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Biochemical changes associated with ascorbic acid–cisplatin combination therapeutic efficacy and protective effect on cisplatin-induced toxicity in tumor-bearing mice

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ABSTRACT

Cisplatin is one of the well-established anticancer drugs being used against a wide spectrum of cancers. However, full therapeutic efficacy of the drug is limited due to development of various toxicities in the host. This study examines the comparative therapeutic effectiveness and toxicities of cisplatin alone and in combination of dietary ascorbic acid (AA) in ascites Dalton's lymphoma-bearing mice. The findings show that the combination treatment of mice with ascorbic acid plus cisplatin has much better therapeutic efficacy against murine ascites Dalton's lymphoma (DL) in comparison to cisplatin alone and this may involve a decrease in reduced glutathione (GSH), catalase activity and increased lipid peroxidation (LPO) in Dalton's lymphoma tumor cells. At the same time, combination treatment indicates a protective role of ascorbic acid against cisplatin-induced tissue toxicities (side effects) in the hosts. Cisplatin-induced histopathological changes in liver, kidney and testes were decreased after combination treatment. The analysis of renal function test (RFT), liver function test (LFT) and sperm abnormalities also suggest an improvement in these parameters after combination treatment. Therefore, it may be concluded that the increased GSH level, catalase activity and decreased LPO in the tissues, *i.e.*, liver, kidney and testes after combination treatment may be involved in its protective ability against cisplatin-induced tissue toxicities in the host.

1. Introduction

Cancer and its treatment are among the most critical health issues. According to World Cancer Report released by the World Health Organization, cancer rates could further increase to 15 million new cases a year by 2020 [1]. Cancer chemotherapy has proven to be an effective treatment approach which is used either singly or in combination with surgery and/or radiotherapy.

Cis-diamminedichloroplatinum-(II) or cisplatin is a platinum-containing inorganic, square–planar complex which is a well-known anticancer agent being used against a wide spectrum of malignancies including testicular, head and neck, ovarian, cervical, non-small cell lung carcinoma, and many other types of cancer [2–4]. The ability of cisplatin to react with DNA and formation of cisplatin-DNA adducts with inter- and intra-strand nuclear DNA cross-links is suggested to be the main mechanism underlying its cytotoxic effect [5]. In addition to its interaction with cellular DNA, the changes in various biochemical/enzymatic parameters, immune response, cell surface structure have also been observed which have led to the proposal of the involvement of multistep and multilevel effects of cisplatin

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in the tumor cells/host [6]. However, the therapeutic efficacy of cisplatin is often hampered by the development of various dose-limiting side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, ototoxicity in the hosts [7] and acquired resistance by cancer cells [8]. In an attempt to overcome these impediments, the use of cisplatin in combination with some modulating agents such as WR2721 [9], quercetin [10] and cordycepin [11] have been tried with varying degree of success. Further, in an endeavour to decrease drug-induced toxicity in the host, the use of anticancer drugs such as cyclophosphamide [12], paclitaxel [13], arsenic trioxide [14] and doxorubicin [15] in combination with vitamin C have also been examined.

Ascorbic acid (L-3-ketothreohexuronic acid lactone) or vitamin C is a water soluble vitamin with antioxidant properties. Ascorbic acid is an active reducing agent involved in various biological effects and plays an important role in the metabolism and detoxification of many endogenous and exogenous compounds [16]. In spite of various reports showing good therapeutic potential of ascorbic acid against cancer [17–19] and supporting a role for increased vitamin C intake in decreasing the risk of cervical cancer [20], its definite use in cancer chemotherapy still remains inadequate [21]. Ascorbic acid has been reported to increase the efficacy of several chemotherapeutic drugs [12,22–24], though few have shown virtually no benefit from its treatment [25,26]. Some contradictory role of ascorbic acid has also been suggested in either inhibiting carcinogenesis [27–29] or enhancing carcinogenesis [30–32]. Some genotoxic effects of vitamin C in *in vitro* test systems have been demonstrated [33,34] but in *in vivo* experiments there are no genotoxic effects by vitamin C.

Thus, considering the inconsistent findings on the significance of vitamin C in cancer chemotherapy and its possible protective implication in the hosts, the present study was undertaken to evaluate the effectiveness of ascorbic acid in minimizing cisplatin-induced toxicities/side effects in Dalton's lymphoma-bearing mice. The findings exhibit that the use of ascorbic acid with cisplatin could be very useful in decreasing cisplatin-induced toxicities/side effects in the host while showing better therapeutic efficacy against murine ascites Dalton's lymphoma.

2. Materials and methods

2.1. Chemicals

Cisplatin solution (1 mg/ml of 0.9%, NaCl) was obtained from Biochem Pharmaceutical Industries, Mumbai, India. L-ascorbic acid (vitamin C) was purchased from HiMedia Laboratories, Mumbai, India. Reduced glutathione, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Ethylenediamine tetra acetic acid (EDTA) and other chemicals used in the experiments were of analytical grade and purchased from SRL Pvt. Ltd., Mumbai, India.

2.2. Animals and tumor maintenance

Inbreed Swiss albino mice colony is being maintained in the laboratory under conventional conditions at room temperature of $24 \pm 2\,^{\circ}C$ with free access to food pellets (Amrut Laboratory, New Delhi) and drinking water *ad libitum*. Ascites Dalton's lymphoma is being maintained in *in vivo* in 10–12 weeks old mice by serial intraperitoneal (i.p.) transplantations of approximately 1×10^7 viable tumor cells per animal (in 0.25 ml phosphate-buffered saline, pH 7.4). Tumor transplanted hosts usually survive for 19–21 days.

The maintenance and use of the mice and the experimental protocol of the present study was approved by the Institutional Ethical Committee of North-Eastern Hill University, Shillong, India.

2.3. Drug treatment schedule and antitumor activity

Therapeutic dose of cisplatin against malignant tumors has been established to be 8–10 mg/kg body weight [2,35]. Thus, based on earlier studies [36] the dose of cisplatin (10 mg/kg body weight) was used in present studies. Similarly, the dose of AA was selected to be 1% in drinking water which has already been standardized as an effective dose [12,37]. Tumor transplanted mice were randomly divided into four groups consisting of 10 mice in each group. Group-I mice served as tumor-bearing control and received normal saline only. Group-II mice were given 1% AA through drinking water for 5 consecutive days starting from the 5th day post-tumor transplantation. Based on the volume of water intake, the AA intake was noted to be about 17.65–19.20 mg/day/per animal. Group-III mice were administered with a single dose of cisplatin (10 mg/kg body weight) on the 10th day post-tumor transplantation. Group-IV mice received AA through drinking water from the 5th day post-tumor transplantation and were administered with cisplatin (i.p., 10 mg/kg body weight) on the 10th day of tumor growth.

The anticancer efficacy was determined as percentage of average increase in life span (ILS) using the formula: $\%ILS = (T/C \times 100) - 100$, where, T and C are the mean survival days of treated and control groups of mice, respectively. The same treatment schedule was followed for the biochemical investigations in DL cells, liver, kidney and testes. Ascites fluid was collected from the peritoneal cavity of mice in different groups and was used to determine the average tumor pH using a pH meter. The ascites fluids were then centrifuged and the pellets were used as DL cells.

2.4. Total reduced glutathione estimation

Total reduced glutathione (GSH) content was determined using the method of Sedlak and Lindsay [38]. Briefly, 5% tissue homogenates of DL cells and tissues were prepared in 0.02 mol/L EDTA (pH 4.7). 100 μl of the tissue homogenate or the pure reduced form of GSH was added 1.0 ml of 0.2 mol/L Tris–EDTA buffer (pH 8.2). To this, 0.9 ml of 0.02 mol/L EDTA (pH 4.7) with 20 μl of Ellman's reagent (10 mmol/L DTNB in methanol) was added. After 30 min of incubation at room temperature, the reaction mixtures were centrifuged and the absorbance of the clear