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Acute and subacute toxicities of biogenic tellurium nanorods in mice

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ABSTRACT

The current study was performed to evaluate the acute and subacute toxicities of biogenic tellurium nanorods (Te NRs). The Te NRs were prepared using *Pseudomonas pseudoalcaligenes* strain Te in a culture medium containing K_2TeO_3 (1 mM) and their physiochemical properties were investigated using TEM, EDX and XRD. The median lethal dose (LD_{50}) of Te NRs and potassium tellurite (K_2TeO_3) were determined in mice and the subacute toxicity was also evaluated. The experimental design involved certain general toxicological, haematological, serum and histopathological investigations. The TEM and XRD analyses showed that the biogenic nanoparticles were rod-shaped and hexagonal. The toxicological evaluation showed that the LD_{50} values of Te NRs and K_2TeO_3 were 60 and 12.5 mg/kg, respectively. Higher doses of Te NRs (6 mg/kg) and K_2TeO_3 (1.25 mg/kg) were accompanied by signs of toxicity, including lower body weight, elevation in MDA and depletion in GSH content, SOD and CAT activity, and changes in biochemistry parameters. No obvious histopathological changes were observed in the treatment with Te NRs. In conclusion, the biogenic Te NRs were less toxic as compared to K_2TeO_3, and the no-observed-adverse-effect level (NOAEL) dose of Te NRs in 14 days subacute toxicity study was lower than 1.2 mg/kg.

1. Introduction

Tellurium (Te) is a metalloid belonging to the chalcogen family of elements in the periodic table, which has common chemical properties with important biological elements such as oxygen, sulfur, selenium (Se) and polonium (Reinoso et al., 2012; Zare et al., 2017). Its biological functions are not clearly established to date, because most of the Te containing compounds are highly toxic. Hence, it was reported that Te is a nearly 'forgotten' element in biology (Ba et al., 2010). However, fungi could incorporate Te to use instead of sulfur and Se in amino acids (cysteine and methionine) (Ramadan et al., 1989). It was indicated that trace elements: copper, zinc and Se are linked together in cytosolic defense against oxidative and nitrosative stress (Klotz et al., 2003). Reactive oxygen (ROS) or reactive nitrogen species (RNS) were subsequently reduced by the selenoenzyme glutathione peroxidase (GPx). In addition to selenocysteine (as in GPx), Se could also be as selenomethionine that catalyzes the reduction in RNS such as peroxynitrite (Klotz et al., 2003). Low-molecular-weight organoselenium and organotellurium compounds also accelerate the reduction of hydroperoxides or peroxynitritein to protect plasmid DNA from their-induced single-strand breaks (Klotz et al., 2003). Stimulating protective cellular stress-signaling pathways such as the antiapoptotic phosphoinositide-3-kinase/Akt cascade is also another antioxidative function of these trace elements to stabilize proteins (Klotz et al., 2003). Incorporation of heavy atoms to selected sites in proteins (telluromethionine), antibacterial (tellurite), cytotoxicity, anticancer, enzymatic, fluorescent and quantum dots (cadmium

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telluride (CdTe) nanoparticles) properties, have also demonstrated the biological potency of Te in pharmacotherapy, imaging and diagnosis (Ba et al., 2010). Furthermore, it has been reported that Te has antifungal, dose- and duration-dependent lipid-lowering and free radical scavenging activities (Zare et al., 2017; Kaur et al., 2003a, b). Lipid-lowering was proposed for inhibition of squalene mono oxygenase activity, an important enzyme involved in the synthesis of ergosterol, cholesterol and phytosterols (Zare et al., 2014).

Toxicity of Te nanostructures and the molecular mechanisms underlying these negative effects have not been elucidated yet. It was found that diphenylditelluride (DPDT) induced neurotoxicity by cytoskeletal disruption resulting from elevation in intracellular calcium ion concentrations (Heimfarth et al., 2017). Oxidative stress (OS) events are another cause of cellular toxicity, cell membrane damage, cell apoptosis and DNA damage of Te compounds (Zhang et al., 2015a, 2015b). DPDT induced apoptosis by increasing caspase 3/7 and 9 activities in HT-29 and CCD-18Co cells, and tellurium tetra chloride ($TeCl_4$) caused necrosis (Vij and Hardej, 2012). Furthermore, liver, kidney and lung toxicity have been reported (da Luz et al., 2015; Zhang et al., 2015b; Pinton et al., 2011). Vascular endothelial toxicity and cardiovascular diseases has also occurred following CdTe quantum dots treatment (Yan et al., 2011). However, further studies are required to be performed on evaluation of biological role and toxicity profile of Te.

Biotechnologically-derived nanoparticles by bacteria species has recently been an attractive subject with increasing attention. There were biological sources for synthesis of nanostructure trace elements. Nanobiosynthesis provides many advantages including uniformity in particle shape, size and less toxicity (Shakibaie et al., 2013). However, little information has been published to establish safety profile for the consumption. Therefore, in the present study, the acute and subacute toxicities of the biogenic Te nanorods (Te NRs) produced by *Pseudomonas pseudoalcaligenes* Te strain in mice were investigated.

2. Materials and methods

2.1. Chemicals and solvents

Potassium tellurite ($K_2TeO_3 \cdot 3H_2O$), nutrient broth, nutrient agar, Ethylene di amine tetra acetic acid (EDTA), n-octanol, malondialdehyde (MDA) bis (diethyl acetal), and disodium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$) were purchased from Merck Chemicals (Darmstadt, Germany). Thiobarbituric acid (TBA), 5,5′-dithio-bis-[2-nitrobenzoic acid] (DTNB), L-glutathione were obtained from Solarbio.Co. Ltd. (Beijing, China). Pyrogallol prepared from Samchun Pure Chemical Co., Ltd. (Seoul, South Korea). Other chemicals and solvents were of analytical grade.

2.2. Biosynthesis, purification and characterization of the Te NRs

Te NRs were biosynthesized using a recently described method (Forootanfar et al., 2015). Briefly, 500 ml Erlenmeyer flasks containing 100 ml of sterile NB medium were supplemented with K_2TeO_3 (final concentration 1 mM) and there were inoculated with 1 ml of the fresh inoculums of *P. pseudoalcaligenes* strain Te (OD_{600}, 0.1). After 80 h incubation in a shaker incubator (30 °C, 150 rpm), the culture media were centrifuged (4000 × g, 10 min) and the obtained biomass were washed using sterile NaCl solution (0.9%). The cells were frozen by liquid nitrogen in a mortar and were disrupted by a pestle. After ultrasonication (100 W, 5 min), the resulting slurry was washed three times by sequential centrifugation (10,000 × *g*, 5 min) with 1.5 M Tris-HCl buffer (pH 8.3) containing SDS (1%) and deionized water, respectively. Then, Te NRs were extracted and purified using an organic-aqueous two partitioning system (n-octyl alcohol–water) as described earlier (Shakibaie et al., 2015). Transmission electron micrographs of NRs were obtained using a transmission electron microscope (TEM) apparatus (Zeiss 902A) operated at an accelerating voltage of 80 Kv and elemental composition of Te NRs samples were analyzed using an EDX (energy dispersive X-ray) microanalyzer. The related size distribution pattern of Te NRs was plotted by the manual counting of 250 individual particles from different TEM images. The XRD pattern of the prepared Te NRs was examined by the X-ray diffractometer (Philips, PW1710) with CuKα radiation (λ = 1.5405 A°) in the 2θ range of 0°–80°.

2.3. Animals

Male NMRI mice (body weight 22 ± 2 g) were used in this study. The mice were obtained from Faculty of Medicine, Kerman University of Medical Science (Kerman, Iran) and housed in plastic cages (six to ten each) in a standard condition with controlled temperature (22 ± 1 °C) and humidity (50 ± 10%) with a 12/12 h light/dark condition for at least a week prior to experimentation. The mice had free access to food and water. The experimental procedures carried out in this study were in compliance with the guidelines of the Kerman University of Medical Science (Kerman, Iran) for the care and use of laboratory animals.

2.4. Acute toxicity

One hundred and twenty mice were randomly divided into twelve groups of ten per group. Various doses of biogenic Te NRs and K_2TeO_3 prepared in distilled water (DW) were administered once daily by oral gavage at doses of 5, 10, 12.5, 15, 40 and 50 mg/kg for K_2TeO_3 and at doses of 10, 25, 50, 60, 75 and 90 mg/kg for biogenic Te NRs. These doses were determined according to the data from the pilot study to estimate the value of the LD_{50}. The animals were observed for general behavior and mortality on the day of dosing (for 6 h) and then daily for 14 days. Cumulative mortality within these days was used for the calculation of median lethal dose (LD_{50}).

2.5. Subacute toxicity evaluation

Thirty mice were randomly distributed into five groups of six mice per group. The first group (control) was administrated DW orally, and the second to forth groups were through oral administration of Te NRs once daily at the doses of 1/50, 1/25 and 1/10 LD_{50} = 60 mg/kg (1.2, 2.4 and 6 mg/kg), respectively, for 14 consecutive days. The fifth group received K_2TeO_3 at the dose of 1/10 LD_{50} = 12.5 mg/kg or 1.25 mg/kg upon oral administration once per day for 14 consecutive days. Furthermore, the mice were weighted once daily for 14 consecutive days.

2.6. Hematological and serum biochemical assessment

At the end of the experiment, all animals were anaesthetized by Ketamine-Xylizine, then the abdomen was opened, and blood samples were collected from cardiac puncture. Blood samples were divided into two aliquot portions. One portion was collected into heparinized eppendorf tube for hematological assessment. Hematological parameters that were measured using hematological autoanalyzer (Mindray, Model BC 5800, China) included white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), and platelet (PLT) count. While the other portion was collected into eppendorf tubes containing no