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Protective effect of riboflavin on cisplatin induced toxicities: A gender-dependent study

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ABSTRACT

The toxicity exerted by the anticancer drug, cisplatin *in vivo* is functional to many factors such as dose, duration, gender and age etc. The present study is aimed to investigate if ameliorative potential of riboflavin on cisplatin induced toxicity is gender dependent. Eighty four adult mice from male and female sex were divided into seven groups ($n=6$) for both sexes. They were treated with riboflavin (2 mg/kg), cisplatin (2 mg/kg) and their two different combinations (cisplatin at 2 mg/kg with 1 mg/kg and 2 mg/kg of riboflavin) under photoillumination with their respective controls for the combination groups without photoillumination. After treatment, all groups were sacrificed and their kidney, liver and serum were collected for biochemical estimations, comet assay and histopathology. In the present investigation, it was evident from antioxidant and detoxification studies (SOD, CAT, GSH, GST, MDA and carbonyl level) that the female mice exhibited better tolerance towards cisplatin inducted toxicity and the ameliorative effect of riboflavin against cisplatin toxicity was found stronger in their combination groups as compared to the male groups as the activity of all antioxidant enzymes were found better concomitant with lower level of MDA and carbonyl contents in the female combination groups than their male counterparts. Furthermore, single cell gel electrophoresis and histopathological examination confirmed that restoration of normal nuclear and cellular integrity was more prominent in female with respect to the males after treatment in the combination groups in a dose-dependent manner. Hence, this study reveals that cisplatin is more toxic in male mice and the ameliorative effect of riboflavin against cisplatin toxicity is stronger in female mice.

Introduction

Cisplatin is one of the most preferred anticancer drugs for treatment of various forms of cancer and solid tumor. Chemotherapy induced toxicities have been major setback to realize the full potential of many anticancer drugs including cisplatin [1]. In spite of its broad clinical application and extensive research over five decades, its exact mechanism of action is still not clear although it is assumed that it derives the anticancer activity from its ability to create inter- and intra-strands cross links in cellular DNA that consequently derail the replication fork and transcription machinery leading to killing of the affected cells [2]. Parallel to this, another widely accepted notion attributive to its antineoplastic action is involvement of heightened oxidative and nitrosative stress leading to apoptosis and necrosis induction in the cancer cells [3,4].

Riboflavin is an essential vitamin that generally acts as a co-factor (flavin adenine mononucleotide and flavin adenine dinucleotide) in numerous enzymatic reactions in all forms of life. Besides, it has an excellent photosensitizing property that has been used under photodynamic therapy for treatment of various diseases. Various contemporary investigations strongly suggest that this vitamin has tremendous potential to be used in improving the chemotherapeutic potential of major anticancer drugs [5–7]. It is also reported that riboflavin deficiency makes human vulnerable to cancers while its sufficiency helps to fight tumors although not well established yet [8,9]. As combinational chemotherapy is cornerstone for treatment of most of the cancers, we have been trying to explore the adjuvant property of riboflavin with cisplatin that has fetched a very promising effect by normalizing cisplatin induced oxidative and nitrosative stress-mediated toxicities *in vitro* and *in vivo* [10,11] which are in agreement with various cell line-based contemporary studies by the different groups of investigators [12–14].

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A great deal of literature suggests that the severity of these toxic effects of cisplatin depends on many factors such as drug dose, frequency of its dosing as well as on gender, age and health status of the patients [15]. Several evidences suggest that both the genders respond differently to the same stress; however, no consolidated explanation or mechanism underlying this sexually dimorphic effect of stress has been reported so far [16]. Many investigators have indicated that females are better responding gender to chemotherapeutic agents such as carboplatin and paclitaxel significantly and have increased chance of receiving second-line therapy with improved quality of life and increased life span as compared to the males. Furthermore, according to a retrospective study on stage III non-small-cell lung cancer showed that the females overall survived longer than males with longer local recurrence-free survival after high-dose hyperfractionated radiation therapy and concurrent chemotherapy [17]. Contemporary studies demonstrate that there are factors such as expression of X chromosome genes and the female sex hormones (estrogen and progesterone) that dictate the outcome and efficacy of any mode of chemotherapy although the exact reasons and mechanism are still not known [18].

Despite extensive research on factors affecting the efficacy of chemotherapeutic drugs for long, no major comprehensive study has been reported yet to address the effect of gender on efficacy of key anticancer drugs such as cisplatin. As female gender has been identified as predictor for treatment response in various cancers, the present study is aimed to investigate whether the ameliorative potential of riboflavin against cisplatin induced toxicities is influenced by the gender *in vivo* or not.

Materials and methods

Materials

Cisplatin, riboflavin, normal melting agarose (NMA), ethidium bromide (EtBr), Histopaque 1077, Hank's balanced salt solution (HBSS), RPMI 1640 and low melting point agarose (LMPA) were purchased from Sigma-Aldrich Chemical Company, USA. Ethylenediaminetetraacetic acid (EDTA), triton X-100, tris-hydrochloric acid, sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and sodium hydroxide (NaOH) were bought from Qualigens Fine chemical company, India. All other chemicals used were purchased from Sisco Research Lab, Mumbai and HiMedia Laboratories Private Limited, Mumbai.

Animals and study groups

Eighty four healthy and adult Swiss albino mice were used in the present study. Among those, half of them were females and rest were males. They were housed in sufficiently large cages (six mice per cage) with maintained $25 \pm 2\,^{\circ}C$ and 12 h day–night cycle. They were acclimatized for 10 days before starting of the treatment on standard pellet mice diet and clean drinking water *ad libitum*. All the experiments conducted on the animals were performed in accordance of "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India).

The mice were distributed randomly into five groups taking six mice per group from both the sexes. They were named as—CN [control group treated with saline only], RF [group treated with riboflavin at the dose of 2 mg/kg body weight], CP [group treated with cisplatin at the dose of 2 mg/kg body weight]. The combination of cisplatin and riboflavin was named as group CB I [treated with the dose of 2 mg/kg body weight of cisplatin with 1 mg/kg body weight of riboflavin (combination I)] and Group CB II [treated with the dose of 2 mg/kg body weight of cisplatin with 2 mg/kg body weight of riboflavin (combination II)], respectively. All the animals were exposed to full-body irradiation under florescent light kept at ~10 cm distance at fluence rate of 38.6 W/m^2 for 12 h daily during daytime from 6 am to 6 pm [19]. Parallel to these, additional two combination groups without photoillumination—CB I' and CB II' were also maintained for both the genders. All the doses were injected intraperitoneally with 1 mL capacity syringe using saline as vehicle solution for all the treatment chemicals. Besides, cisplatin was injected followed by riboflavin administration after a gap of 30 min in all the combination treated groups [CB I, CB I', CB II and CB II']. The dorsal surface of all the mice was mildly shaved for maximum absorption of light through the skin. The mice were given a daily injection for 3 days followed by a gap of a week; then again a daily dose for 3 days with a week's gap and finally three more daily injections were given. This treatment schedule was implemented in accordance of the currently employed cancer treatment strategy for the patients. The treatment strategy, dose and the duration of treatment were chosen to study the chronic effect of the treatment at moderately toxic dose of the drug. All the mice were healthy during the whole treatment. All the animals were sacrificed on the same day by cervical dislocation method on the next day to the final dose given.

Preparation of samples

After the sacrifice, kidney and liver of the treated mice were washed with ice-cold phosphate buffer saline. Their blood was centrifuged at $1500 \times g$ for 10 min to collect the serum. Their kidneys and livers were also homogenized separately at $3000 \times g$ in potassium–phosphate buffer (pH 7.36, 0.1 M) and their supernatants were kept for biochemical assays and estimations. Prior to it, 1 mL of homogenate of each mouse was saved for estimation of reduced glutathione (GSH) and total malondialdehyde (MDA) estimation. All the samples were labeled properly and kept at $-20\,^{\circ}C$ till further analysis. Besides, organs from each groups were also saved for histopathology and comet assay.

Assay of superoxide dismutase, catalase and glutathione-S-transferase

The activity of superoxide dismutase and catalase was assayed with the standard protocols [20,21]. The enzymatic activity of Cu–Zn superoxide dismutase (Cu–Zn SOD) was assessed by auto-oxidation of pyrogallol in presence of 100 μL of sample in succinate buffer (pH 7.36). Their readings were recorded as per minute change at 412 nm by double-beam UV–visible spectrophotometer (UV-3600, Shimadzu, Japan). For the assay of catalase (CAT), extent of inhibition of decomposition of hydrogen peroxide by 50 μL of sample in potassium–phosphate buffer (pH 7.0) was read at 240 nm per minute by double-beam UV–visible spectrophotometer (UV-3600, Shimadzu, Japan). Their specific values were expressed in units per milligram of protein in the sample.

Glutathione-S-transferase (GST) was chosen to assess toxic burden on the organs by the method of Habig et al. [22]. In this assay, 100 μL of sample was allowed to react with 2.7 mL of reduced glutathione (30.73 mg% in potassium–phosphate buffer at pH 6.5) and 200 μL chlorodinitrobenzene (1 mM in acetone). After vortex, the reading was taken at 340 nm per minute against distilled water, as blank, by double-beam UV–visible spectrophotometer (UV-3600, Shimadzu, Japan).