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The influence of cisplatinum on blood, some blood enzyme activities, magnesium level in rat serum and oxygen consumption in liver and kidneys

Wpływ cisplatyny na krew obwodową, enzymy, poziom magnezu i oddychanie tkankowe wątroby i nerki u szczura

In 1965, B. Rosenberg discovered that the platinum compounds that dissolved out of the platinum electrodes used in his experiments inhibited the growth of *Escherichia coli* and in anticipation of their inhibiting the mitosis of rapidly growing cancer cells. As a result, it was demonstrated that cisplatinum (cis – diaminedichloroplatinum) exerted the most potent antitumor influence, and after various preclinical studies, clinical trials of the compound were begun in 1972. In 1978, its usefulness in the treatment of malignant tumors of the urinary tract was demonstrated. In 1983, Carlson et al. (1) treated nine women with germ – cell tumors of the ovary (3 endodermal sinus tumors, 4 immature teratomas and 2 mixed germ–cell tumors) with cisplatinum, vinblastine and bleomycin after cytoreductive operations. All of the patients remained free of disease with a median follow–up of 26 months after diagnosis and 23 months after completion of chemotherapy. All 6 patients with intact ovary were menstruating, 1 bore a normal infant.

Ozoles et al. (3) reports of the effects on renal function of high–dose cisplatinum administered in hypertonic (3%) saline with concomitant hydration in previously untreated patients with advanced nonseminomatous testicular cancer and in 6 patients with ovarian cancer refractory to standard–dose cisplatinum therapy. In the testicular cancer patients there was no statistically significant decrease in creatinine clearance or elevation of serum creatinine.

It seemed to be interesting to learn cisplatinum influence, an antineoplastic drug, on blood elements, some enzyme activity and magnesium levels in plasma as well as respiration in kidney and hepatic tissues.

The aim of this study was to investigate the effect of cisplatinum on blood morphology, some blood enzyme activities, magnesium level in rat serum and oxygen consumption in liver and kidneys.

MATERIAL AND METHODS

The experiments were carried out using 40 Wistar rats, 200–250g of body weight. The animals were divided into 2 groups, 20 rats in each. Cisplatinum (Rhone–Poulenc Rover Laboratoire, Roger Bellon, France) was administered (dissolved in physiological saline, intraperitoneally) in such concentrations so that the single dose was 100mg/1m^2 of body surface. In order to state the dose of the drug the body weight was converted into the surface, using Fisher formula logS=100W*0.425+logL, where S = body surface in m^2, W = body weight, L = body length. Control group (20 rats) was supplemented with 0.1 ml of physiological saline.

The experiments were carried out in three stages, each of them lasted five days. There was one twenty–hours break in the drug administration between the stages. After completion of the three stages – the cycle, rats were killed by chloroform and blood and pieces of liver and kidney were taken off to further examinations.

The following parameters were determined: hemoglobin concentration (g/100ml), erythrocyte number in µl, leukocyte number in µl, lymphocyte and monocyte numbers (in %), lactate dehydrogenase activity (LDH–U/l), a–hydroxybutyrate dehydrogenase activity (a–HBDH–U/l), alanine aminotransferase activity (ALAT–U/l), and aspartate aminotransferase activity (AspAT–U/l). Magnesium concentrations (mg/100ml) were determined photometrically. The enzyme activities were determined by the kinetic methods using kits made by Cormay (Lublin).

The oxygen consumption was measured by Wartburg method in pieces of liver and kidney. These experiments lasted 30 min. and the manometers were read three times every ten minutes. The final evaluation of oxygen consumption was made from mean values of 15 measurements and was expressed as O_2mm^3/g/min.

Statistical analyses were performed by Student's test for unpaired data with $p < 0.05$ as statistical limit.

RESULTS

Table 1 shows circulating blood elements in both groups. Statistically significant leukocytose, erythrocytose, lymphopenia and monocytopenia are evident in rats supplemented with cisplatium. A significant rise in segmented nutrophil number is also observed in this group.

No significant differences are seen between both groups in LDH–4 activity. Similar behaviour is observed in other enzymes (Figs 1, 2). Magnesium concentrations show no significant differences, either (Fig. 3).

Both in liver and kidneys there was a statistically significant diminution in oxygen consumption in cisplatinum–treated rats (Figs 4, 5).

Tab. 1. Blood elements in control and cisplatinum-supplemented rats
Mean values ± SD * - $p < 0.05$

	Control	Cisplatinum
Leukocytes	8200 ± 750	12000 ± 660*
Hb	7.8 ± 0.6	13.5 ± 0.9*
Erythrocytes	3320000 ± 2500	4531000 ± 2750*
Lymphocytes	74 ± 6	57 ± 5*
Monocytes	4.8 ± 0.3	3.6 ± 0.3*
Polymorphonuclear leukocytes	27 ± 3	48 ± 4*

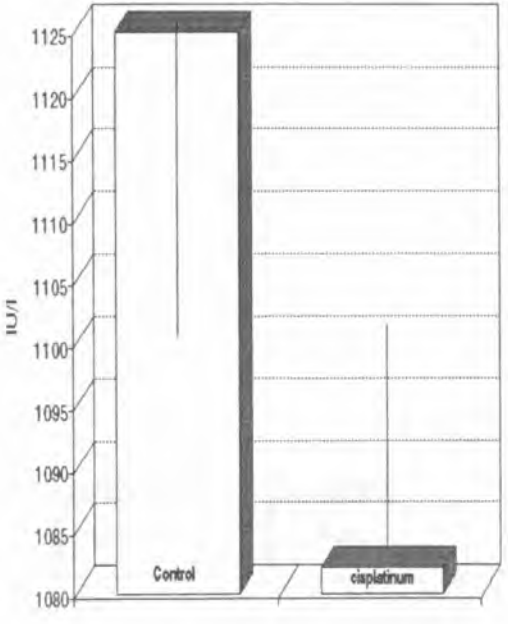

Fig. 1. Lactate dehydrogenease (isozyme 4) activity in control and cisplatinum-supplemented rats. Mean ± SD

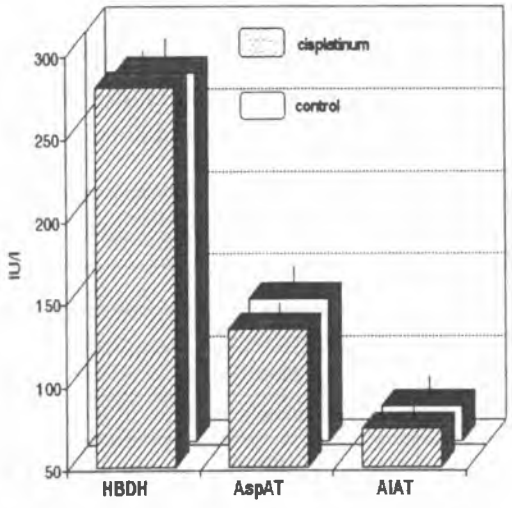

Fig. 2. α-Hydroxybutyrate dehydrogenase (α-HBDH), aspartate aminotransferase (AspAT) and alanine aminotransferase (AlAT) activities in control and cisplatinum-supplemented animals. Mean±SD

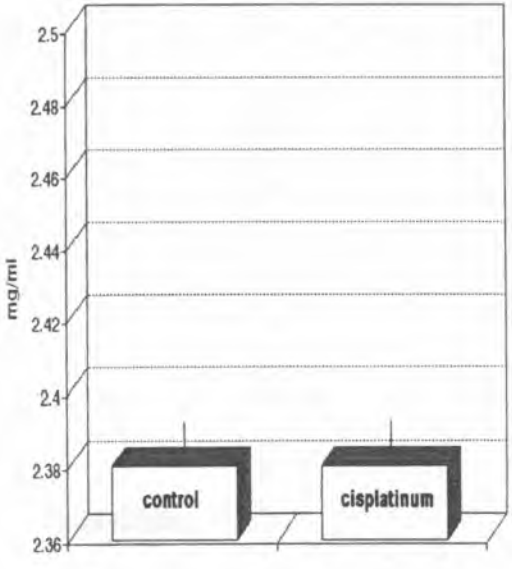

Fig. 3. Magnesium level in plasma of both groups of rats thus investigated. Mean±SD

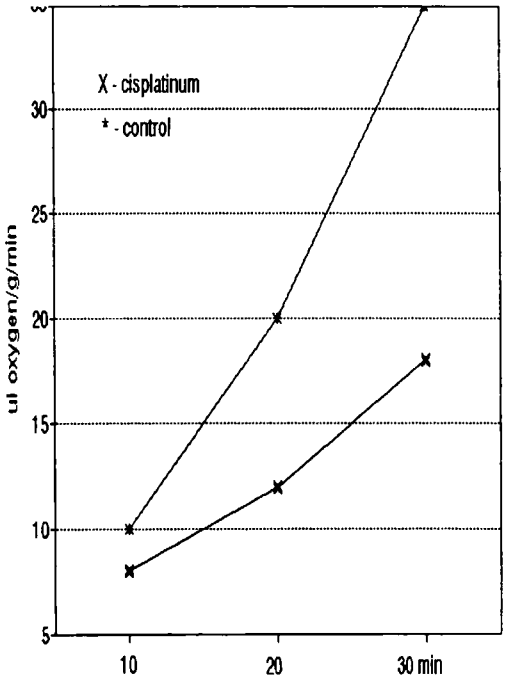

Fig. 4. Oxygen consumption in rat hepatic tissue

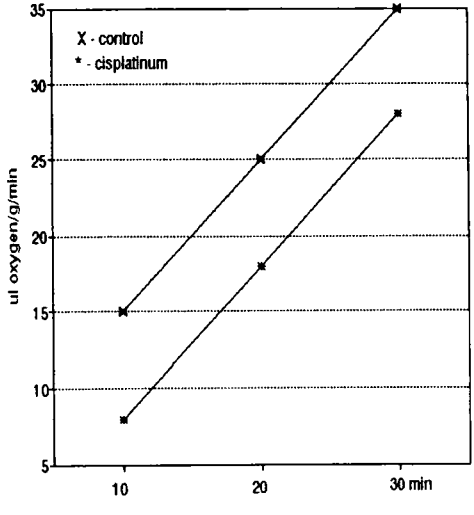

Fig. 5. Oxygen consumption in rat kidneys

DISCUSSION

In literature there is a general lack of data concerning the influence of cisplatinum on enzymatic activity or blood morphology. The major toxic effect of cisplatinum seemed to be myelosuppression. All patients developed leukocyte counts below 1,000/μL and platelet counts below 20,000/μL. All testicular cancer patients had at least one episode of a granulocytopenic fever (2). In this study we observed statistically significant leukocytose, erythrocytose, lymphopenia and monocytopenia, however the above–mentioned cell counts were in physiological range.

There is very sparse information about the mechanism of cisplatinum influence. Perego et al. (4) have investigated the relationship between p53 gene and the development of resistance to cisplatinum in ovarian carcinoma cell systems which included two cisplatinum–resistant variants selected *in vitro* after prolonged drug exposure of the cisplatinum–sensitive parental cell line. Resistance to cisplatinum paralleled a reduced cell susceptibility to cisplatinum–induced apoptosis. DNA single–strad conformation polymorphism analysis of exons 5–9 demonstrated the presence of two mutant alleles at exon 8 in the two resistant cell lines. These observations support a role for mutations of the p53 gene in the development of cisplatinum resistance in ovarian cancer as a consequence of loss of ability of p53 to transactivate bax, an apoptosis–inducing gene.

Murphy et al. (2) show that cisplatinum in a concentration corresponding to the therapeutic plasma concetration for cancer patients cause a marked enhancement of magnesium efflux and uptake. The effect of cisplatinum on magnesium transport is attributed to an increase in the charged form of cisplatinum that accumulates inside the cell.

Schiffer et al. (5) show that contrary to the effects exerted by cisplatinum on brain tumors, general clinical and neurological condition of the rabbits to which intraarterial cisplatinum was administered was unaffected and histopathological examination of the rabbit's brain was normal.

Based on the data thus obtained, the above-mentioned parameters seemed inadequate in clinical monitoring cisplatinum therapy.

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STRESZCZENIE

W pracy badano wpływ cisplatyny na krew obwodową i oddychanie tkankowe u szczura rasy Wistar. Z przeprowadzonych doświadczeń wynika, że stosowany cytostastyk wywoływał leukocytozę, limfopenię i monocytopenię. Obserwowano także statystycznie zmienny wzrost poziomu hemoglobiny i ilości erytrocytów. Stężenie magnezu i badanych enzymów: LDH, a–HBDH, AIAT i AspAT nie różniło się od wartości kontrolnych.

W odniesieniu do oddychania tkankowego wykazano statystycznie zmienny wzrost zużycia tlenu przez tkanki.

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