimulation. Culture plates were incubated for 3 days at 37 °C in a humidified atmosphere with 5% CO_2 in air. At 6 h before harvesting, 1 μCi of tritiated thymidine was added to each well. The cells were harvested onto glass filters and the incorporation of tritiated thymidine was measured in a liquid scintillation counter.

Agents. DDP was dissolved in RPMI 1640 medium immediately before use. To examine the effect on proliferative response, the drug was added to wells of culture plates 24 h after the initiation of culture to give a final concentration of 10^{-7} *M* to 5×10^{-5} *M*. STS was also dissolved in RPMI 1640 medium and added to the stimulation culture at the same time or at various times after exposure to DDP, the molar STS/DDP ratios used ranging from 1 to 1000.

Results

The effect of DDP alone on the proliferation of PBM was examined using the PHA stimulation assay. DDP inhibited the proliferation, and the dose-response relationship for this action is shown in Fig. 1. At the concentration of 5×10^{-5} *M* the response was almost completely suppressed by the drug. On the other hand, after the addition of STS alone no inhibition was seen even with a concentration of 5×10^{-2} *M*.

To determine the ability of STS to block the antiproliferative effect of DDP, STS was added to the stimulation

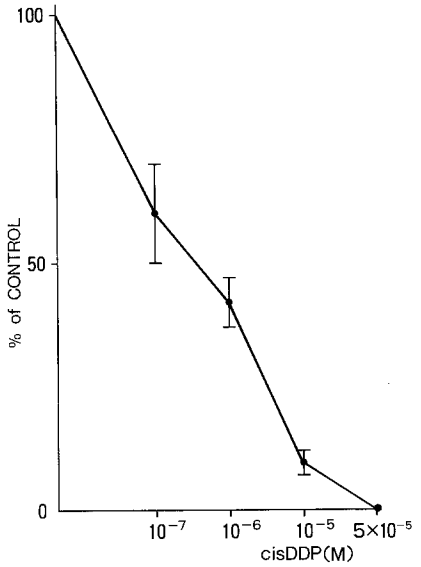

Fig. 1. Effect of DDP on PHA-induced proliferation of PBM. Each *point* = mean ±SEM of measurements recorded in five experiments

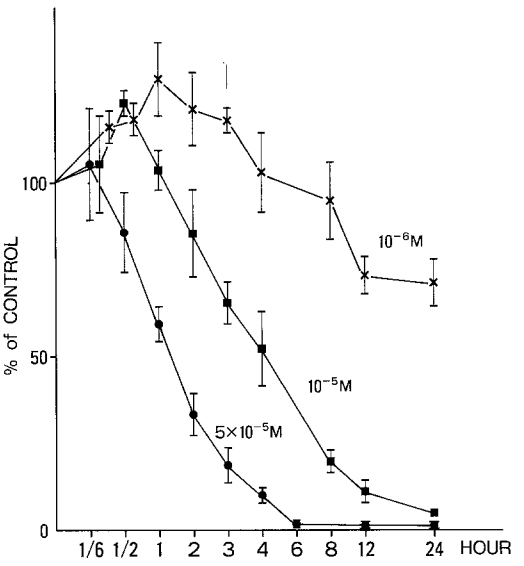

Fig. 3. Temporal aspects of STS protection against the antiproliferative action of DDP. Each *point* = mean ±SEM of measurements recorded in five experiments

culture at molar STS/DDP ratios of 1–1000 at the time of exposure to various concentrations of DDP. As shown in Fig. 2, STS at the ratios of less than 100 induced relatively slight protection against the antiproliferative effect of DDP over the entire concentration range of 10^{-7} to 5×10^{-5} *M*. When STS was added at the molar ratio of 500, the cytotoxicity of DDP was significantly inhibited, and the proliferation of PBM was restored to almost 70% of control at any concentration. The protection was at almost the same level up to the ratio of 1000.

To examine the temporal aspects of STS protection, STS was added to the culture at a molar STS/DDP ratio of 1000 at various times after exposure to DDP (Fig. 3). Addition of STS within 10 min after DDP exposure induced protection of almost the same level as that seen following concurrent addition of STS even at the highest concentration of DDP (5×10^{-5} *M*). However, the drug cytotoxicity could be significantly blocked 30 min after exposure of this concentration of DDP. At this concentration of 10^{-5} *M*, protection by STS was effective within 1 h after drug exposure. Then, cytotoxicity of DDP at 10^{-6} *M* was protected by STS within 6 h after exposure.

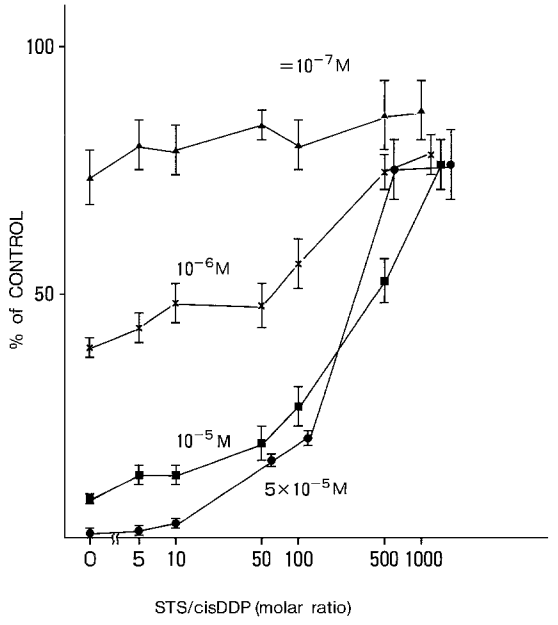

Fig. 2. Protective effect of STS on the antiproliferative action of various concentrations (10^{-7} to 5×10^{-5} *M*) of DDP. Each *point* = mean ±SEM of measurements recorded in seven experiments

Discussion

The results of the present study demonstrate that the antiproliferative effect of DDP was significantly inhibited by STS at molar STS/DDP ratios of more than 500, whereas the cytotoxicity of the drug was slightly blocked at molar ratios of less than 100. With such low ratios STS had to be added within 10 min after drug exposure to induce effective protection.

Several sulfur-containing compounds have been reported to be capable of forming a DDP–DNA complex and reducing the cytotoxicity of DDP [1, 2, 13]. STS was shown to produce a dose-related decrease in the toxicity of DDP in the mouse, which was reflected in a reduction in weight loss, nephrotoxicity, and toxicity for marrow colony-forming units in culture and in increased survival after an otherwise lethal dose of DDP [5, 9]. The protective effect against the lethal toxicity of DDP was also investigated in bacteria [9].

To examine the availability for clinical use, the protective effect of STS on DDP cytotoxicity in human cells must be investigated. The antagonism by STS of the DDP-induced decrease in growth rate of a human lymphoblastoid cell line was reported by Howell et al. [6]. These authors showed that molar STS/DDP ratios of 280–950 were required to restore the growth rate of these cells to 50% of that in control cells. In the present study we chose

the experimental system of nucleic acid synthesis in human PBM under mitogenic stimulus of PHA in vitro, since DDP has been shown to be a persistent and selective inhibitor of DNA synthesis [4, 7] and also a potent inhibitor of PHA-induced proliferation of PBM [3, 8]. Our results showed that STS at molar STS/DDP ratios of more than 500 significantly blocked the antiproliferative effect of DDP and restored proliferation to almost 70% of control. However, the protection was minimal when STS was added at molar ratios of less than 100.

Burchenal et al. [1] reported that thiourea reduced the overall toxicity when injected into the mouse 2 h after DDP administration. Under some in vitro conditions the thio compound can dissociate established DNA interstrand crosslinks and reverse the lethal lesions caused by DDP [2]. However, Howell and Taetle [5] showed that STS was effective in providing protection against nephrotoxicity in the mouse only over a rather limited time span extending from 1 h before to 30 min after DDP. The present results demonstrated that the addition of STS within 10 min after the drug exposure in vitro was necessary to achieve the effective protection of DDP cytotoxicity against human PBM, especially in the presence of a high concentration of DDP. Thus, the cytotoxicity caused by DDP was not reversed by STS. This fact is consistent with the hypothesis that STS reduces toxicity by combining directly with DDP to form a complex no longer capable of crosslinking macromolecules, rather than by displacing DDP that was already bound [5].

Effectiveness of regional DDP chemotherapy in combination with the inactivation of DDP reaching the systemic circulation by STS has been reported in animals [10–12] and humans [6]. The present results appear to show that the simultaneous presence of STS in the systemic circulation at molar STS/DDP ratios of more than 500 is necessary to achieve effective protection against DDP toxicity in humans. Furthermore, the suppression of DDP toxicity in the target area of regional chemotherapy may be minimal in the presence of STS at molar ratios of less than 100. These findings may provide useful information for clinical application of the combination of regional DDP chemotherapy and systemic STS. These results have been taken account of in the design of a clinical trial that is now under way.

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