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EXPERIMENTAL DATA REGARDING SECONDARY EFFECTS OF CARBOPLATIN ON THYMUS AND MYOCARDIUM IN RAT

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Carboplatin (Paraplatin), an analog of Cisplatin, is an alkylating agent belonging to the family of platinum-containing products, widely used in the chemotherapy of many types of malignant diseases in humans. According to the previous studies concerning its toxicity, this anticancer drug has a significant myelotoxicity, nephrotoxicity and cardiotoxicity, but its mechanism of action is not completely understood. Therefore, we investigated the histological and ultrastructural modifications at the level of thymus and myocardium of rat, induced by a single therapeutic dose of Carboplatin (400 mg/m^2 body surface administered i.v.), 24 hours, 4, 11 and 18 days after the treatment. Our investigations demonstrated that Carboplatin seriously affects thymocytes, epithelio-reticular cells and vessels of the thymus. In myocardium, the drug induced a slight congestion, haemorrhage, a diffuse oedema, lysis of the sarcolemma and myofibrils. All these modifications, already appeared 24 hours after the treatment, aggravated progressively, and started decreasing after 11 days.

Key words: Carboplatin, thymus, myocardium.

INTRODUCTION

Carboplatin (Paraplatin) is a heavy metal coordination compound, used as a chemotherapeutic agent for the treatment of a wide spectrum of human malignancies, especially for ovarian, testicular, bladder, pulmonary and other solid tumours (Holleran and DeGregorio, 1988). Although the mechanism of action of Carboplatin is not completely known, its effect on cancer cells has been attributed to direct damage to the DNA with subsequent cell death. It is generally accepted that DNA adduct formation with Carboplatin is involved in the mechanism of antitumour activity (Sherman and Lippard, 1987). Meanwhile, various adverse effects of Carboplatin have been attributed to its association with proteins and enzymes in normal tissues (Levi et al., 1980). Cysteine, methionine and histidine, which are important aminoacid residues constituting proteins, are very reactive toward Carboplatin because of its nucleophilic displacement reaction for the

chlorides (Howe-Grant and Lippard, 1989). In addition, the efficacy of Carboplatin on suppression of graft rejection (Khan et al., 1971) on adjuvant-induced arthritis in rats (Bowen et al., 1974; Fairlie et al., 1987), as well as on refractory rheumatoid arthritis in a patient treated for a concomitant adenocarcinoma (Sanchez-Bursan et al., 1989) has been described. The mechanisms of its immunomodulatory effects of Carboplatin has not been elucidated. However, it has been demonstrated that Carboplatin complexes inhibit B-cell surface membrane immunoglobulin capping (Tsokos and Choi, 1980), suppress chemotaxis of monocytes (Nielsen, 1984), and inhibit the proliferation of peripheral lymphocytes in response to mitogens or allogenic cells, as well as the growths of T and B cells in culture (Ohnuma et al., 1978; Calvert, 1994).

Unfortunately, this alkylating agent has a significant hematoxicity. The dose limiting effect of Carboplatin is mainly myelotoxicity, particularly leucopenia. The drug may cause anemia and thrombocytopenia (Walker et al., 1989; Hruban et al., 1991).

The histological and ultrastructural modifications induced by these two anticancer drugs on some different organs and tissues are still incompletely known. Our experimental investigations tried to emphasise these aspects in concordance with the moment of the sacrification after the administration of some therapeutic doses of these cytostatics on thymus and myocardium of rats.

MATERIALS AND METHODS

Our experiments with Carboplatin were carried out with the following five groups of healthy male Wistar rats, weighing 190 ± 10 g, and maintained under bioclimatic laboratory conditions, with no food for 18 hours before the treatment, but having water *ad libitum*.

– group U – untreated (control) animals;

– groups CA1, CA2, CA3 and CA4, treated i.v. with 400 mg Carboplatin/m^2 body surface, and sacrificed 24 hours, 4, 11 and 18 days after the treatment. Immediately after the scarification of animals, we took fragments from the thymus and myocardium.

For histological examination, the fragments were fixed in 10% neutral formol, processed by the paraffin technique and the sections of 5–6 μm were stained by the hematoxylin-eosin and Masson-Goldner trichrome methods (Muresan et al., 1974). Examination were performed with an Olympus BX51 microscope, a CCD Media Cibernetics camera, and Image Pro Plus software (Kuo 2007; Crăciun and Horobin, 1989; Florea and Crăciun, 2012). For ultrastructural investigations, fragments of organs were prefixed in 2.7% glutaraldehyde solution and postfixed in 2% osmic acid solution. The fragments of 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° 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 Soft Imaging Analysis software (Kay 1967; Ploaie and Petre, 1979; Pavelka and Roth, 2005).

The histological and ultrastructural modifications induced by Carboplatin in the treated groups were compared to the control and also to each other, according to the time between drug i.v. administration and killing, to establish a time-lapse dynamics of the effects.

RESULTS AND DISCUSSIONS

1. Histological and ultrastructural investigations of the rat thymus after Carboplatin treatment

Light and electron microscopy investigations showed that Carboplatin caused certain histological and ultrastructural modifications, the intensity of which depended on the time elapsed after the treatment, as compared with normal thymus.

Thus, even after 24 hours after the treatment, clear histological modifications could be noticed, especially in the cortex of the thymic lobules, where a marked thymocyte depletion and an agglutination of these cells contributed to the homogeneous tendency of the whole lobule, all over the thymic surface. In addition, a few groups of lymphocytes, which were in different necrosis stages, appeared in the peripheral areas of the lobules (celular and nuclear picnosis, chromatin condensation or fragmentation etc.). In this moment of the experiment the vascular component of the thymus was not visibly affected yet.

Four days later, we could see that the cell depletion in this central lymphoid organ got worse and, as a consequence of this process, the medullarisation of the lobules aggravated. The cell number decreased especially in the cortex, so that a structural inversion of the two zones of the thymic lobules appeared. Because of the massive lymphocyte death, the epithelio-reticular cells could be easily seen. The necrosis process had a zonal character, so, the lobules got a particular aspect, designated as “starry sky”. The vascular component of the thymus was affected too. Both interlobular and intralobular, a vascular congestion and many microhaemorrhages could be noticed.

At 11 days from the treatment, thymic lobules had relatively normal dimensions, but the cortex significantly got thin. Between the cortex and medulla, thymocytolisis and many grave haemorrhages were present (Fig. 1). Besides, a sclerosis of the thymic structures, especially in the medulla could be seen (Fig. 2).

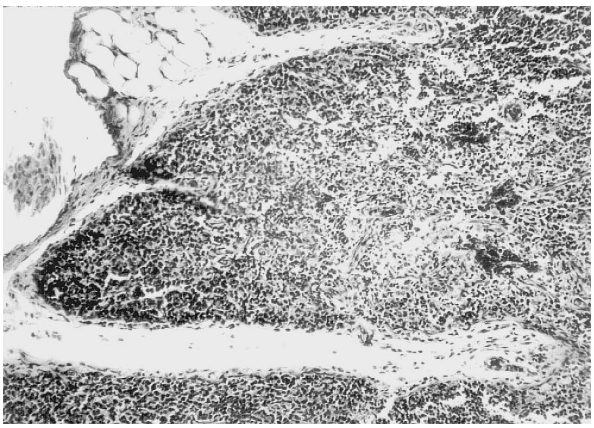

Fig. 1. Thymic lobule 11 days after the treatment. Thymocytolysis and many intralobular haemor-rhages can be seen (HE, oc. x8, ob. x10).

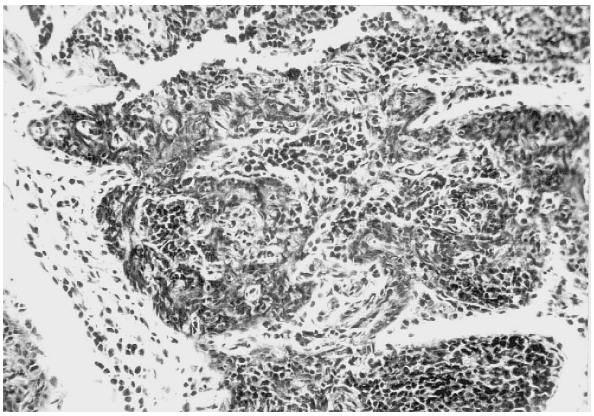

Fig. 2. At 11 days after the treatment, sclerosis of the thymic structures, especially in the medulla of the lobules were noticed (HE, oc. x8, ob. x 10).

At 18 days after the chemotherapy, the thymocyte agglutination and lysis could be still noticed almost all over the lobule surface, and so did the haemor-rhages. These histological aspects demonstrated that the toxic effect of Carboplatin still persisted.

The electron microscopy investigations, as compared with normal thymus (Fig. 3), showed that this cytostatic, after 4 days of the treatment, blocked immediately and seriously the cell mitosis in the thymus. In addition, Carboplatin induced many changes in the shape, dimension and ultrastructure of both the thymocytes and the epithelio-reticular cells. Thus, many thymocytes had a swollen and rarefied cytoplasm, picnotic or retracted nuclei (some of them having a peculiar shape and an abnormal disposition of their chromatin), a pronounced

decrease of the ribosome number, and a discreet swelling of the mitochondria. In a few cells it could be noticed a gradual karyolitic process. The cell density decreased significantly, especially in the cortex of the lobules, and in the lysis areas a lot of apoptotic corpuscles were present (Fig. 4). Lysis of a large number of thymocytes in both the cortex and medulla emphasized the epithelio-reticular cells, which were affected too by a degenerative transformation consisting of: vacuolisation of the cytoplasm (more evident along their processes), the appearance of many nuclei modified in shape and dimensions.

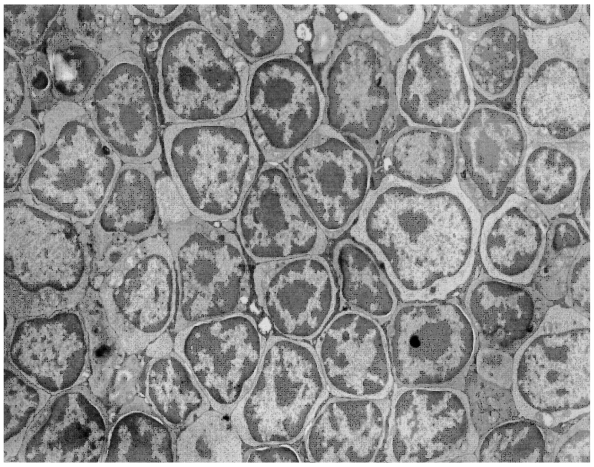

Fig. 3. Normal tymocytes in the thymus of control rats (x 7200).

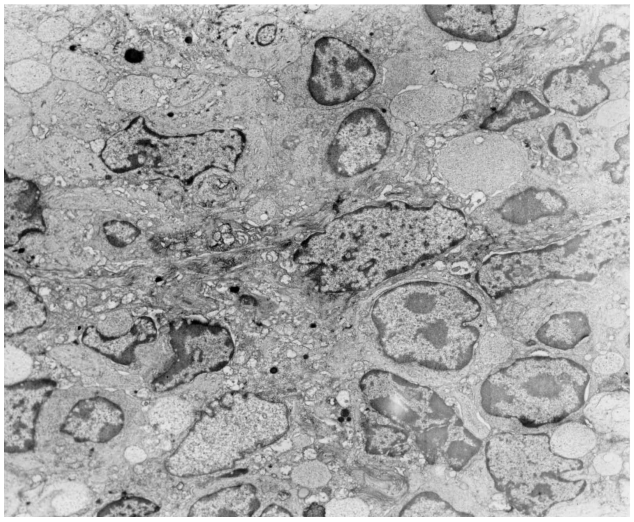

Fig. 4. Thymocyte depletion, nuclear picnosis and karyolysis processes could be noticed 4 days after Carboplatin treatment (x 5250).

Besides, this alkylating antitumoral agent determined a serious congestion of the vessels both in the medulla and cortex, aspect correlated with a significant modification of the vascular permeability and even integrity, with a massive extravasation of the erythrocytes which appeared disposed in wide groups between the thymocytes (Fig. 5). A significant enough collagen proliferation could be observed especially pericapillary, and in the lysis areas, which demonstrated the existence of a fibrosis tendency of some thymic lobules (Fig. 6).

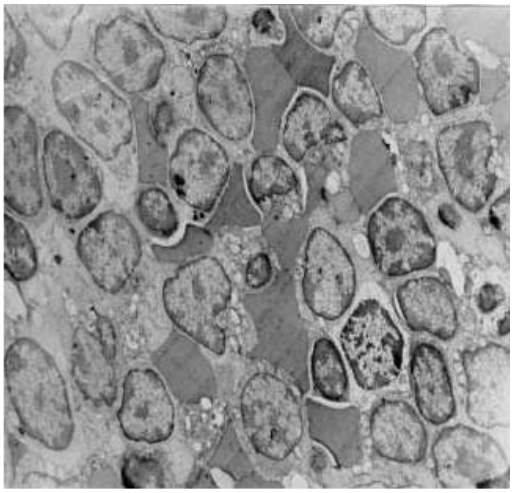

Fig. 5. Four days after the treatment the integrity of the vascular endothelium was deeply altered, many erythrocytes being seen among the thymocytes (x 5880).

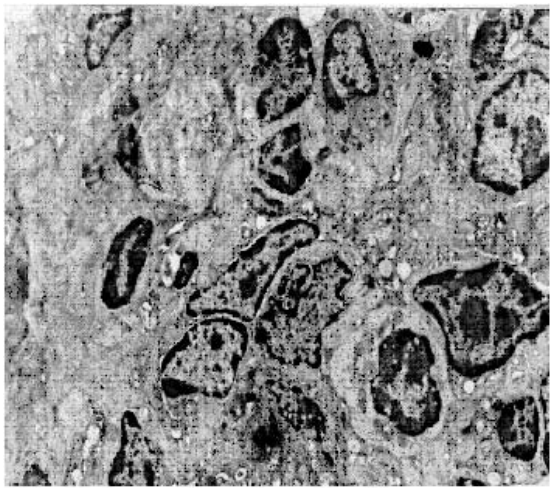

Fig. 6. A proliferation of collagen fibers in the cortical-medulla area was evident 4 days after Carboplatin administration, showing a tendency to fibrosis (x 4560).

The large number of altered thymocytes and epithelioreticular cells were correlated with a very intense activity of the macrophages, as a specific defense reaction (Fig. 7).

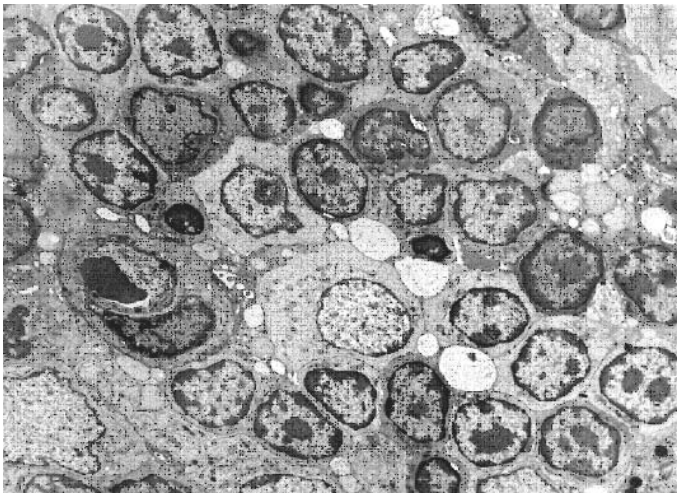

Fig. 7. Four days after Carboplatin treatment, an intense activity of macrophages could be seen (x 10200).

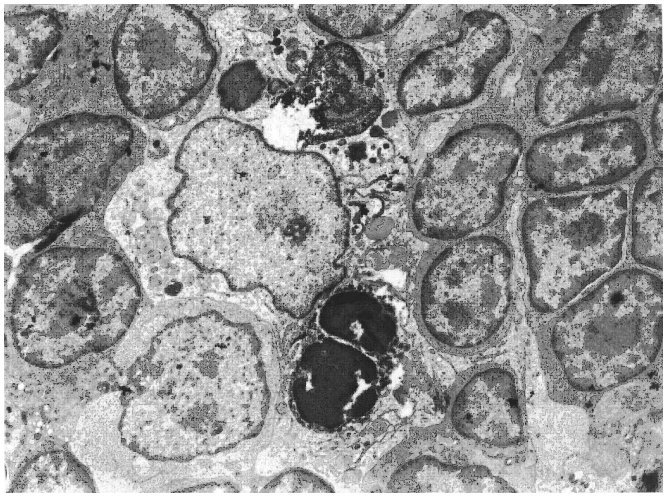

Fig. 8. At 18 days after the treatment, a recovery tendency could be noticed (x 6200).

Both the light and electron microscopy researches showed that this anticancer drug, administered in a single therapeutic dose alters immediately and seriously enough both the cellular and the vascular component in the thymus. But the most sensitive were the thymocytes, on which the toxic effect could be observed as early

as 24 hours from the treatment, then it got worse during the next 10 days. Just at the end of the experiment, 18 days after the treatment, a recovery tendency could be noticed concerning the number, density, shape, and structure of the lymphocytes in the thymic lobules (Fig. 8). The massive decreasing of the myocyte number was a consequence of the necrotic, necrobiotic and apoptotic processes correlated with a drastic antimitotic effect of Carboplatin.

According to the previous studies, Paraplatin has a serious thrombocytopenic effect (Bowen et al., 1974; Fairlie et al., 1987; Holleran and DeGregorio, 1988; Walker et al., 1989; Hruban et al., 1991).

This effect correlated with the toxic action of the drug determined a grave modification of the permeability and even integrity of the vessels, which contributed to the starting, and aggravation of the degenerative processes involving the thymocytes and epithelio-reticular cells.

Conclusions

– The toxic effect of Carbplatin on the rat thymus was significant, but it had a reversible character.

– Carboplatin seriously affected both the cells (thymocytes and epithelio-reticular cells) and the vessels of the thymus.

– The massive thymocyte depletion in the thymus was a consequence of the necrobiotic, necrotic and apoptotic processes correlated with the antimitotic effect of this alkylating agent.

– The sensitivity of the thymocytes depends on their age, the lymphoblasts being the most affected cells.

– Just in some areas, Carboplatin induced an obvious sclerosis of a few number of thymic lobules.

– The permeability and integrity of the thymic vessels were significantly affected by Carboplatin; this vascular alterations seem to contribute to the degenerative processes which involved both the thymocytes and the epithelio-reticular cells.

– The modifications induced after the treatment with Carboplatin were similar with those induced by Cisplatin (Crăciun and Pasca, 2013).

2. Structural and ultrastructural studies of the rat myocardium after the treatment with Carboplatin

Our investigations evaluated the histological and ultrastructural modifications induced by a single therapeutic dose of Carboplatin (400 mg/m^2 body surface) administered i.v., on the left ventricle of the rat myocardium, after 24 hours, 4, 11 and 18 days of the treatments.

By light microscopy it could be seen that Carboplatin determined certain histological modifications which already appeared 24 hours after the treatment.

They consisted of a slight congestion and a diffuse oedema which was not correlated with the presence of a cellular infiltration. Many myocytes had nuclear modifications consisting of: hypertrophy, an increased number of nucleoli and peculiar arrangement of chromatin in groups. A few nuclei were spherical in shape and very intensely stained, all these apects being very well pointed out on the sections stained with hematoxylin-eosin, and the oedema was obvious on the sections stained with Masson-Goldner dye.

All the modifications evolved progressively, so that 4 days after the treatment the stasis, congestion, oedema were more serious and, in addition, many interfascicular hemorrhages appeared. The circulatory disturbances correlating with a significant perivascular and interfascicular oedema persisted in the group sacrificed 11 days after the treatment. Here and there, microfocuses of myolysis could be noticed. A small number of myocytes were affected by a granulo-hyaline dystrophy.

The electron microscopy investigations showed that Carboplatin, as compared with the normal myocardium (Fig. 9), induced certain ultrastructural alterations consisting of the appearance of a serious modification of the vascular and sarcolemmal permeability correlated with a modified flux of electrolytes and water.

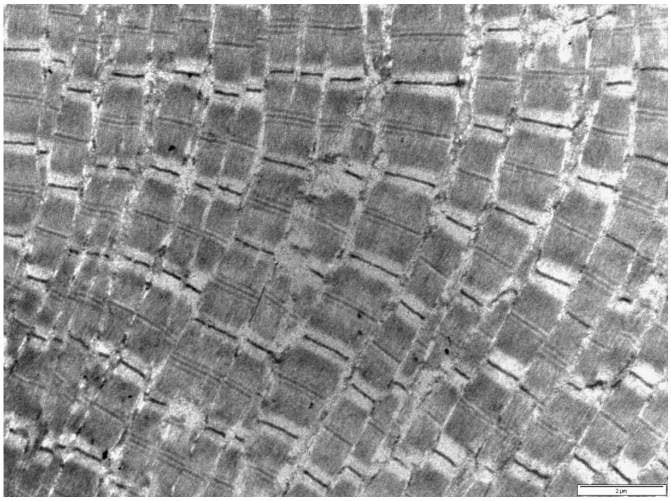

Fig. 9. Appearance of the control myocardium (barr, 2 μm).

After 4 days of the treatment, these leading to cell swelling, disorganization of the cell ultrastructure, especially at the periphery of the myocytes, lysis of the sarcolemma and myofibrils (Fig. 10), breaking of the Z-lines, increasing of the interfascicular spaces (Fig. 11), swelling and degeneration of the mitochondria, and swelling of the nucleus. The massive oedema between the myofibrils determined a myocyte disorganisation. In the areas with an advanced lysis process, an obvious collagenous proliferation appeared.

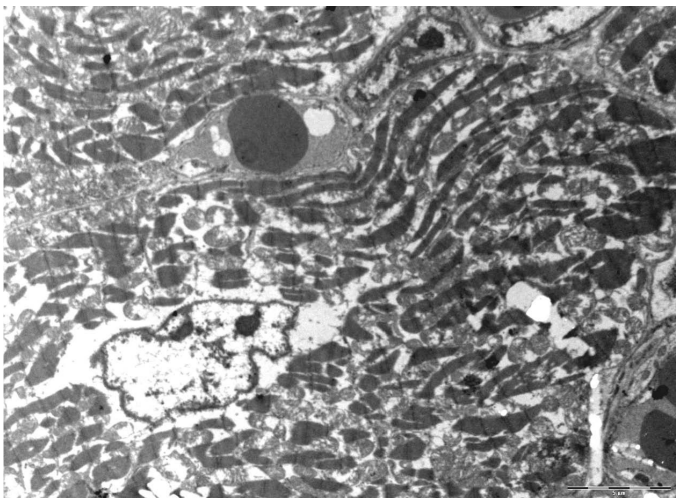

Fig. 10. Cell swelling, lysis of the sarcolemma and myofibrils were evident 4 days after the treatment (barr, 5 μm).

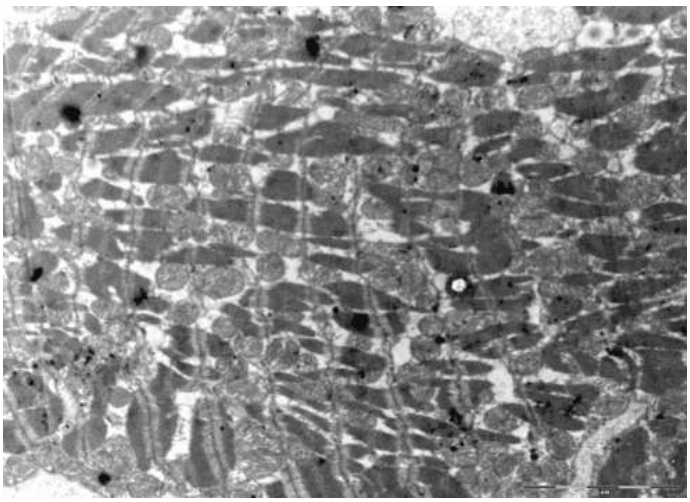

Fig. 11. Breaking of the Z-lines and increasing of the interfascicular spaces were noticed 4 days after the treatment (barr, 5μm).

At the end of the 18 days of the experiment, the granular myocardosis, correlated with myolysis microfocuses, congestion, stasis, oedema and haemorrhages, had a zonal character and was very discreet, an obvious recovery process of the myocardium being noticed (Fig. 12).

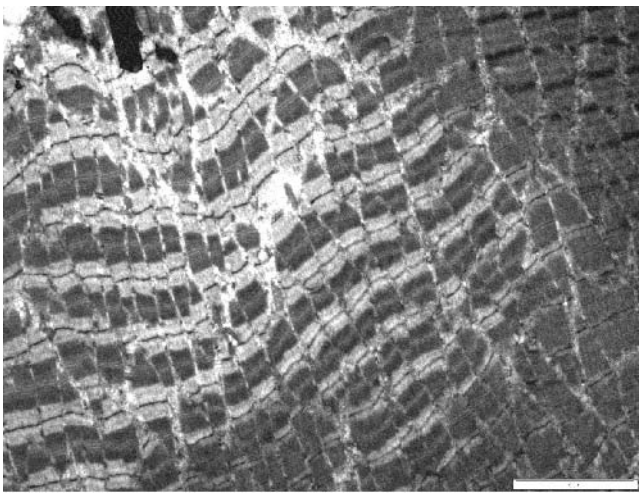

Fig. 12. An obvious recovery process of the myocardium was observed 18 days after Carboplatin treatment (barr, 5 μm).

The histological and ultrastructural aspects confirmed the cardiotoxic effect of this anticancer drug, which, according to the previous studies, appears after high doses of Carboplatin or after a long chemotherapy and consists of certain histological modifications: stasis and haemorrhages at the level of the capillaries, very grave microvascular disturbances and a slight hypertrophy of the myocardium mass (Hruban et al., 1991; Sakai et al., 1993; Walker et al., 1989). In severe Carboplatin treatment, muscle cells are irreversibly damaged, the sarcolemmal permeability being seriously affected. The functional consequences of the altered sarcolemmal permeability involve a modified flux of electrolytes and water, leading to cell swelling and increased transport of Ca^{2+} from the extracellular space to the cardiac muscle cell (Fleckenstein et al., 1973). Besides, dilatation of the tubules of the sarcoplasmic reticulum, increased number of irregularly shaped lysosomes containing heterogenous deposits of electron-dense material, dehiscence of intercalated discs, mitochondria with distroyed cristae, and intramitochondrial amorphous inclusion bodies were observed (Dietrich, 1993; Katz et al., 1985; Kino, 1981). Thus, a myocardial Ca^{2+} overload developed and the ultrastructural modifications of the myocytes were correlated with certain devastating effects on the cardiac cell functions.

The histological and ultrastructural modifications noticed by us demonstrated that Carboplatin, administered i.v. in a single therapeutic dose of 400 mg/m^2 body surface, had a significant cardiotoxic effect, which already appeared 24 hours after the treatment, aggravated progressively, and started decreasing after 11 days; in the 18th day of our experiment, an obvious recovery tendency was noticed, all the modifications having a zonal character and altering especially isolated myocytes or small groups of myocardial cells.

Conclusions

– A single therapeutic dose of 400 mg Carboplatin/m^2 body surface had a moderate toxic effect of the rat myocardium, consisting of the appearance of both histological and ultrastructural modifications.

– All the modifications induced by this anticancer drug seemed to be due to the grave circulatory disturbances and the thrombocytopenia.

– Carboplatin determined certain histological alterations: capillary stasis, interfascicular haemorrhages, oedemas, nuclear modifications (hypertrophy, the presence of an increased number of nucleoli, a peculiar arrangement of the chromatin in groups) and an obvious collagenous proliferation in the lysis areas.

– The ultrastructural modifications induced by Carboplatin consisted of cell swelling, disorganisation of the cell ultrastructure, especially at the periphery of the myocytes, lysis of the sarcolemma and myofibrils, breaking of the Z-lines, swelling of the interfascicular spaces, swelling and degeneration of the mitochondria, myocyte disorganisation and myolysis.

REFERENCES

1. Bodrogi I., Baki M., Horti J., Liszka G., Otto S., Hindy I., Sugar J., Eckhardt S. – Vinblastine, cisplatin and bleomycin treatment of advanced nonseminomatous testicular tumors, Neoplasma, 1990, 37, 445-450.
2. Bowen J.R., Gale G.R., Gardner W.A., Bonner W.A. – Carboplatin: Suppression of adjuvant arthritis in rats, Agents Action, 4, 108-118, 1974.
3. Burkitt H.G., Young B., Heath J. W. – *Functional Histology*, 3rd edition, Churchill Livingstone, Edinburgh, 1993.
4. Calvert H. – Dose optimisation of carboplatin in adults. Anticancer Res., 1994, 14, 2273-2278.
5. Chan A. S. – Effects of hydrocortisone on the ultrastructure ot the thymic cysts of the chick embryo, Poultry Sci., 1991, 70, 631-640.
6. Chiricuţă I. – *Cancerul şi chimioterapia*, Ed. Medicală, IMF Cluj, 1979.
7. Cowan W. C., Sorensen G. D. – The effect of hydrocortison on the mouse thymus: an electron microscopic study, Anat. Rec., 1963, 145, 120-122.
8. Crăciun C., Miclăuş V., Crăciun V. – Studiu ultrastructural al timusului la vârste diferite, *Rev. Med. Oradeană*, 1996, 3, 1-8.
9. Crăciun C., Paşca C. – Structural and ultrastructural data on side effects of cisplatin in spleen, kidney and liver of rats. Acta Metallomica, 2013, XII, 1, 5-16.
10. Csaba G., Dunay C., Olah J., Toro I. – Effect of glucocorticoids on the thymus. I. Histological and electron-microscopic investigation of mast cell formation. Acta Biol. Acad. Sci. Hung., 1967, 18(3), 345-361.
11. Dietrich W.S. – The ultrastructure of cardiac muscle in health and disease. Micron, 1993, 24(1), 47-73.
12. Fairlie D.P., Withehouse M.W., Broomhead J.A. – Irritancy and anti-inflamatory activity of bs(cyclopentadienyl)titanium(IV) complexes in rats, Chem-Biol. Interact., 61, 277-291, 1987.
13. Flekenstein A., Janke J., Doring H.J., Pachinger O. – Calcium overload as the determinant factor in the production of catecholamine-induced myocardial lesions, Cardiomyopathies, 1973, 2, 455-466.
14. von Gaudecker B. – Ultrastructure of the age-involuted adult human thymus. Cell Tiss. Res., 1978, 186, 507-525.
15. Ghadially F.N. – *Ultrastructural pathology of the cell*. Ed. Butterworths, London and Boston, 1978.
16. Grigg A., Szer J., Skov K., Barnett M. – Multi-organ dysfunction associated with high-dose carboplatin therapy to autologus transplantation. Bone Marrow Transplantation, 1996, 17, 67-74.

17. van Haelst V. – Light and electron microscopic study of the normal and pathological thymus of rat. II. The acute thymic involution. Z. Zellforsch., 1967, 80, 153-182.
18. Hayat M.A. – *Principles and techniques electron microscopy: Biological Applications,* 4th edition, Cambridge University Press, 2000.
19. Holleran W.M., DeGregorio M.W. – Evolution of high-dose carboplatin, Invest. New Drug, 6, 135-142, 1988.
20. Howe-Grant M.E., Lippard S.J. – Aqueous platinum(II) chemistry; Binding to biological molecules, pp.63-125, in *Metal Ions in Biological Systems, Vol. 11. Metal complexes as anticancer agents* (Sigel H., Ed.), Marcel Dekker, New York, 1980.
21. Janeway C. A., Travers P. – Immunobiology. Blackwell Sci. Publ., Oxford, 1994.
22. Khan A., Albayrak A., Hill J.M. – Effect of paraplatin on graft rejection: Prolonged survival of skin grafts against H2 histocompatibility, Proc. Soc. Exp. Biol. Med., 1971, 141, 7-17.
23. Katz A. M., Freston J. W., Messineo F.C., Herbette L.G. – Membrane damage and patogenesis of cardiomyopathies, J. Molec. Cell. Cardiol., 1985, 17, 11-20.
24. Kino M. – Chronic effects of some drugs under partial inhibition of catalase activity in the rat heart: light and electron microscopic observation, J. Molec. Cell Cardiol., 1981, 13, 5-25.
25. Lesson C.R., Lesson T.S., Paparo A.A. – *Textbook of Histology*, W. B. Saunders, Philadelphia, 1985.
26. Levi J., Jacobs C., Kalman S.M., McTigue M., Weiner M.W. – Mechanism of carboplatin nephrotoxicity, Pharmacol. Exp. Ther., 1980, 213, 545-550.
27. Marcelis A.T. M., Reedijk J. – Binding of platinum compounds to nucleic acids with respect to the anti-tumor activity of cis-diamminedichloroplatinum (II) and derivates, Recl. Trav. Chim. Pays-Bas, 1983, 102, 121-129.
28. Millward M. J., Webster L. K., Toner G.C., Bishop J.F., Rischin D., Stokes K.H., Johnston V.K., Hicks R. – Carboplatin dosing based on measurement of renal function – experience at the Peter MacCallum Cancer Institute, Cancer Res., 1996, 26, 372-379.
29. Morel G., Deschaux P., Cavot E., Fontanges R. – Caryometrie des cellules reticulaires thymique chez le rat. Influence de L'hydrocortisol et de la surrenalectomie, Biomedicine, 1976, 24, 6, 417-425.
30. Nielsen H. – Effect of paraplatin on human blood monocyte function in vitro, Cancer Immunol. Immunother., 1984, 18, 223-225.
31. Ohnuma T., Arkin H., Minowada J., Holland J.F. – Differential chemotherapeutic susceptibility of human T-lymphocytes and B-lymphocytes in cultures, J. Cancer, 1978, 60, 749-752.
32. Paşca C., Crăciun C., Ardelean A. – *Efecte secundare ale unor citostatice asupra organismului*, Ed. Risoprint, Cluj-Napoca, 2000 a.
33. Paşca C., Miclăuş V., Crăciun C., Kis E., Sandu D., Crăciun V., Papuc I., Munteanu C. – Histological and ultrastructural studies of the rat myocardium after a single therapeutic dose of Carboplatin administred intravenously, Studia Univ. "Babeş Bolyai", Ser. Biol., 2000 b, XLV (2), 49-55.
34. Paşca C., Miclăuş V., Crăciun C., Ardelean A., Kis E., Sandu D., Crăciun V., Papuc I. – Structural and ultrastructural aspects of the rat thymus after Carboplatin chemotherapy, Chap.III, pp. 276-284, in Curr. Probl .Cell. Mol. Biol., Vol. V, 2000 c.
35. Paşca C., Crăciun C., Kis E. – Structural and ultrastructural modifications induced by two therapeutic doses of cis-diamminedichloroplatinum (II) (cisplatin) on some lymphoid organs in white Wistar rats, Studia Univ. Babeş-Bolyai, Biologia, 2001, XLVI (2), 65-74.
36. Sakai T., Yoshikawa K., Nosaka S., TakenoshitaM. – A case report of cardiac failure caused by the new anti-neoplasic agent, carboplatin. Cancer, 1993, 72 (5), 756-760.
37. Sanchez-BursonJ., Aparicio L.A., Mendez M., Galdo F. – Paraplatin, a treatment for refractory rheumatoid arthritis? Br. J. Rheumatol., 1989, 28, 358-367.
38. Sherman E. Suzanne, Lippard S.J. – Structural aspects of platinum anticancer drug interactions with DNA, Chem. Rev., 1987, 87(5), 1153-1181.
39. Stevens A., Lowe J. – *Histology*. Gower Medical Publ., London-New York, 1992.
40. Tsokos G.C., Choi D.D. – Inhibition of capping of immunoglobulin and concavalin A receptors by carboplatin in mouse spleen cells, Cancer Lett., 10, 261-267, 1980.
41. Walker R.W., Rosemblum M.K., Kempin S.J., Christian M.C. – Carboplatin associated thrombotic microangiopathic hemolytic anemia, Cancer, 1989, 64 (5), 1017-1020.