STRUCTURAL AND ULTRASTRUCTURAL DATA ON SIDE EFFECTS OF CISPLATIN IN SPLEEN, KIDNEY AND LIVER OF RATS

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Most of the cancer drugs used in chemotherapy induce strong immunosuppressive states and lymphotoxic effects, characterized especially by alterations of lymphoid tissues. Among alkylant anti-cancer drugs, the platinum compound Cisplatin is frequently used in chemotherapy of different cancer forms, because of their huge chemotherapeutic potential. Knowing the secondary effects of this antineoplastic agent on different organs has a significant role for establishing the treatment strategy. This should be adapted to organism's response to the antitumor action and to the tolerance of different organs regarding the immunosuppressant state induced by the chemotherapy. Our structural and ultrastructural experimental investigations established that administration of this anticancer drug to rats in therapeutical doses induce lymphotoxicity, nephrotoxicity and hepatotoxicity. These effects were determined mainly by its antimitotic activity, correlated with drastic necrobiotic, necrotic and apoptotic effects.

Key words: Cisplatin effects – ultrastructure, rats spleen, kidney, liver.

INTRODUCTION

As cytostatic drugs interact in the same way with the tumor cells and with the rapidly dividing normal, healthy cells, the non-selectivity of the antitumor chemotherapy represents a major issue in treating cancer disease. The harmful effects will interest especially the tissues whose cells divide fast and continuously, especially the germinal and hematopoietic tissues (Daicoviciu and Chiricuță, 1983; Terezholmy, 1996; Moran, 2000; Paşca et al., 2000 a; Bischin et al., 2012). The disturbances induced by the administration of the cancer drugs are tightly connected to the setting of some serious structural and functional damages within central and peripheral lymphoid tissues (Hruban et al., 1991). These unwanted side effects are altering the metabolic functionality of certain tissues and organs (immunotoxicity, neurotoxicity, immune-

suppression). The administration of cytostatics with immunosuppressive and strong lymphotoxic effects, as cyclophosphamide, cisplatin, vincristin, carboplatin etc., can induce several side effects on the immune system (Goodman et al, 1990; Kelly and Rosencweig, 1990; Yoshiruni et al., 1990; Blank et al., 1991; Luster et al., 1993; Milicevic et al., 1994; Crăciunaş et al., 1995; Crăciun et al., 1996; Terezhalmy et al., 1996; Moran, 2000; Paşca et al., 2000 a, b, c, d).

Cisplatin or *cis*-Diaminedichloroplatinum (II) is an antineoplastic agent with vast therapeutic potential, especially against solid tumours, such as bladder, testicular, ovarian, brain etc. The major side effects of this anticancer drug are its nephrotoxicity and mielotoxicity (Paşca et al., 2000 a, c, d; Paşca et al., 2001).

Cisplatin (Platinol, *cis*-DDP) is a platinum alkylating agent, successfully applied in the chemotherapy of several types of cancer. Its antineoplastic activity is generally believed to result from the interaction of the drug with the DNA in the tumor cells, this interaction leading to the formation of different types of adducts through the reaction of the bifunctional platinum compound with N7 atoms of the nucleobases guanine and adenine. Inside cells, *cis*-DDP can form DNA-protein cross-links (Fightinger et al., 1982, 1985, 1987). The DNA adducts are known to be responsible for its therapeutic activity. Cisplatin has been shown to be incorporated into proteins through methionine and cysteine (Koikawa et al., 1993; Olas and Wachowicz, 1996; Sfikakis et al., 1996). Also, platinum binding through disulfide (S-S) bond cleavage can cause marked effects on the secondary structure of the proteins as well as on their biological properties (Ohta et al., 1992).

The histological and ultrastructural modifications induced by this anticancer drug on different organs and tissues are still incompletely known. Our research aimed to highlight the alterations produced in spleen, kidney and liver, by administration of Cisplatin in therapeutical dose, and to search for a time-dependent dynamics of these modifications, as compared with untreated rats.

MATERIALS AND METHODS

Our experiments were carried out on healthy, adult male Wistar rats, weighing around 190 ± 10 g and maintained under bioclimatic laboratory conditions; the animals were starved for 18 hours before the treatment, but had free access to water.

For spleen investigations were organized seven groups, as follows:
- group C – untreated (control);
- groups C1, C2 and C3 were treated with a single i.v. dose of 10 mg Cisplatin/m^2 body surface and were sacrificed 24 hours, 10 and 18 days after the treatment;
- groups C4, C5 and C6 received 20 mg i.v. Cisplatin/m^2 body surface and were killed 24 hours, 10 and 18 days after the treatment.

For kidney investigations, four groups of Wistar rats were treated with 20 mg Cisplatin/m^2 body surface/day, administered i.v. for three days, and sacrificed 24 hours, 4, 11 and 18 days after the treatment and compared with untreated group.

For liver investigations, two groups of rats were tested: a control group and a group that received i.v. 4 mg/kg b.w./24 h, Cisplatin for 5 days.

The animals were not fed for 18 hours before their sacrification. Spleen and kidney samples were taken for histological and electron microscopy examinations and liver sample only for electron microscopy investigations. For light microscopy, the tissue fragments were fixed in 10% neutral formalin, processed by the paraffin technique and sections of 5-6 μm were stained by the hematoxylin-eosin and Masson-Goldner trichrome methods (Muresan et al., 1974). Examination was performed with an Olympus BX51 microscope, a CCD media Cibernetics camera, and Image Pro Plus software (Kuo 2007). For ultrastructural investigations, fragments of the same tissues were prefixed in 2.7% glutaraldehyde and postfixed in 2% osmic acid. The biological samples were dehydrated in acetone and then embedded in epoxyde resin, Epon 812. The ultrathin sections were obtained using a Leica UC6 ultramicrotome with a Diatome Ultra 35^0 diamond knife, and were double contrasted with uranyl acetate and lead citrate. Examination of the sections was performed in a JEOL JEM TEM 1010 electron microscope using a Megaview III camera and a Soft Imaging Analysis software (Kay 1967; Ploaie and Petre, 1979; Crăciun and Horobin, 1989; Pavelka and Roth, 2005; Florea and Crăciun, 2011, 2012).

RESULTS AND DISCUSSIONS

1. Spleen of the rats treated with Cisplatin

The two therapeutical doses of Cisplatin used, but especially the high dose, induced significant histological and ultrastructural modifications in the spleen. Evident histological alterations already appeared 24 hours after the treatment, a serious congestion and stasis, correlated with a decrease of lymphocyte count in the germinal centers in the white pulp, so that all the lymphatic nodules appeared smaller. These modifications were more evident in the animals treated with the high dose. Besides, as a consequence of the zonal necrotic and necrobiotic processes, a "starry sky" aspect of the nodules could be noticed. Ten days later, these nodules appeared very small, and having an homogenized structure (Fig.1). Many of them were atrophied, and the germinal centers were difficult to observe. Splenic follicles had a vestigial appearance (Fig. 2). 18 days after the treatment, although the lymphatic nodules were still smaller, a certain recovery process could be observed. Thus, a large number of lymphoblasts, granulocytes in different developing stages and megakaryocytes appeared in the centre of the nodules.
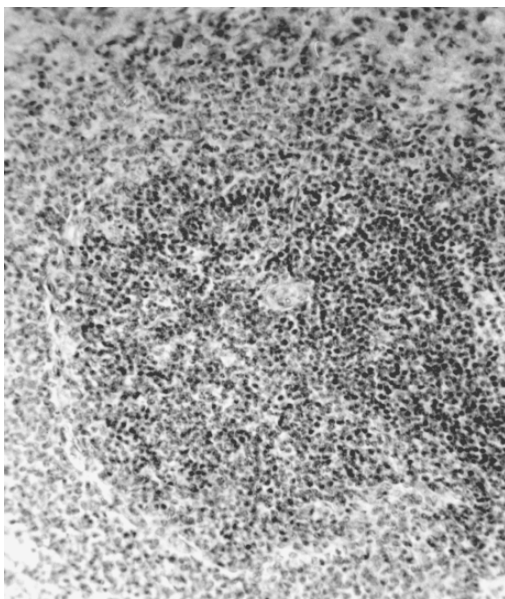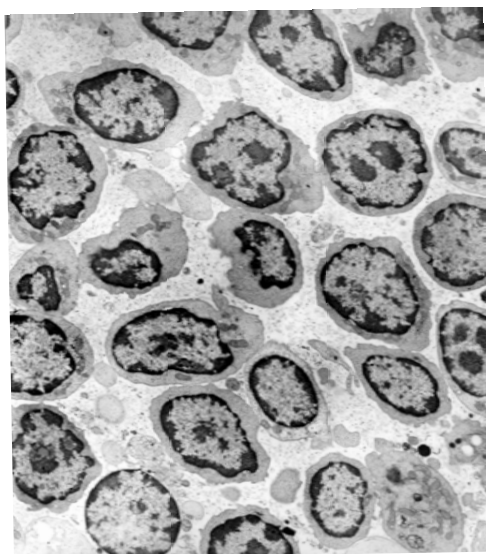
Fig. 1. Ten days after the treatment with 10 mg Cisplatin, lymph nodes had homogenized centres. (oc. x8; ob. x16).
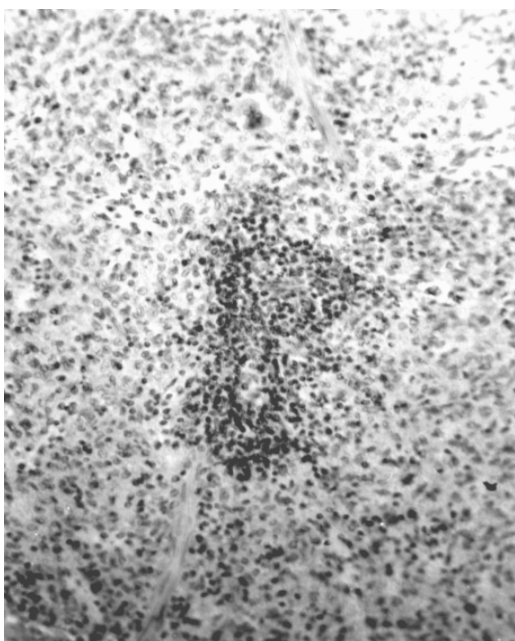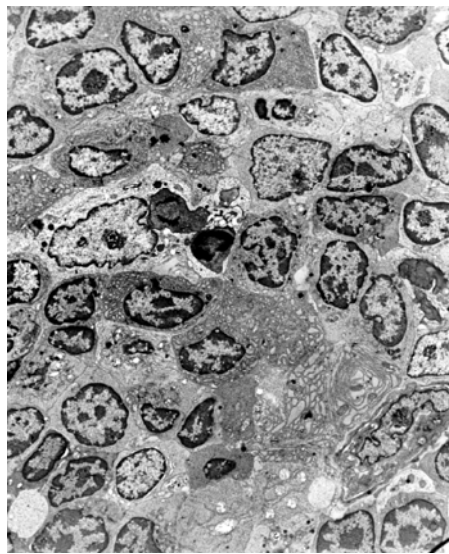

Fig. 2. Ten days after the treatment with 10 mg Cisplatin, splenic follicles showed a vestigial aspect. (oc. x8; ob. x16).

At ultrastructural level, 24 hours after treatment with 20 mg Cisplatin, there was a depletion of lymphocytes in the white pulp (Fig. 3), and after ten day, abnormal aspects of lymphocytes and picnosis processes were seen (Fig. 4).

In the groups treated with 10 mg

Fig. 3. TEM image 24 hrs after treatment with 20 mg of Cisplatin: a depletion and rarefaction of lymphocytes took place. X 7,140

Fig. 4. TEM image 10 days after treatment with 20 mg of Cisplatin. Abnormal aspect of lymphocytes, many of them suffering a picnosis process; very active macro-phages were also noticed. X 5,400

Cisplatin/m^2 body surface, the light and electron microscopic investigations of the spleen showed a less drastic toxic effect. Although all the histopathological aspects previously described appeared immediately after the chemotherapy, they were less intense, and 10 days after the treatment a recovery processes could be noticed, so that finally period of the treatment the spleen of the treated rats appeared almost completely recovered. Besides, in these groups, we could not see any reactivation of the extramedullary hematopoiesis at the level of the spleen. We may conclude that this dose of cytostatic didn't have a high hematotoxic effect, so that the hematopoietic marrow was still able to produce enough blood cells all along the experimental time.

The histological and ultrastructural studies showed that both doses of Cisplatin, but especially the high one, had a significant toxic effect, in both central (thymus) and peripheral (spleen) lymphoid organs.The ultrastructural alterations induced in spleen after ten days of treatment with Cisplatin suggest not only the noxious action of the drug, but also the reaction of the affected tissue, revealed by the increased activity of macrophages, plasmocytes, monocytes, eosinophils and neutrophils, indicating a protective reaction of the spleen to the noxious action of the drug (Crăciunaş et al., 1996).

Conclusions

- Cisplatin had a dose-dependent toxic effect on the spleen.
- The two doses induced significant, but reversible histological and ultrastructural modifications.
- Cisplatin had a significant toxic effect on both cellular and vascular components of the spleen.
- The toxic effect of Cisplatin on the cellular component was determined by its antimitotic activity, but was less intense than in the case of thymus.

2. Kidney of the rats treated with Cisplatin

Histological and ultrastructural investigations of the kidney showed the appearance of some significant modifications which seemed to evolve gradually, starting from the moment of treatment.

The first histological alterations could be noticed as soon as 24 hours after the treatment, but they were very discreet, had a zonal character affecting small areas, especially between the cortex and medulla of the kidney. In few uriniferous tubules, there was a polymorphous proteic granular material which appeared as a consequence of some incipient necrosis process of the tubule epithelium. Just a small number of renal corpuscles were affected, they presenting a discreet oedema of the Bowman's space.

Four days after the treatment, certain uriniferous tubules in the cortex of the kidney were swollen and the cytoplasm of the epithelial cells was homogenous. The granular material inside the lumen of the tubules persisted and had an obvious merging tendency. All these alterations persisted and aggravated in the group sacrificed 11 days after the treatment, in which could be noticed renal tubules that showed a urinary stasis, their walls having an atrophied epithelium, and here and there suffering serious necrosis processes. As a result of these processes, many cells of the tubular epithelium detached from the basement membrane, and made up the granular material noticed in a larger number of tubules than in the group sacrificed 4 days after the treatment.

The necrosis process progressively got worse, so that in the group sacrificed 18 days after the treatment the urinary stasis was significantly increased, more tubules being very distended and having an advanced necrosis process of the epithelial cells. Besides, many cells were very swollen, having an abnormal, peculiar aspect, their dimensions being three times larger compared to normal cells.

In addition, in this group, a proliferation of the mesangial and endothelial cells and an accumulation in the capillary lumen of certain circulating elements (polymorphonuclears, monocytes and lymphocytes) were obvious. The interposition of mesangial and monocyte cells determined the parietal thickening and the striking "double outline" aspect of the basement membrane.

Electron microscopy showed that Cisplatin, induced grave nuclear alterations, vacuolization of the cytoplasm, intracellular lysis phenomena in the nephrocytes of the proximal tubules (Fig. 6), as compared with normal nephrocytes of proximal tubules (Fig. 5). These modifications started 4 days after the treatment, and became
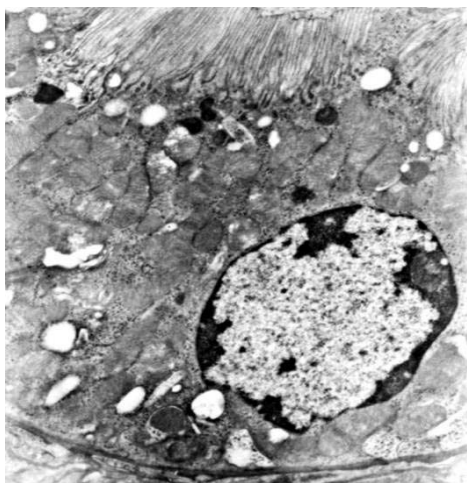
Fig. 5. Normal nephrocyte in the proximal tubule. X 12,600

Fig. 6. Vacuolization of the cytoplasm and intracellular lysis in a nephrocyte of the proximal tubule, 4 days after the treatment.

worse until the 18th day of the experiment. After 11 days, the mitochondria became swollen and had rarefied matrix and cristae (Fig. 7). Many epithelial cells had a seriously affected brush border, the microvilli being swollen and destroyed here and there, and their basal infoldings appeared hypertrophied. The pronounced cellularity of the glomeruli was due to mesangial cell proliferation and especially to monocytic infiltration. Monocytes appeared in the mesangial zones, between the basement membrane and the endothelium or in the lumen of some capillaries. Many granular structures were found in the subendothelial area and in the lumen of some capillaries, too.

Both the histological and ultrastructural modifications previously presented, confirm the nephrotoxicity of Cisplatin. According to the previous investigations, the nephrotoxicity of this anticancer drug is minimal when it is administered in low doses, but high doses induce a significant glomerular and tubular toxicity which could seriously affect the function of the kidney (Knox et al., 1986; Brock et al., 1981; Fightinger et al, 1982, 1985, 1987; Daicoviciu and Chiricuță, 1983; Ettinger et al, 1993; Frenkel et al, 1995; Stretkowicz et al., 1996; Ardelean et al., 2002; Hermenean et al., 2008 a, b).

Fig. 7. Swollen mitochondria with rarefied matrix and cristae in a nephrocyte
of renal proximal tubule, 11 days after the treatment. X 24,150.

Its toxic effect consists of certain alterations, both in the renal corpuscle structure (glomerular capillaries, endothelium, mesangium and Bowman capsule) and the uriniferous tubules, especially between the cortex and medulla.

As a consequence of the affecting of the glomerular capillaries, the ultrafiltration process was significantly disturbed. It has also been suggested that mesangial cells can contract when stimulated by angiotensin, with consequent decrease of blood flow in the glomerular capillaries (Farquhar et al., 1961). The mesangial cell proliferation could be another cause for the alteration of the ultrafiltration process at the level of the kidney of the rats treated with Cispaltin. Because of the alterations in the uriniferous tubules, the excretion and absorption processes were affected, too.

Conclusions

- Cisplatin had a nephrotoxic effect which got worse during the 18 days of the experiment.
- Cispaltin injured the structures involved in both the ultrafiltration process (glomerular capillaries, Bowman capsule, mesangium) and in the absorption and excretion processes (epithelium of the proximal convoluted tubule, loop of Henle and distal tubule).
- Light microscopy examination showed that Cisplatin induced necrosis processes of the tubular epithelium, urinary stasis, proliferation of the mesangial cells, the appearance of some granular proteic material inside the lumen of the tubules, a few epithelial cells getting an abnormal, peculiar aspect, and a significant monocyte infiltration.
- Electron microscopy investigations demonstrated that Cisplatin produced grave nuclear alterations, a vacuolization of the cytoplasm in the epithelial cells, the swelling of the mitochondria and a grave alteration of the brush border and of the basal infoldings.

3. Liver of the rats treated with Cisplatin

Two groups of rats were tested: a control group and a group that received i.v. 4 mg/kg b.w./24 h, Cisplatin for 5 days. Fragments of the liver were processed for electron microscopy investigations.

After 5 days of treatment with Cisplatin, several ultrastructural modifications were visible in hepatocytes, as compared with the control rats (Fig. 8). The nuclei, no longer spherical, had an irregular outline and a larger amount of heterochromatin, which reveals the direct action of Cispaltin upon nuclei. The vesiculated appearance of RER cisternae and the large amount of SER, suggest the specific reaction of these organelles to the drug (Crăciun and Rusu, 1980; Orlandi and Yezequel, 1972; Philips et al., 1987; Tanikawa, 1968; VanBezooijen, 1990). We have also noticed a significant increase of glycogen stores, occurring in hepatocytes under two forms already described by Cardell and Cardell (1990), namely as particles spread within the cytoplasm matrix and aggregate particles, respectively. The increase of glycogen stores was in agreement with the high level of blood sugar, which we have also recorded.

The occurrence of SER vesicles among the glycogen particles reveals a functional relationship between them (Man and Blok, 1966) and suggest also a glycogenesis process (Crăciun, 1985). The large number of peroxisomes were identified in the liver of rats treated with Cisplatin. No significant alterations of mitochondria were visible. The amount of lipid droplets was reduced, which correlated with the absence of lipid deposits in the Ito cells, reflecting an altered lipid synthesis (Fig. 9).
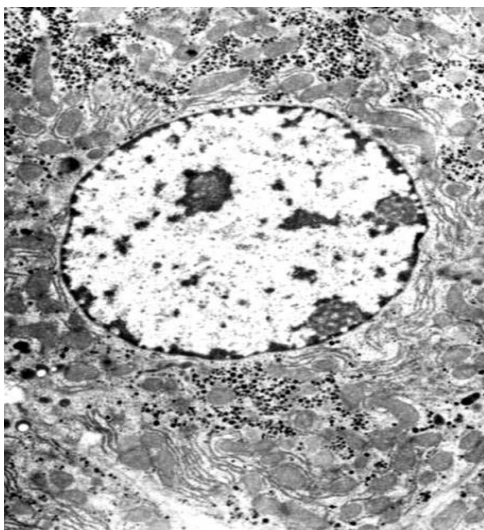

**Fig. 8.** Hepatocyte of control group. X 12,000
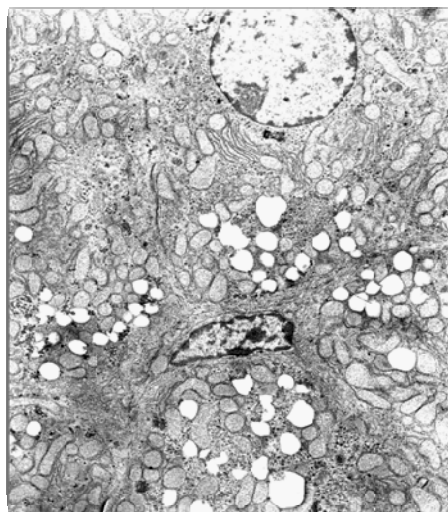
Fig. 9. Absence of lipid droplets in the Ito cells of treated rats. X 7,200

Certain bile canaliculi were swollen and have less microvilli, this suggesting an alteration of bile transit and reabsorption of substances.

The hepatocytes contain numerous Golgi complexes which elaborate lysosomes around biliary canaliculi (peribiliary bodies). Noteworthy was also the occurrence of multivesicular bodies towards the biliary pole of some hepatocytes. The above-mentioned alterations are in agreement with the major and minor modifications of hepatocytes following drug administration described by Phillips et al. (1987). Yet, a few hepatocytes displayed deep ultrastructural modifications that were irreversible and led to their destruction. In such hepatocytes the matrix of cytoplasm was rarefied, the endoplasmic reticulum and Golgi complexes were degraded and the nuclei were destroyed, or the Disse space was filled with compact collagen which affected the absortive process at the level of vascular pole of hepatocytes. In such highly affected parts of the liver, the Kupffer cells were active, being filled with phagocytic products to be eliminated or diminished (Muto and Fujita, 1997). Also, capillary congestion and stasis (Fig. 10), and microhaemorrhages followed by extravasation of erythrocytes (Fig. 11) were observed.
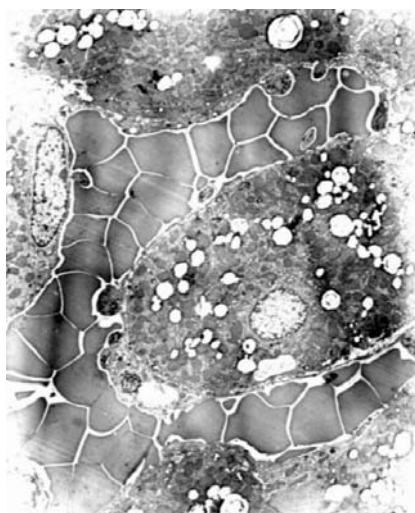
Fig. 10. Stasis at the level of capillary vessels, in the liver of treated rats. X 5,400.
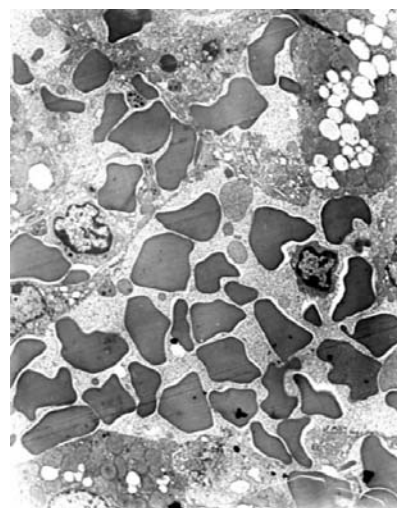
Fig. 11. Microhaemorrhage followed by extravasation of erythrocytes, in the liver of treated rats. X 5,400.

Obviously Cisplatin induced many ultrastructural modifications in hepatocytes. The irreversible ones occured in centrolobular hepatocytes of the 3rd streaming zone (Zajicek et al, 1985) and might be accounted for by the formation of linkers between DNA strands. Thus, the multiplication of hepatocytes in the 1st streaming zone could have been blocked and followed by an untimely "ageing" and a decrease in their metabolic activity.

Evidently, such hepatocytes which have lost their protein synthesis capacity, will be phagocytized by Kupffer cells and probably belong to the 3rd zone of hepatocellular streaming.

Conclusions

- Cisplatin has been found to induce ultrastructural modifications of different degrees. The irreversible ones were evident in centrolobular hepatocytes of the 3rd streaming zone, being the consequence of the formation of linkers between the two DNA strands. Consequently, the multiplication of hepatocytes from the first streaming zone was blocked, which caused a premature "ageing" and a diminishing of their metabolic activity.
- In the dose and period of treatment used, Cisplatin induced irreversible alterations that destroyed a number of hepatocytes. Taking into account the high liver regeneration capacity, we consider that shortly after the end of the treatment (5-6 days) most of the alterations will be gradually eliminated.
- We may conclude that the dose and period of treatment with dose experimented, represent a tolerable limit level.

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