Effect of Cisplatinum on The Liver of The Adult Albino Rat and the Possible Protective Role of Vitamin E (Histological and Ultrastructural Study)
Saber Shona, Abdel-Wakeel Essawy, Sherif Mohamed Zaki* and Tarek Ibrahim Abd EI-Galil

Department of Anatomy, Faculty of Medicine, Cairo University, El-Kasr Al Aini Street, Cairo, Egypt

Abstract

Background: Cisplatinum is one of the older chemotherapy drugs which is been used for decades. In spite of its significant anticancer activity, its clinical use is often limited by its undesirable side effects. The liver is known to accumulate significant amounts of cisplatinum thus hepatotoxicity could be associated with cisplatinum treatment.

Aim: The aim of the present study is to detect the histopathological effect of cisplatinum on the liver of the adult albino rat and the possible protective role of vitamin E prevention of cisplatinum-induced hepatotoxicity.

Material and methods: Thirty adult male albino rats were used in the current study. The rats were divided into 3 groups; control, cisplatinum and cisplatinum + vitamin E groups. Histological, transmission electro-microscopic as well as morphometric studies were used in the study.

Results: Histological examination of the liver of the cisplatinum treated rats exhibited the presence of congestion of the central veins and hepatic sinusoïds. Massive intracytoplasmic vacuolations, margination of chromatin with the occurrence of karyorrhexis, pyknosis in the nuclei were also constant features. Kupffer cells were observed with the presence of hemosiderin granules. The ultrathin sections exhibited degeneration of the nuclei with margination of the nucleoli and irregularities of the nuclear membranes.

Slight improvement of the histological findings was observed in the liver of the cisplatinum and vitamin E treated rats. Degeneration of the hepatocytes, margination of the nucleoli, karyorrhexis, apoptotic nuclei and apoptotic bodies were observed in this group.

Conclusion: Thus, it is recommended to investigate the liver capacity before the use of cisplatinum and throughout the course of treatment. Once, the liver changes start to develop, shifting to another more safe antitumor drug must be considered. As regard using vitamin E to reduce the toxic effect of the cisplatinum on the liver, it was found that vitamin E had a minimum protective role.

Keywords: Cisplatinum; Vitamin E; Liver

Introduction

Cisplatin, cisplatinum, or cis-diaminedichloroplatinum (II) is a platinum-based chemotherapeutic agent that is highly effective in the treatment of cancer [1]. It is one of the older chemotherapy drugs which is been used for decades. It is used to treat various types of cancers, including sarcomas, some carcinomas (e.g., small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors [2]. Cisplatin is the most commonly used chemotherapy drug in the USA [3].

In spite of the significant anticancer activity of cisplatinum, its clinical use is often limited by its undesirable side effects [4]. Hepatotoxicity rarely occurred at standard doses. However, at higher doses, hepatotoxicity is frequently observed and could alter the clinical situation of the patients [5]. The liver is known to accumulate significant amounts of cisplatinum, second only to the kidney; thus hepatotoxicity could be associated with cisplatinum treatment [6]. The lipid peroxidation is crucial in the pathogenesis of cisplatinum-induced hepatic injury [7].

Vitamin E is a fat-soluble vitamin. It has high affinity to phospholipids, cholesterol and triglycerides (the three main structural elements of the cell membrane, mitochondria and endoplasmic reticulum) due to its hydrophobic lipid solubility. Thus, vitamin E appeared to be the first line of defense against peroxidation of the polyunsaturated fatty acids of the cell membrane [8].

The aim of the present study is to detect the histopathological effect of the higher dose of cisplatinum on the liver in the adult albino rat and the possible protective role of vitamin E in prevention of cisplatinum-induced hepatotoxicity. This will be done through histological, morphometric and Transmission Electro-microscopic studies (TEM).

Material and Methods

Chemicals

Cisplatinum was supplied as vials 10 mg/10ml (Merck Company-Germany) and was given as a single dose of 45 mg/kg intraperitoneally. This dose induces hepatotoxicity in rats without lethality [9].

Vitamin E (tocopherol) was supplied as capsules 200 mg (Pharco Pharmaceutical Company) and was given orally by a special blunt-tipped syringe (Animal house, Faculty of Medicine, Cairo University) once/day for 21 consecutive days, pre-cisplatinum treatment in a dose of 100 mg/kg diluted in olive oil [10].
Animals

Thirty adult male Sprague Dawley rats (Animal house, Faculty of Medicine, Cairo University), weighing 250-300 grams were used. Five animals were housed per cage, and the animals were acclimatized to standard laboratory conditions (12:12-hour light-dark cycle, temperature 20°C, fed ad libitum and allowed free water supply). The experiment was performed according to the Helsinki agreement on the guiding principles for research involving animals and human beings.

The rats were divided into three equal groups; group I (control), group II (cisplatinum group) and group III (cisplatinum and vitamin E). They were sacrificed after sixteen hours [9] by inhalation of high dose of ether. Then, the livers were extracted.

Light microscopic study

Part of the livers was fixed in buffered formol saline, processed for paraffin sections of 5µm thickness and sections were stained with Haematoxylin and Eosin (H&E) and Masson’s trichrome stains [11] for histological study. The sections were examined and photographed using a Canon digital camera, attached to IBM computer system.

Ultrastructural study

The other part of the livers was cut into small pieces, fixed in 4% glutaraldehyde then washed in phosphate buffer and post fixed in 1% osmium tetraoxide. Fixation was followed by dehydration and embedding in epoxy resins. Semithin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate [12] then examined and photographed using a transmission electron microscope (Faculty of Agriculture, Cairo University).

Morphometric study

Sections stained with Masson’s trichrome stain were examined by the use of the image analyzer computer system to measure the area % of fibrosis in each group (software Leica Qwin 500, England). In each chosen field the hepatic tissue was enclosed inside the standard measuring frame and then the Connective Tissue area (CT) was masked by a blue binary color to be measured (Figure 1).

Ten slides, every 7th from each experimental tissue sample, were used from each group. Ten readings were obtained in each specimen and their mean values were obtained. The statistical package for the social science (SPSS version 17) was used on data analysis. Data was expressed as mean ± SD. Two way analysis of variance (ANOVA) was used.

Results

Light microscopic results

Results of the group I: Histological examination of the liver of the control rats exhibited the normal architecture of the liver. The parenchyma of the normal liver specimens was remarkably uniform in appearance all through. It was consisted of hepatic lobules formed of plates of hepatocytes arranged radially around the central veins. The hepatic sinusoids, lined with endothelial cells, Kupffer cells were found occupying the spaces between the radially arranged hepatic plates (Figure 2).

Results of the group II: Histological examination of the liver of the cisplatinum treated rats exhibited congestion of the central veins, hepatic sinusoids with the presence of massive intracytoplasmic vacuolations (figures 3a, figure 3b). Margination of chromatin with the occurrence of Karryorehxis, pyknosis in the nuclei was constant.
features (figures 3b, 3c and 3d). Kupffer cells were obviously observed with the presence of hemosiderin granules (figure 3c).

Results of the group III: Histological examination of the liver of the cisplatinum and vitamin E treated group showing: (a) Congestion of the central vein (C) and sinusoids (S). Note the presence of massive intracytoplasmic vacuolations (V) and kupffer's cells (arrows). (H & E x400) (b) Massive intracytoplasmic vacuolation (V), karryorexhis (kr) and margination of chromatin inside the nucleus (arrow). Note kupffer's cells (kc) and sinusoidal congestion (C). (H & E x1000) (c) Karryorexhis (kr), pyknotic nuclei (p) and hemosiderin granules (arrow). (H & E x 1000) (d) Intracytoplasmic vacuolations (black v) and intracytoplasmic vacuolations (red v). Note the presence of karryorexhis (kr) and pleomorphism of the hepatic cells (figure 4b). Degeneration of the nuclei with margination of the nucleoli was also observed (figure 4c).

Electron microscopic results

The ultrathin sections of the liver of the control rat showed regularly-shaped euchromatic nucleus with finely dispersed chromatin. The mitochondria appeared normal. The rough endoplasmic reticulum appeared as parallel arrays of fine tubules situated in the cytoplasm on the side of the nucleus (figure 5a).

The sections of the cisplatinum treated rats exhibited degeneration of the nuclei with margination of the nucleoli. The nuclear membranes were also irregular (figure 5b).

Finally, the ultrathin sections of the cisplatinum and vitamin E treated rats exhibited degeneration of the nuclei with margination of the nucleoli. The nuclear membranes were also irregular (figure 5b).

Morphometric results (Table 1)

The mean area % of fibrosis of cisplatinum treated group increased significantly in comparison with the corresponding control group. With treatment with vitamin E, there was a significant decrease in the mean area % of fibrosis in comparison to the control group. The p-value in vitamin E treated group is 0.49 compared to the cisplatinum treated group which does not confer strength in role of vitamin E.

Discussion

Although the therapeutic doses of cisplatinum is 14 mg/kg [2], we use in the current study much higher dose 45 mg/kg as our study was designed to examine the effect of cisplatinum in the patients with refractory solid tumours and with advanced germ cell tumors refractory to first-line chemotherapy. Many of these are cured with high-dose chemotherapy with stem cell rescue [13].

Congestion in the current study was obvious in the cisplatinum treated group as compared to the control group; similar finding was previously reported [14].

Congestion in the central veins and hepatic sinusoids was observed in the cisplatinum treated group and this finding is not clearly understood as about more than 95% of cisplatinum is protein bound (inactive) so as to produce such hepatic congestion. Congestion might be attributed to direct irritant effect of the drug on the wall of the blood vessels [15] or might be secondary to the fibrotic changes in the perportal areas affecting the intrabiliiary system. This would lead to obstruction of the duct system especially the Hering duct with concomitant obstruction to the dual blood supply to the liver.

Cytoplasmic vacuolations were also observed in this group which is caused by the oxidative damage of cisplatinum to the membrane lipids and other cellular component of the liver cells [16]. Dissolution of hepatic cords by the effect of cisplatinum is another explanation to these vacuolations [17].

<table>
<thead>
<tr>
<th>Area percent Mean (± SD)</th>
<th>SER</th>
<th>P value</th>
<th>F value</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.41 ± 0.137</td>
<td>± 0.043</td>
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</tr>
<tr>
<td>Cisplatinum treated group</td>
<td>3.8 ± 0.425</td>
<td>± 0.14</td>
<td>0.009*</td>
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<tr>
<td>Cisplatinum + Vitamin E treated group</td>
<td>2.75 ± 0.13</td>
<td>± 0.13</td>
<td>0.02*</td>
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*: in comparison to control. **: Cisplatinum in comparison to cisplatinum + Vitamin E treated group

Table 1: % of fibrosis in the studied group after 16 hours.
Degeneration of the hepatocytes was noticed in the cisplatinum treated group which is attributed to Reactive Oxygen Species (ROS) [18]. Apoptosis occurred as the cisplatinum kills the liver cells through the induction of apoptosis [9]. The appearance of apoptotic hepatocytes, in the current study, indicated very recent hepatocyte damage as scavenger kupffer’s cells engulfed the apoptotic cell fragments within few hours [19].

The lipid peroxidation is crucial in the pathogenesis of cisplatinum-induced hepatic injury [7], so vitamin E had been used in the current study to detect the possibility of its protective role which might be due to the inhibitory effect of lipid peroxidation on cell proliferation.

With the use of vitamin E in conjunction with cisplatinum, slight partial improvement of the histological findings of the hepatic cells occurred as compared to cisplatinum treated groups, this may be due to the antioxidant effect of vitamin E in preventing lipid peroxidation [20].

The presence of the apoptosis was obviously observed in cisplatinum and vitamin E treated group as vitamin E might enhance antineoplastic activity by inducing apoptosis [21].

Also the liver in the cisplatinum and vitamin E treated group showed degeneration of the hepatocytes, margination of the nucleoli, karyorehxis, apoptotic nuclei and apoptotic bodies, all these indicated that vitamin E has a very minimum protection.

Thus, it is recommended to investigate the liver capacity before the use of higher dose of cisplatinum and throughout the course of treatment. Once, the liver changes start to develop, shifting to another more safe antitumor drug must be considered.

As regard using vitamin E to reduce the toxic effect of cisplatinum on the liver, it was found that vitamin E had a minimum protective role.

References


