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Establishment of drug delivery system nanocapsulated with an antioxidant (+)-catechin hydrate and sodium meta borate chelator against sodium fluoride induced oxidative stress in rats

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ABSTRACT

Oxidative stress a major cause of fluoride induced toxicity and mitochondrial impairment in common in experimental rats during chronic exposure of fluoride. Attempts have been made in the present experiment to diminish oxidative damage, combined therapy with (+)-catechin hydrate (an antioxidant) and sodium meta borate (chelator) were used. Fluoride intoxication in rats was performed by using 13 mg/kg NaF and both antioxidant CH and chelator SMB were used at a concentration of 8.98 μM/kg body weight. Mixture of CH and SMB in free or in PLGA nanocapsule encapsulated form were prepared. The efficacies of those formulations were tested in combating free radical mediated oxidative insult produced by sodium fluoride (NaF). The amalgamated therapy used in this experiment was shown to reduce fluoride levels in liver, brain and kidney from 9.5, 5.5, 6.3 μg/g to 4.6, 2, 2.6 μg/g, respectively. Our result indicated that the combined chelator and antioxidant therapy in nanocapsulated drug delivery system could provide a projection in combating fluoride induced mitochondrial impairment in rat model.

1. Introduction

Fluorine is a necessary trace element for human health. However, fluoride accumulation leads to cascading effects resulting in altered physiological functions in human being. About 50% of ground water sources in India have been identified as fluoride contaminated. Excessive ingestion of fluoride causes fluoride intoxication or fluorosis in animal system. In recent decades extensive information has accumulated on the role of fluoride in cellular respiratory processes and associated free radical reactions [1]. The adverse effect of fluoride on haematopoietic organs have been reported [2] that suppress the ability of white blood cells [3].The development of bone cancer by fluoride has also been reported [4]. Fluoride causes premature aging in human body also [5]. It has been demonstrated that fluoride induces a huge amount of free oxygen radical generation in humans [6] and causes a down regulation of the activity of SOD and CAT [7]. Superoxide anion arising through metabolic processes is considered to be the primary reactive oxygen species (ROS) and can either directly or prevalently through enzyme or metal catalysed processes further interact with other molecules to generate secondary ROS [8]. Fluoride intoxication also decreases the levels of mitochondrial enzymes such as ICDH, SDH, MDH, α-KGDH and NADH in cardiac tissue of rats [9].

Lewis acid boron compounds typically interact with fluoride anions to form corresponding fluoroborate species. Cataionic boranes form chelate complexes with fluoride anions [10]. Catechin hydrate, a polyphenolic flavonol exhibit its antioxidant properties against many diseases including ischemic neurodegeneration, atherosclerosis, stomach cancer [11–13]. Our previous report already discussed the potency of combined therapy by

Abbreviations: ROS, reactive oxygen species; NaF, sodium fluoride; H_2O_2, hydrogen peroxide; ATP, adenosine tri phosphate; ADP, adenosine di phosphate; CH, catechin hydrate; SMB, sodium meta borate; NP, nanoparticle; NPCH, nanoparticulatecatechin hydrate; NPSMB, nanoparticulate sodium meta borate; NP (CH + SMB), nanoparticle encapsulated with catechin hydrate and sodium meta borate; ICDH, isocitrate dehydrogenase; SDH, succinate dehydrogenase; MDH, malate dehydrogenase; αKGDH, alpha ketoglutarate dehydrogenase; GR, glutathione reductase; GSSG, oxidised glutathione; TISAB, total ionic strength adjustment buffer; PLGA, poly lactide-co-glycolide; DMAB, didodecyldimethylammonium bromide; DPH, diphenylhexatriene; EDTA, ethylenediaminetetraacetic acid; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester; BBB, blood brain barrier; HE, hematoxylin and eosin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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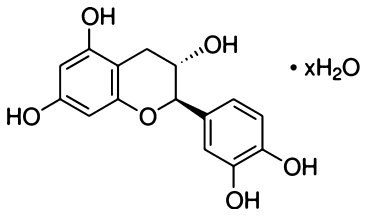

Fig. 1. Chemical structure of (+)-catechin hydrate ($C_{15}H_{14}O_6 \cdot xH_2O$)

chelator and antioxidant drug to prevent arsenic induced chronic toxicity [14]. Hence an improved combinatorial delivery mode for (+)-catechin hydrate and sodium metaborate could be expected an effective approach to reduce oxidative stress mediated by sodium fluoride (Fig. 1).

The use of nanoparticles as drug carriers has an obvious advantage over existing approaches to circumvent the blood brain barrier. Loaded with both hydrophobic and hydrophilic drugs, this colloidal carrier has the ability to cross both the GI tract mucosal barrier and the BBB to enhance drug bioavailability via particle uptake mechanisms and therefore, it appears to have significant promise in delivering therapeutic molecules [15].

In this study we noticed that the potency of coencapsulated catechin hydrate and sodium metaborate to fight against fluoride induced fluorosis is more as compared to nanocapsulated catechin hydrate or, nanocapsulated sodium metaborate in animal model. Thus, the combined therapy is proved to be more advanced in rat model against fluoride toxicity by improving oxidative stress related damage and deposition of fluoride.

2. Materials and methods

2.1. Chemicals

Poly (lactide-co-glycolide) (PLGA) (Resomer RG 50:50H) and didodecyldimethylammonium bromide (DMAB) were purchased from Aldrich (St. Louis, MO, USA) and Sigma (St. Louis, MO, USA), respectively. Ethyl acetate (AR Grade) was purchased from Rankem Fine Chemicals (New Delhi, India). Sodium fluoride (Merck, Darmstadt, Germany) was used for experimental purposes. Fast green FCF and Sirius Rose BB were acquired from Fluka. Chloroform and methanol were purchased from E. Merck. (+)-catechin hydrate was purchased from Sigma Chemicals (St. Louis, MO, USA). ALT, AST determination kits were purchased from Span Diagnostic Ltd., India. All other reagents were of analytical grade.

2.2. Preperation of CH and SMB nanocapsules

A modified emulsion–diffusion–evaporation method [16] was used to make nanocapsules of catechin hydrate and sodium metaborate. 5 mg CH was dissolved in 1 ml ethyl acetate and 5 mg SMB in 1 ml water. In brief, 70 mg of PLGA was dissolved in 5 ml of ethyl acetate at room temperature. The organic solution of PLGA and drug or PLGA and SMB in ethyl acetate was then separately emulsified with 5 ml of an aqueous phase containing 50 mg of DMAB. The resulting organic/water emulsion was stirred at room temperature before being homogenized at 15,000 rpm for 5 min with a high-speed homogenizer (Polytron PT4000; Polytron Kinematica, Lucerne, Switzerland). The organic solvent was removed by constant stirring on a water bath set at 40 °C. Nitrogen gas was used to remove further traces of organic solvent. The suspension was ultracentrifuged at 35,000 rpm in a Sorval RC 5B Plus using the Sorval T-865 rotor for 1 h. The pellet of nanocapsules was washed with phosphate-buffered saline (PBS) twice and suspended in 0.25 M PBS.

2.3. Characterization of nanocapsule using atomic force microscopy and scanning electron microscopy

5 μl of the sample were deposited onto Ruby mica sheet (ASTM V1 Grade Ruby Mica from MICAFAB, Chennai) for 15–30 min. Samples were gently washed with 0.5 ml MilliQ water to remove the molecules that was not firmly attached to the mica. Mica sheets are basically negatively charged so molecule binds strongly on the mica surface, after drying the sample by using vacuum dryer (Fig. 2).

AAC mode AFM was performed using a Pico plus 5500 ILM AFM (Agilent Technologies USA) with a piezoscanner with 9 μm maximum range. All images were obtained in aquatic mode with 225 μm length cantilever used from nanosensors, USA, having 150–300 kHz resonance frequency. Images were processed by flatten using Pico view1.1 version software (Agilent Technologies, USA). 3D imaging has been done through Pico Image Advanced version software (Agilent Technologies, USA).

For SEM, lyophilised samples were coated with a thin layer of carbon, for high resolution electron imaging applications. High energy beam of electrons systematically scans across a surface of samples to reveal signal that gives information about sample topography, morphology by SEM.

2.4. Animal experiment

Female Sprague dawley rats, each weighing approximately 80–120 g, were reared and acclimatized to conditions in the animal house (20–22 °C, 60–80% relative humidity, 12-h light/dark cycle) for 7 days before the commencement of the treatment, during which they received food (purchased from Hindustan Lever Ltd., Maharashtra, India) and drinking water. The 50% acute lethal dose (ALD50) of sodium fluoride (NaF) via the oral route in rats was found to be 52 mg/kg body wt. The rats were divided into seven groups and each group contained five animals. Animals in group 1 were kept as untreated normals, and group 2 animals were considered fluoride-treated experimental controls, and one-fifth the ALD50 of sodium fluoride (i.e., approximately 13 mg/kg body weight) was given daily for 16 weeks by oral gavage. All animals except group 1 were treated identically for 16 weeks, and then various treatments were started for 8 weeks. After 16 weeks of NaF administration the animals in group 3 were fed free CH (8.98 μmol/kg body weight of CH in 0.2% Tween 80 aqueous solution). Group 4 received a SMB solution orally (8.98 μmol/kg body weight of SMB in water); groups 5 and 6 were fed a nanocapsulated formulation of CH or SMB (containing 8.98 μmol/kg body weight of either CH or SMB), respectively, for 8 weeks. Group 7 received coencapsulated CH: SMB in equimolar proportions (8.98 μmol total) in nanocapsulated form. The animals were treated with drugs twice a week for four weeks. For last week animals were treated with normal water and food. At the end of 26 weeks rats were anesthetized with ether and sacrificed. Blood is collected through cardiac puncture and serum and plasma were separated by centrifugation at low speed to prevent haemolysis maintained at 4 °C. Serum was stored in 4 °C for further clinical enzyme assay. After dissection rat liver, brain and kidney tissues were isolated, wash with cold physiological saline and processed for mitochondrial particle preparation as soon as possible to avoid tissue damage. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, OIST, Vidyasagar University and the animals were cared in accordance with the "Guide for the care and use of laboratory animals" and "Committee for the purpose of control and supervision on experimental animals."