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Original Contribution

Efficacy and safety of selenium nanoparticles administered intraperitoneally for the prevention of growth of cancer cells in the peritoneal cavity

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ABSTRACT

Peritoneal implantation of cancer cells, particularly postoperative seeding metastasis, frequently occurs in patients with primary tumors in the stomach, colon, liver, and ovary. Peritoneal carcinomatosis is associated with poor prognosis. In this work, we evaluated the prophylactic effect of intraperitoneal administration of selenium (Se), an essential trace element and a putative chemopreventive agent, on peritoneal implantation of cancer cells. Elemental Se nanoparticles were injected into the abdominal cavity of mice, into which highly malignant H22 hepatocarcinoma cells had previously been inoculated. Se concentrations in the cancer cells and tissues, as well as the efficacy of proliferation inhibition and safety, were evaluated. Se was mainly concentrated in cancer cells compared to Se retention in normal tissues, showing at least an order of magnitude difference between the drug target cells (the H22 cells) and the well-recognized toxicity target of Se (the liver). Such a favorable selective distribution resulted in strong proliferation suppression without perceived host toxicity. The mechanism of action of the Se nanoparticle-triggered cytotoxicity was associated with Se-mediated production of reactive oxygen species, which impaired the glutathione and thioredoxin systems. Our results suggest that intraperitoneal administration of Se is a safe and effective means of preventing growth of cancer cells in the peritoneal cavity for the above-mentioned high-risk populations.

Peritoneal carcinomatosis is a life-threatening complication of gastric, colorectal, liver, and ovarian cancers [1–3]. Peritoneal seeding of cancer cells frequently occurs during operation as a result of surgical trauma to the tumors, which are exposed to the abdominal cavity [4,5]. In addition, spontaneous rupture of a hepatocellular carcinoma (HCC)[2] results in intraperitoneal spillage of the cancer cells [2,3]. If peritoneal implantation of cancer cells cannot be effectively suppressed at an early stage, it can lead to the formation of malignant ascites, which are refractory to treatment, or numerous small tumor nodules and tumor masses of various sizes, which are too widespread for resection to be performed on them [1].

Intraperitoneal chemotherapy (IPC), approved by the U.S. Food and Drug Administration [6], is the most efficient modality for treatment of peritoneal carcinomatosis because the peritoneal–plasma barrier significantly inhibits the transport of certain kinds of drugs from the peritoneal cavity to systemic circulation, thereby generating a high drug concentration around the cancer cells located in the peritoneal cavity and maintaining low systemic drug levels [7,8]. Currently, either intraoperative hyperthermic IPC or early postoperative IPC is used to prevent peritoneal dissemination of cancer cells after a cytoreductive surgery. Unfortunately, the adverse effects of these IPC approaches have been frequently noted [9]. For example, IPC with cisplatin after debulking surgery causes hematologic toxicities, emesis, and nephrotoxicity [10]. Therefore, safe and effective approaches for preventing peritoneal carcinomatosis are needed.

Selenium (Se) is an essential trace element with antioxidant effects at nutritional doses, primarily through selenoproteins, such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) [11,12]. On the other hand, certain types of Se compounds at high-dose levels have potent cell killing capacity by stimulating the formation of reactive oxygen species (ROS) [13,14]. Evidence supporting the cancer-preventive effects of Se has mainly been obtained from animal experiments using high levels of Se that

Abbreviations: BSA, bovine serum albumin; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione *S*-transferase; HCC, hepatocellular carcinoma; IPC, intraperitoneal chemotherapy; NADPH, nicotinamide adenosine dinucleotide phosphate; NPFT, nonprotein free thiol; PBS, phosphate-buffered saline; ROS, reactive oxygen species; Trx, thioredoxin; TrxR, thioredoxin reductase.

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closely approach Se toxicity [15,16]. In this regard, Se seems not to be a suitable chemopreventive agent for long-term application because the potential risks overwhelm any possible benefits [17]. It is worth noting that Milner's team [18,19] achieved strong Se-dependent inhibition in L1210 leukemic or Ehrlich ascitic cells inoculated in the peritoneal cavity of mice with no apparent ill consequences to the host by using a regimen of intraperitoneal injection of sodium selenite. However, the mechanism of action of sodium selenite and why it exhibited high efficacy and impressive safety in these models remain unclear. The poor performance of Se in cancer prevention by intragastric administration and the encouraging results of Se in inhibiting peritoneal cancer cells by intraperitoneal administration lead us to raise the hypothesis that IPC with Se could be used for prevention of peritoneal implantation of cancer cells. Therefore, the goal of our present work was to investigate the effect of IPC with elemental Se nanoparticles on proliferation of murine hepatocarcinoma 22 cells (H22 cells) implanted in the peritoneal cavity of mice and elucidate the potential mechanism. The results suggest that timely treatment through IPC with Se is a safe and highly efficient modality for eradicating peritoneal H22 cells. The mechanism of Se action is mainly ascribed to a selective and substantial Se accumulation, which causes drastic elevation of ROS in peritoneal cancer cells, but not in normal tissues.

Materials and methods

Chemicals

Glutathione (GSH), glutathione reductase (GR; from baker yeast), TrxR (from rat liver), bovine serum albumin (BSA), and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were all obtained from Sigma (St. Louis, MO, USA). Auranofin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Other chemicals were of the highest grade available. Elemental Se nanoparticles with an average size of 50 nm, estimated using transmission electron microscopy (Fig. 1A) or a dynamic light scattering instrument (Fig. 1B), were prepared according to our previously reported procedure using sodium selenite as a selenium source, GSH as a reductant, and BSA as a disperser of Se nanoparticles [20].

Animals and cells

Healthy male Kunming mice (20–22 g) and their diet (0.14 μg Se/g), as well as H22 cells, were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. The mice were housed at controlled temperature (25 ± 1 °C) and humidity (50 ± 10%) and in a 12-h day/night cycle. The mice were allowed ad libitum access to food and water. All animal treatment procedures complied with the guidelines of Anhui Agricultural University for the care and use of laboratory animals. H22 cells were maintained in our laboratory once weekly. Briefly, viable H22 cells in ascitic fluid were adjusted to 100 million/ml with saline. Then 0.2 ml was injected into the peritoneal cavity of the mice.

To evaluate the efficacy of proliferation suppression by Se nanoparticles, mice inoculated with 2 million viable H22 cells into the peritoneal cavity only once on day 0 were randomly grouped (n=6). One hour after the inoculation, the mice were intraperitoneally injected with Se nanoparticles (0.20, 0.35, or 0.70 mg Se/kg body weight, bw) once daily for 7 consecutive days (experiment 1) or at a dose of 1.4 mg Se/kg bw once daily for 1, 2, or 3 days (experiment 2). In experiment 3, mice were intragastrically administered Se nanoparticles (3 or 4.5 mg Se/kg bw) once daily for 7 consecutive days. Mice were sacrificed 24 h after the last treatment in experiments 1 and 3 and on the 7th day for experiment 2. In each experiment, mice in the control group received saline treatment intraperitoneally or intragastrically.

To estimate the safety of Se nanoparticles, 21 mice were randomly divided into three groups (n=7). Mice were intraperitoneally injected with saline or Se nanoparticles (0.7 or 1.4 mg Se/kg bw) for 7 consecutive days. All mice were sacrificed 24 h after the last treatment.

To measure Se distribution, mice inoculated with 100 million viable H22 cells in the peritoneal cavity were randomly grouped (n=6). One hour after inoculation, mice were administered Se nanoparticles at a dose of 0.7 mg Se/kg bw intraperitoneally (experiment 1) or 4.5 mg Se/kg bw by gavage (experiment 2). Mice in the control group were sacrificed at 6 h posttreatment, and mice in the Se groups were sacrificed 3, 6, and 12 h (experiment 1) or 3 and 12 h (experiment 2) posttreatment.

To determine the mechanism of proliferation suppression, mice inoculated with 100 million viable H22 cells in the peritoneal cavity were randomly grouped (n=5). One hour after inoculation, the mice were intraperitoneally administered saline (control) or Se nanoparticles at a dose of 4.5 mg Se/kg bw, and then the mice were sacrificed at 1, 3, or 9 h posttreatment.

Sample preparation and biomarker assessments

At the end of each set of experiments, the mice were sacrificed by cervical dislocation. Peripheral blood from the ophthalmic veins was collected into Eppendorf tubes with or without heparin for preparation of either whole blood or serum, respectively. Hematological parameters of the whole blood were analyzed using a hematology analyzer. Serum biomarkers were determined using commercially available kits.

To count H22 cells in the peritoneal cavity, a sufficient amount of H22 cells was collected from the peritoneal cavity. For this purpose, the ascitic fluid was first collected as much as possible, and then the peritoneal cavity was further washed three times with ice-cold saline to fully obtain cells. The ascitic fluid and the washed saline were merged for counting viable cells in a hemocytometer using the trypan blue dye exclusion method. The pooled ascitic fluid was centrifuged to remove H22 cells, and the resulting supernatants were used to estimate the total extracellular activity of GPx, GR, and glutathione *S*-transferase (GST) in the peritoneal cavity. One unit of GPx or GR activity is defined as nanomoles of nicotinamide adenosine dinucleotide phosphate (NADPH) oxidized per minute [21,22], and 1 unit of GST is defined as nanomoles of 1-chloro-2,4-dinitrobenzene conjugate formed per minute [23]. Se levels in tissues or cells were determined using a 2,3-diaminonaphthalene fluorescence-based method [24]. To assess the levels of Se in H22 cells, samples were centrifuged at 300*g* for 5 min, to precipitate H22 cells thoroughly while leaving the potential extracellular Se nanoparticles in the supernatant. To detect intracellular alterations in TrxR, thioredoxin (Trx), and GSH, the H22 cells were subjected to ultrasonic treatment. The resultant homogenates were centrifuged (15,000*g* at 4 °C) for 15 min. The supernatants were used to assess TrxR and Trx activities (1 unit of TrxR or Trx activity was defined as 1 nmol of NADPH oxidized/min) [21,25], as well as nonprotein free thiol (NPFT) levels, with GSH being a predominant component [26].

ROS detection

ROS levels were evaluated using DCFH-DA as a probe in a spectrofluorophotometer with fluorescence intensity excited at 488 nm and recorded at 525 nm. Each sample containing 5 million viable H22 cells was incubated in 1 ml 1640 serum-free medium with 50 μM DCFH-DA at 37 °C for 20 min. Next, the solution was centrifuged (500*g* at 4 °C) for 5 min and then washed with saline three times to thoroughly remove extracellular probe.

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