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Amifostine increases cure rate of cisplatin on ascites hepatoma 22 via selectively protecting renal thioredoxin reductase

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Abstract

It has been demonstrated via in vitro experiments that the anti-cancer drug cisplatin (CDDP) can inactivate thioredoxin reductase (TrxR), a molecular target for cancer therapy. The present study in mice revealed that CDDP at pharmacological doses inhibited TrxR activity in both ascitic hepatoma 22 (H22) cells and kidney, leading to suppression of H22 cells proliferation along with nephrotoxicity. Amifostine, a clinical used cytoprotective agent, protected against CDDP-induced TrxR inactivation in kidney but not in H22 cells. Such an excellent selective modulation of amifostine on TrxR led us to exploit the potential of amifostine in increasing cure rate of CDDP on cancer. In mice, CDDP at the doses of 5 and 7.5 mg/kg once weekly for 4 weeks could not completely control H22 ascites development and the cure rate was no more than 12.5%; CDDP 9 mg/kg by the same schedule prominently suppressed the ascites development, but finally resulted in 87.5% mortality caused by CDDP toxicity. Thus, these dose-dependent therapeutic results well recapitulated the clinical dilemma of chemotherapy on cancer. However, co-treatment of CDDP (9 mg/kg) and amifostine largely reduced CDDP toxicity, and obtained a cure rate as high as 87.5%. Overall, the present study demonstrates both pharmacological and toxicological effects of CDDP involve TrxR inactivation, and the large enhancement on CDDP cure rate in H22 ascites model by using amifostine is, at least in part, ascribed to its selective modulation on TrxR.

Keywords: Thioredoxin reductase; Cisplatin; Amifostine; Selectivity; Cure rate

1. Introduction

Mammalian thioredoxin reductase (TrxR) is a member of pyridine nucleotide disulfide oxidoreductases, catalyzing NADPH-dependent reduction of the redox-active disulfide in thioredoxin (Trx) which serves a wide range of functions in cellular proliferation and redox homeostasis [1,2]. In addition, TrxR has wide substrate specificity, due to easy access to the redox-active amino acid selenocysteine at the penultimate carboxyl terminal position [3,4].

TrxR incites pro-survival effects, enhances tumor proliferation and resistance to therapeutic modalities [5]. TrxR is over-expressed in many tumor cells,

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accounting for about 0.5% of total soluble proteins in human lung adenocarcinoma cells [6]. TrxR1 knockdown by using small interfering RNA technology in mouse Lewis lung carcinoma (LLC1) cells can convert the biological profiles of these cells similar to normal cells. Furthermore, mice injected with the TrxR1 knockdown LLC1 cells showed a dramatic reduction in tumor progression and metastasis [7]. In vitro experiments have demonstrated that TrxR inactivation is involved in the mechanism of several anti-cancer drugs [8]. In mice, we reported that cyclophosphamide, one of the most extensively used anti-cancer drugs, potently inhibited TrxR activity of tumors [9]. More recently, it was reported that targeting TrxR is a basis for cancer therapy by arsenic trioxide [10]. Thus, TrxR provides an excellent molecular target for cancer therapy [11].

Amifostine (WR-2721) is a pharmacological antioxidant used as a broad-spectrum cytoprotective agent in cancer chemotherapy and radiotherapy [12]. It is an inert phosphorylated thiol prodrug that must be dephosphorylated to the active metabolite WR-1065 by membrane-bound alkaline phosphatase, which has a much higher expression in normal tissues than tumors [13,14]. Thus, the dephosphorylation rate of amifostine is higher in normal tissues than tumors. In addition, low pH and hypoxia in tumors, may also reduce the formation of WR-1065 [15]. Basic experiment and clinical studies demonstrated that amifostine selectively protected some normal tissues against cytotoxicity of chemotherapeutic agents and radiation therapy, but brought no benefits to neoplastic tissues [15,16]. Selectivity of protection may be attributed to a preferential formation and uptake of WR-1065 by normal cells. Once inside cells, WR-1065 acts as an antioxidant, scavenging free radicals, decreasing the DNA interstrand cross-links caused by alkylating agents and decreasing platinum–DNA adducts [17]. Based on large randomized studies, FDA approval was granted in 1995 for a substantial attenuation of cumulative nephrotoxicity in patients receiving repeated doses of cisplatin (CDDP) for advanced ovarian cancer or non-small-cell lung cancer [18].

It has been demonstrated via in vitro experiments that CDDP can inactivate TrxR [19], but in vivo whether CDDP at pharmacological doses inhibits TrxR activity has not been reported hitherto. If CDDP suppresses TrxR activity of cancer cells in vivo, it might be extrapolated that CDDP-induced inactivation of renal TrxR acts as a pivotal mechanism of nephrotoxicity, a dose limiting side effect of CDDP. Given that pharmacological and toxicological effects of CDDP both involve TrxR inactivation, whether amifostine could selectively protect renal TrxR is of particular interest. The present study is aimed at elucidating these presumptions.

2. Materials and methods

2.1. Chemicals and drugs

NADPH, HEPES, insulin, 5,5′-dithiobis(2-nitrobenzotic acid) (DTNB), Trx (*Escherichia coli*), guanidine hydrochloride, reduced glutathione (GSH), bovine serum albumin, CDDP, and amifostine were all purchased from Sigma (St. Louis, MO, USA). Other chemicals were of the highest grade available.

2.2. Animals

Healthy male Kunming mice (body weight 20–22 g, 4–5 weeks) and diet were purchased from Shanghai SLAC Laboratory Animal Co. Ltd., PR China. The mice were housed in plastic cages (6–8/each) in a room with controlled temperature (22 ± 1 °C) and humidity ($50 \pm 10\%$) and 12 h light/dark circle. The mice were allowed *ad libitum* to obtain food and water. All experiments involved in mice were performed strictly complying with the ethical guidelines issued by the University of Science and Technology of China.

2.3. Tumor cell inoculation

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 H22 cells/mL was injected into peritoneal cavity of mice for ascitic cells growth, and the transplantation of H22 cells was carried out once weekly.

2.4. Animal treatments

In the first experiment, to observe time course of TrxR activity in ascitic H22 cells and kidney after CDDP treatment, 24 H22 ascites-bearing mice were randomly divided into four groups with six mice in each group. Treatment was initiated 3 days after cell inoculation. Group I was treated with saline as control, groups II–IV were intraperitoneally (i.p.) injected with CDDP at the dose of 10 mg/kg body weight. Mice in groups II–IV were sacrificed by cervical dislocation at 1, 3, and 5 h, respectively, after CDDP treatment. Mice in group I were sacrificed at 5 h after saline injection. Ascitic fluid was collected from the peritoneal cavity and then was centrifuged to obtain H22 cells, which were further washed with ice cold saline