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Cytotoxicity and therapeutic effect of irinotecan combined with selenium nanoparticles

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ABSTRACT

Although chemotherapeutic drugs are widely applied for clinic tumor treatment, severe toxicity restricts their therapeutic efficacy. In this study, we reported a new form of selenium, selenium nanoparticles (Nano Se) which have significant lower toxicity and acceptable bioavailability. We investigated Nano Se as chemotherapy preventive agent to protect against toxicities of anticancer drug irinotecan and synergistically enhance the anti-tumor treatment effect *in vitro* and *in vivo*. The underlying mechanisms were also investigated. The combination of Nano Se and irinotecan showed increased cytotoxic effect with HCT-8 tumor cells likely by p53 mediated apoptosis. Nano Se inhibited growth of HCT-8 tumor cells partially through caspases mediated apoptosis. *In vivo* experiment showed Nano Se at a dose of 4 mg/kg/day significantly alleviated adverse effects induced by irinotecan (60 mg/kg) treatment. Nano Se alone treatment did not induce any toxic manifestations. The combination of Nano Se and irinotecan dramatically inhibited tumor growth and significantly induced apoptosis of tumor cells in HCT-8 cells xenografted tumor. Tumor inhibition rate was about 17.2%, 48.6% and 62.1% for Nano Se, irinotecan and the combination of Nano Se and irinotecan, respectively. The beneficial effects of Nano Se for tumor therapy were mainly ascribed to selectively regulating Nrf2-ARE (antioxidant responsive elements) pathway in tumor tissues and normal tissues. Our results suggest Nano Se is a promising selenium species with potential application in cancer treatment.

1. Introduction

Although the broad-spectrum clinical applications of chemotherapeutic drugs for tumor treatment, as a double-edged sword, severe toxic side effects, such as myelosuppression, severe damage to digestive tract, liver, kidneys and other vital organs, restrict their therapeutic efficacy, even directly or indirectly lead to death by reducing body's natural anti-tumor immunity [1]. Pharmacologically speaking, to overcome the side effects of chemotherapy, a variety of drugs, such as elevated white blood cell drug, painkillers, antiemetic and so on are employed for clinic use to improve patients' quality of life. However, with increased administration of adjuvant drugs which can not fundamentally change cachexia of cancer patients, some new side effects will come into being. One potential therapeutic strategy to solve the problem is concomitant use of adjuvant agents which can decrease toxicity of chemotherapy drugs without compromising their efficacy. However, we face a great challenge that drugs reduced the toxicity of chemotherapy drugs inevitably reduce the efficacy of chemotherapy drugs. In view of the drawbacks of chemotherapy agents, there is a critical need to develop new treatment strategies that reduce the toxicity of anti-tumor drugs, while reversing drug resistance and enhancing therapeutic selectivity.

Recently, selenium (Se) has attracted a great interest due to its important health effects, particularly the effects related to the immune response and cancer prevention activity [2–6]. Results of epidemiological, preclinical and clinical studies have shown Se decreases the risk of a series of cancers, such as mammary, prostate, lung, colon and liver cancer [7–13]. Research results also suggest

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both dosage and Se forms are critical for its anticancer activity [14]. Some molecular Se compounds, such as selenomethionine (SeMet), sodium selenite, methylselenocysteine etc, have more effective anticancer activity at high dosage [13,15–18]. However, high doses of selenite give rise to great concerns about its toxicity [19–22] In this regard, nanoparticles of elemental selenium (Nano Se) as a new form of selenium appears to be more effective than that of other Se sources at inducing selenoproteins, scavenging of free radicals, preventing oxidative DNA damage through potent antioxidant activity with low toxicity and acceptable bioavailability [21,23–28]. Moreover, Nano Se can be given by various routes, such as p.o. or i.v. routes and is convenient for administration. These advantages enable Nano Se a suitable adjuvant agent for tumor chemotherapy.

In this study, we designed new therapeutic strategies which combined Nano Se with anticancer drug irinotecan and investigated whether Nano Se can protect against toxicities of irinotecan without compromising its antitumor activity, or even improve the therapeutic effect *in vitro* and *in vivo*. As apoptosis is an important mechanism to suppress cancer growth, and caspases are critical for inducing apoptosis, we quantified apoptosis and detected the activated caspases forms in human ileocecal adenocarcinoma HCT-8 cells. The tumor suppressor p53 mediate multiple biological functions of apoptosis, cell cycle arrest and DNA repair by interacting with other proteins. We analyzed the expression of p53 protein in HCT-8 cells and IEC6 rat gut epithelial cells after irinotecan treatment with or without Nano Se combination. Moreover, we examined apoptosis by TUNEL assay in tumor tissue. Peroxiredoxin 1 (Prx1), thioredoxin 1 (Trx1) and their upstream regulator nuclear factor erythroid-2 related factor 2 (Nrf2) protein expression in liver as representative of normal tissue and tumor tissue were examined by immunohistochemistry. We analyzed the possible mechanism of Nano Se as selective modulators of the therapeutic selectivity and efficacy of chemotherapy agent irinotecan. These results obtained will have great potential to provide a new type of adjuvant agent for tumor chemotherapy.

2. Materials and methods

2.1. Materials

Sodium selenite, glutathione (GSH), bovine serum albumin (BSA), sodium hydroxide, hematoxylin and eosin were obtained from Sigma–Aldrich. Irinotecan hydrochloride was purchased from Beijing BaijiXinyao Pharmacy. TUNEL Apoptosis Assay Kit was obtained from Promega. Rabbit monoclonal Anti-Peroxiredoxin 1 (Prx1) antibody and rabbit monoclonal Anti-phospho-Nuclear factor erythroid 2-related factor 2 (Nrf2) (Ser40) antibody were purchased from Abcam. Rabbit polyclonal Anti-Thioredoxin 1 (Trx1) antibody and polymerized HRP-Goat Anti-rabbit IgG were purchased from Beijing Biosynthesis Biotechnology Co. Ltd.

2.2. Preparation and characterization of Nano Se

The Nano Se was fabricated according to our previously reported method [14]. Briefly, 40 mL 10 mM sodium selenite was mixed with 160 mL 10 mM GSH solution containing 1.25 mg/mL BSA. The pH of the solution was adjusted to 7.1 by addition of 1.0 M NaOH, Nano Se and oxidized glutathione (GSSG) were formed instantly. The resulting red product solution was dialyzed against double distilled water for 72 h, the water was changed every 6 h to separate GSSG from the Nano Se. The final solution containing Nano Se was stored at 4 °C.

The morphology and structure of the resulting Nano Se was observed with a JEOL-200CX transmission electron microscope (TEM). The mean size and size distribution were measured at 25 °C by dynamic light scattering (DLS) technique (NICOMP 380/ZLS (PSS)) and analyzed by ZPW388 software.

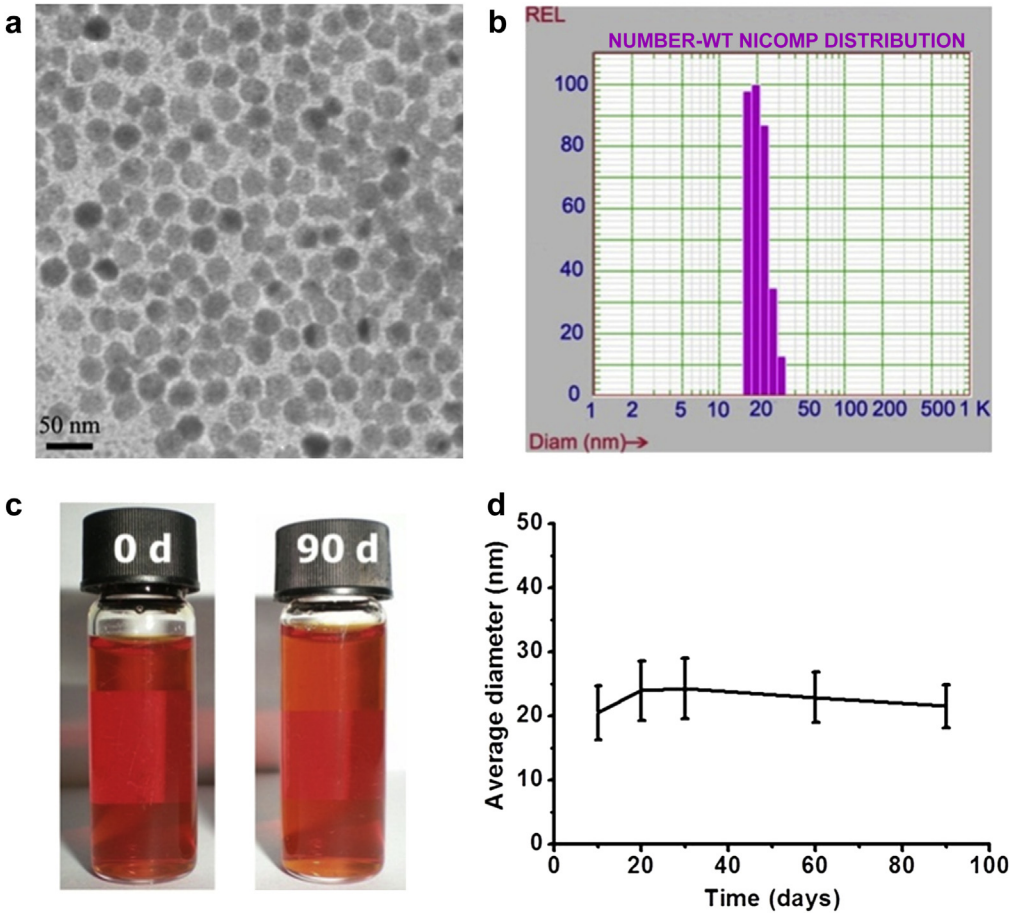

Fig. 1. The characterization of Nano Se. a. TEM image of Nano Se. b. Size distribution of Nano Se by dynamic light scattering (DLS). c. Light image of Nano Se solution at different storage time. d. The stability of Nano Se in deionized water observed using DLS particle size analyzer.