Chemico-Biological Interactions 245 (2016) 90–99

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Tetracycline hydrochloride: A potential clinical drug for radioprotection

Amit Alok, N.K. Chaudhury*

Division of Radiation Biodosimetry, Institute of Nuclear Medicine & Allied Sciences, Delhi, 110054, India

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
28 December 2015
Accepted 1 January 2016
Available online 5 January 2016

Keywords:
Tetracycline hydrochloride
Radiation
Free radicals
DMF

ABSTRACT

Radiation exposure in planned scenario necessarily requires radioprotector for protection against radiation injuries in tissues and organs. A large number of potential radioprotectors have been investigated but no approved radioprotector is available. Hence, in quest for radioprotector, repurposing of clinical drug is an approach which aims at finding the radioprotective potential of known drugs so that in case of untoward accident the knowledge could be translated to drug usage. In this study, we have investigated the radical scavenging properties of tetracycline pertaining to radioprotection. Our study suggests that tetracycline hydrochloride efficiently scavenges free radicals in ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays. Hydroxyl radical scavenging assay has demonstrated its ability to scavenge gamma radiation induced free radicals by lowering the formation of malondialdehyde. Radiation causes damage to macromolecules and hence the protection offered by tetracycline hydrochloride to DNA and protein shows its radioprotective potential. Plasmid DNA relaxation study with pBR322 has shown that tetracycline hydrochloride confers dose modification factor (DMF) of 2 and 4 at 100 μM and 250 μM concentration respectively. Tetracycline hydrochloride has also protected bovine serum albumin (BSA) from radiation induced degradation. The *ex vivo* studies for lipid peroxidation and mitochondrial membrane potential further substantiate our findings. The whole body animal survival study has shown the drug to offer 20% protection at a lethal radiation dose of 9 Gy. This study demonstrates the radioprotective potential of the drug by providing some insight into *ex vivo* and *in vivo* efficacy.

1. Introduction

Radiation exposure is known to cause various harmful effects which can lead to morbidity and mortality. Reports from several accidental sites have reiterated the need for radioprotectors for first responders. Radiation countermeasure agents are necessary for management of radiation emergency in the event of accidents in nuclear power plant, nuclear industries, military operations in nuclear warfare and dirty bomb attack by terrorists. The administration of radioprotectors prior to radiation exposure is expected to reduce excess free radicals in the cellular system [1]. The members of first responders for radiation emergency will require radioprotector before undertaking rescue operation for protection against risks of radiation injuries. In addition, radioprotectors could be useful in radiotherapy for protecting tissues surrounding target volume from the effects of radiation. In accidental scenarios, radiomitigators are necessary for administration after exposure for reducing progression of radiation injuries and thereby mitigate the burden of radiation exposure. Radiation causes damage to tissues and organs and the severity of radiation effect depends on the absorbed dose of radiation. All organs are affected by radiation, however, hematopoietic and gastrointestinal systems are among the most sensitive. Radiation countermeasure agents are necessary as prophylactic, mitigating and therapeutic agents. Since, radiation induced organ injuries are known to cause spreading of infections and lowering of immune function, therefore, a number of supportive medicines will be necessary for the management of radiation victims. Many potential radioprotectors have been discovered by various researchers but none have reached the drug development stage. Amifostine developed by Walter Reed Army Research Institute in 1980's has shown strong radioprotection in animal

* Corresponding author. Division of Radiation Biodosimetry, Institute of Nuclear Medicine & Allied Sciences, Brig S K Mazumdar Marg, Timarpur Delhi, 110054, India.
E-mail address: nkcinmas@rediffmail.com (N.K. Chaudhury).

http://dx.doi.org/10.1016/j.cbi.2016.01.001
0009-2797/

model against lethal radiation dose, but because of systemic toxicity in human amifostine was not found suitable as a radioprotector. However, it was approved by US Food and Drug Administration (FDA) as cytoprotecatnt for the treatment of head and neck cancer in radiotherapy patients [2,3]. The major limitations of all investigating molecules are poor bioavailability and efficacy [4] which gets compounded by the non availability of human cohort for clinical trials due to ethical reasons.

Tetracycline hydrochloride (Fig 1 inset) is a wide spectrum antibiotic against many gram-negative and gram-positive bacteria [5,6]. Recent report has suggested the usage of tetracycline as a radiation countermeasure agent [7]. Tetracycline has been shown to protect hematopoietic stem/progenitor cell populations from radiation damage in C3H mice and provided 87.5% survival when administered before radiation and 35% when administered 24 h after lethal radiation dose of 8 Gy. A broad spectrum antibiotic like tetracycline hydrochloride, if effective as a radioprotecor, can serve as multipurpose drug to contain infection which is prevalent after radiation injury. Free radicals interact with all biomolecules resulting in damage to DNA, proteins and lipids [8]. Thus, compounds having free radical scavenging activity can lower the impact of radiation at cellular level [9].

Tetracycline hydrochloride is clinically used as 250 mg and 500 mg capsules for oral administration in human [10]. There are reports of the contraindications of tetracycline hydrochloride [11–20]. All classes of tetracyclines form a stable calcium complex in bone forming tissues. A decrease in fibula growth rate has been reported in premature infants treated with oral tetracycline in doses of 25 mg/kg every 6 h [11]. Studies in animal model have shown that tetracycline crosses the placenta and can have toxic effects on the developing fetus. Tetracyclines may cause permanent discoloration of teeth, enamel hypoplasia, and inhibition of skeletal growth in the fetus. This drug is to be used with optimum caution in case of renal impairment. The long-term use of tetracyclines was reported to cause microscopic brown-black discoloration of the thyroid gland; however, abnormal thyroid function has not been reported [12–15]. There are selective case studies on the side effects of tetracycline in gastrointestinal, nervous, renal, hematological and hepatic system in which drug has been shown to cause metabolic acidosis as well. [11, 16–20]. However, radioprotective dose envisaged is likely to be single dose therefore; much of the contraindications mentioned may not be of serious concern.

One of the reasons of not having a approved radioprotector as yet is the long time taken during drug development process which involves human clinical trial. A repositioned drug does not require initial years typically required for the discovery stage of drug development. The repositioning strategy involves reinvestigating drug candidates for potential new therapaeutic application by applying drug's known pharmaceutical properties like toxicity, pharmacokinetics and pharmacodynamics. Recycling old drugs and rescuing shelved drugs make drug repositioning an attractive form of drug discovery and many drugs have been discovered for different clinical conditions [21,22]. The report of potential radioprotection of tetracycline hydrochloride and observed radioprotection in C57BL/6 mice opens up a challenging task to take the lead further to develop it for radioprotective application. In the present study, we have assessed radical scavenging properties of tetracycline hydrochloride using free radical scavenging assays like DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and FRAP (Ferric reducing antioxidant power). Detailed analysis of the observed radioprotection on macromolecules like plasmid DNA (pBR322) and bovine serum albumin (BSA) along with other *ex vivo* studies on tissue samples have suggested its strong radioprotective potential. Finally, the survival study with pre administration of tetracycline hydrochloride in whole body irradiated C57BL/6 mice [23] at lethal dose establishes its potential as radioprotector *in vivo* system.

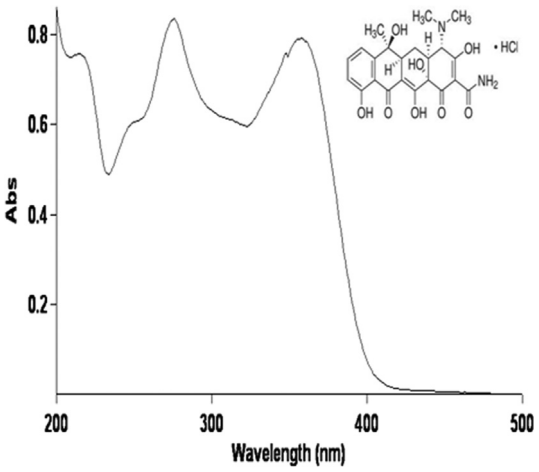

Fig. 1. UV absorption spectra of tetracycline hydrochloride (50 μM) in water. Structure of tetracycline hydrochloride (inset).

2. Materials and method

2.1. Materials

The chemicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, 2-Deoxy-D-ribose, propidium Iodide, N,N,methylene-bisacrylamide, bovine serum albumin (BSA), ammonium persulfate, 2-Mercaptoethaol, methanol, acrylamide, propidium iodide, low melting agarose, thiobarbituric acid (TBA), rhodamine 123, ferrous sulphate, ethidium bromide, normal melting agarose, methanol, brilliant blue were obtained from Sigma Chemical, USA. Tetracycline hydrochloride was from Fluka, USA. Sodium acetate and sodium hydroxide (NaOH), was procured from Merck, Germany. N-Lauroylsarcosine sodium, Dulbecco's phosphate buffered saline (PBS), trichloroacetic acid (TCA), hydrochloric acid (HCl) EDTA disodium salt hydrate, Tris Hydrochloride and Guanidine Hydrochloride was obtained from Himedia, India. Protein estimation kit by Lowry method and pBR322 were obtained from Genei, India.

2.2. Experimental animals and whole body survival study

C57BL/6 male mice of age 6–8 weeks were used for *in vivo* and *ex vivo* animal experiments. The whole body survival study was carried out at lethal radiation dose of 9 Gy. Briefly, the mice were issued, acclimatized and divided into three groups i.e. (i) Control, (ii) Radiation dose of 9 Gy, (iii) Tetracycline hydrochloride 150 mg/kg + 9 Gy. Tetracycline hydrochloride (150 mg/kg) was prepared in sterilised water immediately before intra peritoneal injection and was administered 1 h and 24 h before radiation. The mice were then kept and maintained in a standard conditions in the animal house and was routinely monitored for bodyweight. The mice used for the survival experiment and all other subsequent experiments involving animals were obtained under ethical clearance from the Institutional Animal Ethics Committee (IAEC).

2.3. Gamma-radiation sources and irradiation

Gamma irradiation of plasmid DNA, BSA and other tissue samples in *ex vivo* studies requiring high doses were carried out using