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Experimental report

A quantitative morphometric study of the kinetics of tissue regeneration after administration of cisplatin

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The effect of the anti-neoplastic drug cisplatin was investigated on several rat tissues using a novel computerized morphometric image analysis system. The rats were sacrificed in pre-determined intervals, ranging from 1 to 36 days after drug administration, and their liver, kidneys and external ears were sampled and sectioned. Tritiated thymidine was injected into each rat 1 h prior to sacrifice to enable autoradiographical explorations. All sections were histomorphometrically studied by a computerized system. The kidneys of the experimental group revealed increased tubular diameters, especially in the corticomedular region. In the ears, decreases of the mean epithelial thickness, in the mean number of nuclei and in the mean nuclear surface were observed. The thickness of the connective tissue also decreased significantly by the end of the first week and returned to its normal size later on. No changes were detected in the ear cartilage. In the liver, no morphometrical differences were noticed in the hepatocyte density, Kupffer cell density or nuclear area. The nuclei of the hepatocytes and Kupffer cells retained a constant ratio to the total number of cells throughout the whole experiment. Liver autoradiography revealed that the hepatocyte and Kupffer cell labeling indexes after cisplatin administration were significantly higher than those of the control group, indicating enhanced replication of cells and increased synthesis of DNA, which in turn is accumulated in the nuclei and turn the cells into polyploids. In addition, the mean radius of the liver acini after cisplatin was diminished, followed by a significant reduction in the size of the progenitor compartment. It is concluded that while the kidneys are the organs most affected by cisplatin, the progenitor compartment of the liver is heavily affected as well. Computerized morphometry was demonstrated as a highly reproducible and accurate quantitative method for evaluation of histopathological changes in various mammalian tissues.

Key words: Cisplatin, morphometry, tissue regeneration, uptake kinetics.

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Introduction

Computerized quantitative morphometry has been used for studying a variety of human and animal tissues in physiological and pathological conditions. Among the organs investigated, some only to a limited degree, were the kidneys,[1] liver,[2] brain,[3] lungs,[4] skin epithelium,[5] bone,[6] prostate,[7] epididymis,[8] eyes[9] and mammary glands[10]. Some of these tissues were studied in healthy animals and some in pathological conditions, under drug treatment. Among the diseases studied were a variety of tumor models, either non-treated or treated with anti-neoplastic agents, e.g. methotrexate and cisplatin.

Scanning of the relevant literature reveals that although ample data has been accumulated on several aspects concerning clinical and pharmacological activities of cisplatin, information on the pathological reactions of healthy tissues exposed to this drug is scarce. Moreover, most of their morphologic analyses presented are not quantitative. In as much as cisplatin has been proven to be nephrotoxic, hepatotoxic, autotoxic, neurotoxic and impairing gastrointestinal and hematological systems,[11] little has been demonstrated on its quantitative histopathology, even in its major target organs, such as the kidney and the liver.

The present study was undertaken in order to evaluate the morphometric and kinetic changes in the behavior of healthy rat tissues as a reaction to the administration of cisplatin. This was performed by a novel method of computerized quantitative morphometric image analysis. Specific tissue elements have been studied and quantified in the kidney, liver, cartilage, connective tissue and squamous stratified epithelium. In addition, cellular kinetics and the size of the progenitory segment

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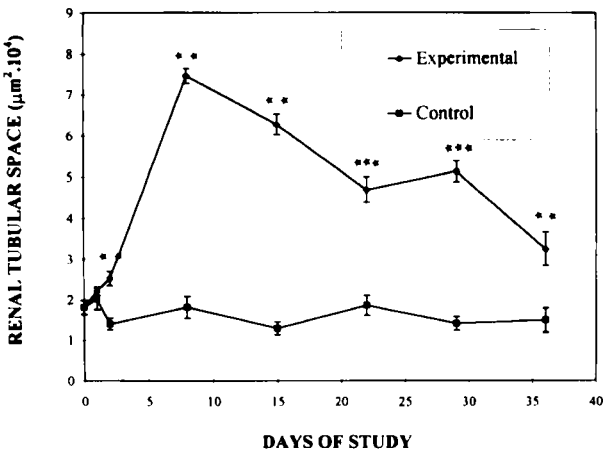

Figure 2. Morphometric measurements of proximal tubular area in the cortico-medullary region of the kidney. $^{**}p < 0.01$; $^{***}p < 0.05$.

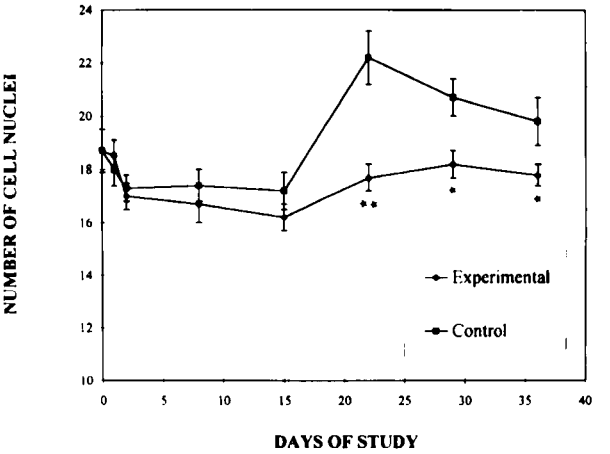

Figure 4. Number of cell nuclei in the epithelium compartment of the ear, as evaluated morphometrically. $^{*}p < 0.05$; $^{**}p < 0.01$.

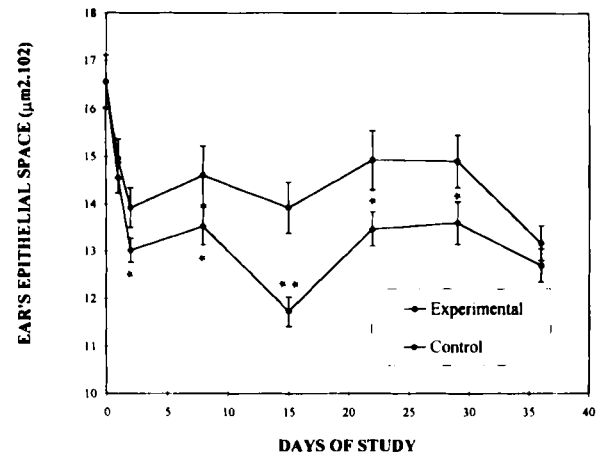

Figure 3. Morphometric evaluation of the ear's epithelium, demonstrating changes in thickness along a constant field length of 100 μm. $^{*}p < 0.05$; $^{**}p < 0.01$.

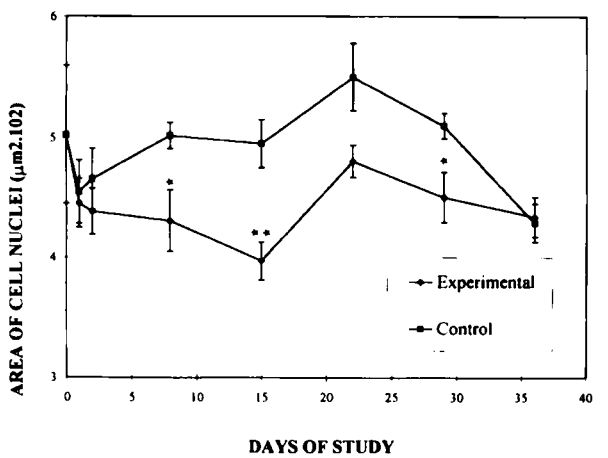

Figure 5. Morphometric measurement of the area of cell nuclei (volume fraction) in the epithelial space of the ear. $^{*}p < 0.05$; $^{**}p < 0.01$.

Ear tissue

Three different measurements were performed on the ear epithelium: (i) thickness, (ii) nuclear cell count and (iii) nuclear zone.

Changes in the mean thickness of the ear's epithelium were recorded and measurements were carried out along a constant length of 100 μm. A significant decrease in the thickness of the ear epithelium was noticed already 2 days after administration of cisplatin, remaining significantly low throughout the 29th day of the study (Figure 3).

Nuclear cell count of the ear's epithelium was significantly decreased after cisplatin administration, from the 15th day after drug administration onwards. The maximal decrease in cell count was observed on or around the 21th day, followed by a partial compensation until the 36th day of the study (Figure 4).

For the ear's epithelium nuclear zone, fluctuations in the volume fraction of the area of cell nuclei started on the eighth day after cisplatin administration and lasted throughout the 29th day of the study. On the 36th day, the area of cell nuclei of the experimental group equalled to that of the control group (Figure 5).

Ear cartilage and connective tissue

Cell nuclear area and the thickness of the cartilage and connective tissue were measured. No signifi-

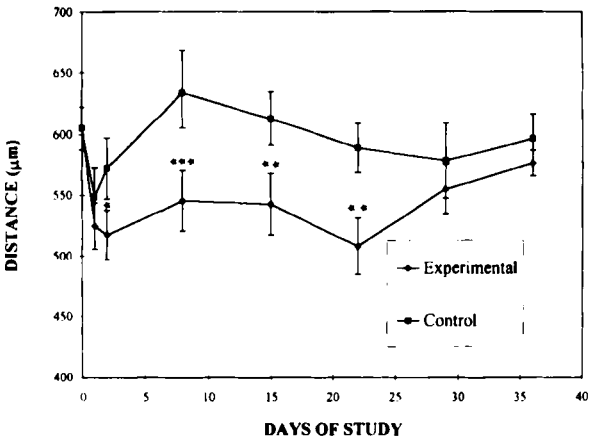

Figure 6. Distance between the portal space and the central vein of the liver, measured morphometrically. $^{*}p < 0.05$; $^{**}p < 0.01$. $^{***}p < 0.005$.

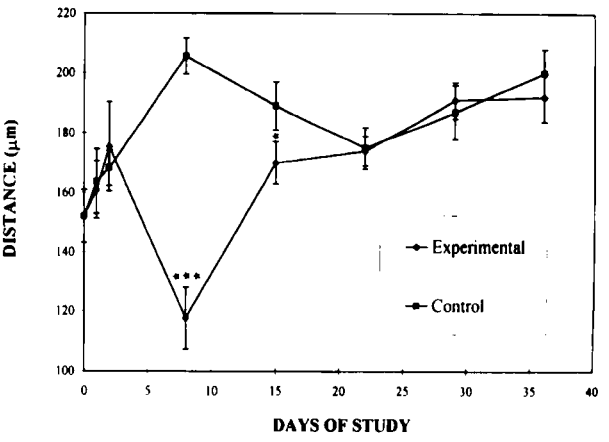

Figure 7. Distance between the portal space and the labeled hepatocytes, evaluated morphometrically. $^{*}p < 0.05$; $^{***}p < 0.005$.

cant differences were observed between the experimental and control groups in these two parameters, in any of the days after cisplatin administration.

Liver

Hepatocyte nuclear count, hepatocyte nuclear area and number of Kupffer cell nuclei were evaluated in both the experimental and control groups, but no significant differences were observed between both groups. In addition, the distance between the portal space and the central vein of the liver was recorded. A remarkable decrease was recorded between days 9 and 29 of the study (Figure 6).

The number of labeled cells

This was counted in each sub-area of the liver by autoradiography. In general, hepatocytes and Kupffer cells followed a similar pattern of behavior, characterized by fluctuations throughout the study. Hence, while the number of labeled hepatocytes decreased during the first days and increased after the 14th day, the number of Kupffer cell nuclei increased during the first week and decreased towards the end of the study. These differences were not significant.

The average distance of labeled hepatocytes from the proximal portal space

The distance of the labeled hepatocytes from the adjacent portal space was measured in both groups. The distances recorded in the experimental group on the eighth day of the study were about half those of the control group and were borderline on the 15th day. No significant differences could be recorded on the other days between the two groups (Figure 7). The relative size of the progenitor compartment was somewhat decreased around the seventh day, but this decrease was not significant.

Discussion

Tissue damage, as a result of cisplatin administration in laboratory animals, has been studied extensively, mainly on a phenomenological basis.[14,15] The effect of the drug on the rat kidney is comprised of degenerative changes, mostly at the cortico-medullary junction and particularly at the proximo-tubular epithelium. The prominent changes include nuclear and cellular metaplasia and degeneration of the proximal tubules. Most reports were based on qualitative histopathological observations, while the scientific methodology of the present study is totally quantitative, i.e. morphometric.

The main quantitative finding in the present study is a significant dilatation of the tubular structures in the cortico-medullary junction area. This dilatation reached maximal values by the end of the first week, with an apparent recovery in the following weeks. The damage to the tissues in this instance can be attributed to a wide-scale necrosis of different lining cells that were shown to exfoliate into the tubular lumen. The study on the effect of

cisplatin on the dermis and epidermis revealed contradictory data, concluding that the drug induced only minor changes in these compartments.[16,17] In the present study we could demonstrate that cisplatin induced a significant decrease in epithelial thickness, that could be directly correlated with the decrease in number of nuclei, suggesting that it is a phenomenon of hypoplastic nature. This decrease was pertinent throughout the entire study.

The dermal connective tissue, which reacted at the beginning of the study in a similar fashion to that of the epithelium, demonstrated a compensatory fibroblastic proliferation towards the fourth week, followed by an increase in dermal thickness, with values significantly higher that those of the controls. The fact that the cartilage of the experimental group did not demonstrate any significant difference when compared with the control group could be attributed to its avascular nature, resulting with its relative stability to the effect of the drug, thus reflecting an extremely slow turnover of cells.

The liver tissues were previously recognized as a highly vulnerable target to chemotherapeutic drugs. Nevertheless, hepatoxicity has hardly been recorded as a major side effect of cisplatin administration. It should be pointed out that high levels of cisplatin have been detected in the liver.[18,19] Several studies reported hepatoxicity as a side effect of cisplatin treatment, manifested by changes in serum levels of liver enzymes and albumin.[20,21] The present morphometric study introduced quantitative techniques that could reveal new data concerning the changes in the liver tissue, i.e. that the hepatocytes and Kupffer cell labeling index was significantly higher after cisplatin administration. This change could be attributed to the difference in cell cycle periodicity. The Kupffer cell cycle is known to be shorter than that of the hepatocytes and the DNA synthesis in the Kupffer cells is significantly enhanced.[22]

An increase in DNA synthesis is not necessarily pronounced as an increased mitotic index, due to the fact that no significant differences have been detected between the experimental and control groups. The increased DNA synthesis is attributed to a compensatory reaction of the hepatocyte to the chemotherapeutic agent. Our observations are in agreement with previous reports on hepatocytic nuclear polyploidity.[23] It is suggested, therefore, that the exposure of the hepatocytes to cisplatin is a trigger for DNA synthesis, resulting in an increased number of polyploid cells. A similar phenomenon was reported in the rat liver as a result of CCl_4 intoxication.[24]

An additional parameter that was investigated in the present study was the change in the progenitor compartment size. This compartment was significantly smaller in the experimental rats as compared with the control ones. The changes in the relative size of the proliferative zone supported the observation of a decrease in the progenitor compartment size after cisplatin administration. This parameter was maximally decreased 1 week after drug administration and disappeared completely 3 weeks later.

Conclusion

This study demonstrates kinetic and morphometric changes in several rat tissues after administration of cisplatin. A computerized morphometric analysis system has enabled quantification of the damage caused by cisplatin to liver, epithelium and connective tissue. This advanced methodology enables characterizing and quantifying side effects of drugs, especially chemotherapeutic agents, at the cellular level.

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