PHARMACOLOGY AND TOXICOLOGY

Early and Delayed Effects of Carboplatin on the Blood System

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Carboplatin injected intravenously in a maximum permissible dose to Wistar rats inhibited erythro- and granulocytopoiesis in the bone marrow, caused hyporegenerative anemia, leukopenia, and thrombocytopenia in the peripheral blood, led to hypoplasia of the thymus and spleen, and produced moderate apoptosis-inducing effects. These effects were observed within the first month after treatment. In mice carboplatin induced chromatid aberrations in the bone marrow and increased the count of erythrocytes with micronuclei. Moderate hypoplasia of erythro- and granulocytopoiesis and accelerated involution of the thymus were observed 3 and 6 months after cytostatic treatment. Our results indicate that carboplatin possesses higher myelotoxic activity compared to cisplatin.

Key Words: *peripheral blood; bone marrow; lymphoid organs; carboplatin; cisplatin*

Carboplatin (cis-diammine[1,1-cyclobutanedicarboxylato] platinum) is a second-generation complex platinum compound. Antitumor activity of carboplatin during mono- and polychemotherapy of various solid tumors compares well with cisplatin (cis-platinum(II) diammine dichloride). Carboplatin is less nephrotoxic, but more myelotoxic than cisplatin [1,11]. Clinical and experimental studies of the peripheral blood indicate that carboplatin causes neutropenia, thrombocytopenia, and anemia [6,10].

Here we evaluated early and delayed responses of rat hemopoietic and lymphoid organs to carboplatin injected intravenously in a single subtoxic dose.

MATERIALS AND METHODS

Experiments were performed on 50 male Wistar rats and 30 BALB/c mice weighing 200-220 and 18-20 g, respectively (Rassvet nursery, Tomsk). The animals were kept under conditions approved by European Convention for the Protection of Vertebrate Animals Used in Experimental and Scientific Studies (Strasbourg, 1986).

The rats and mice received single injections of carboplatin in a maximum permissible dose (MPD, 60 and 50 mg/kg, respectively) into the caudal vein. These doses were determined by a 30-day graphic probit analysis [7]. Control animals (15 rats and 8 mice) received an equivalent volume of physiological saline. Peripheral blood tests in rats (hematocrit and contents of erythrocytes, reticulocytes, platelets, and leukocytes) were performed 2, 5, 10, 15, and 30 days and 3 and 6 months after carboplatin injection (5 animals per point). Parameters of the bone marrow (total cell count and myelogram) were determined after decapitation. The weight, cytogram, and morphological parameters of the thymus and spleen were determined after fixation in Carnoy fluid and hematoxylin and eosin staining. In mice, metaphase spreads of bone marrow cells were examined 6, 12, 24, 48, and 72 h postinjection using the method of Ford with modifications [5]. The count of abnormal metaphases, chromosomal aberrations, chromatid fragments, and exchanges per 100

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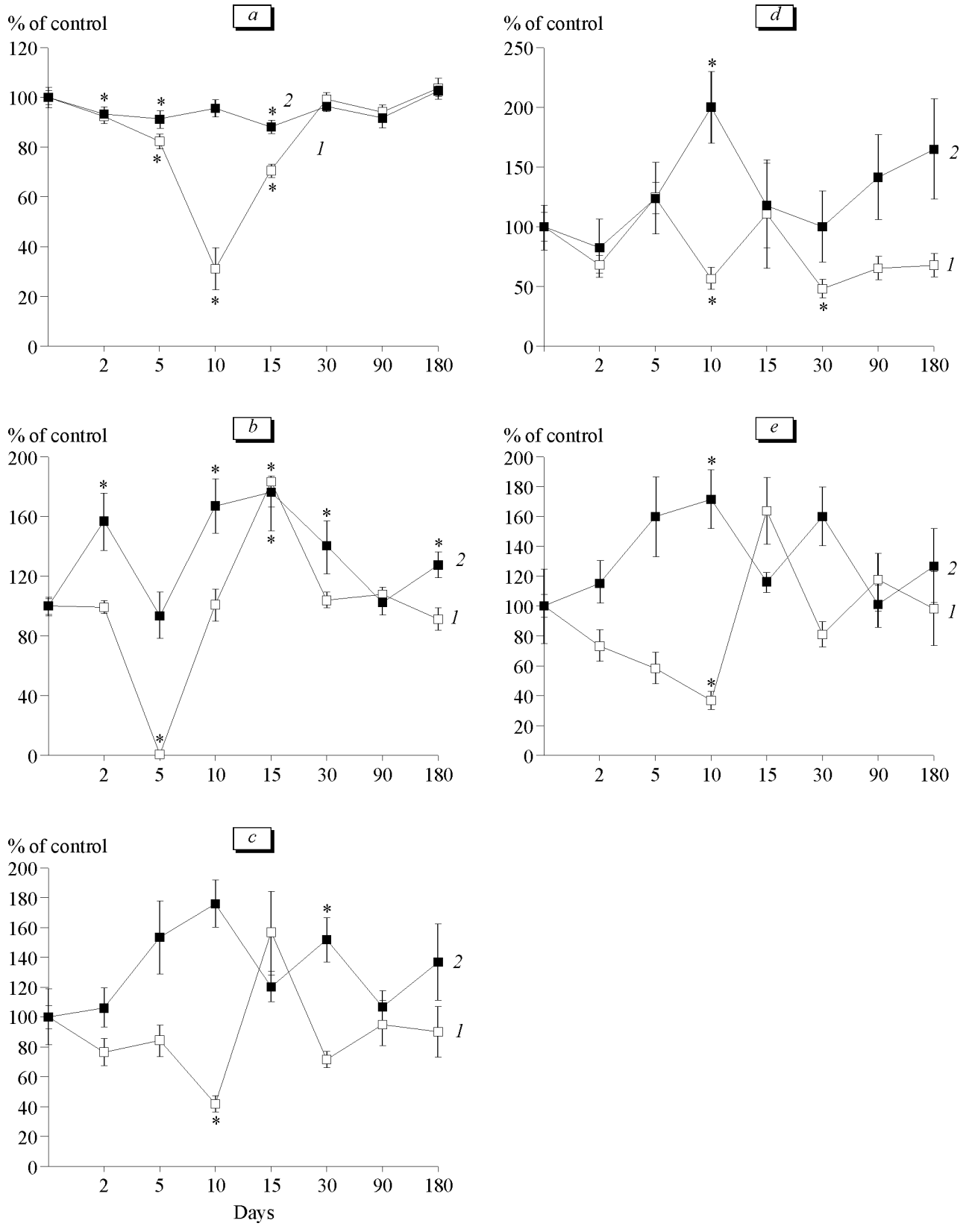

Fig. 1. Hematocrit (*a*), reticulocyte number (*b*), and total counts of leukocytes (*c*), segmented neutrophils (*d*), and lymphocytes (*e*) in the peripheral blood of rats receiving carboplatin (*1*) and platidiam (*2*) in MPD. Here and in Fig. 2: *$p<0.05$ compared to the control.

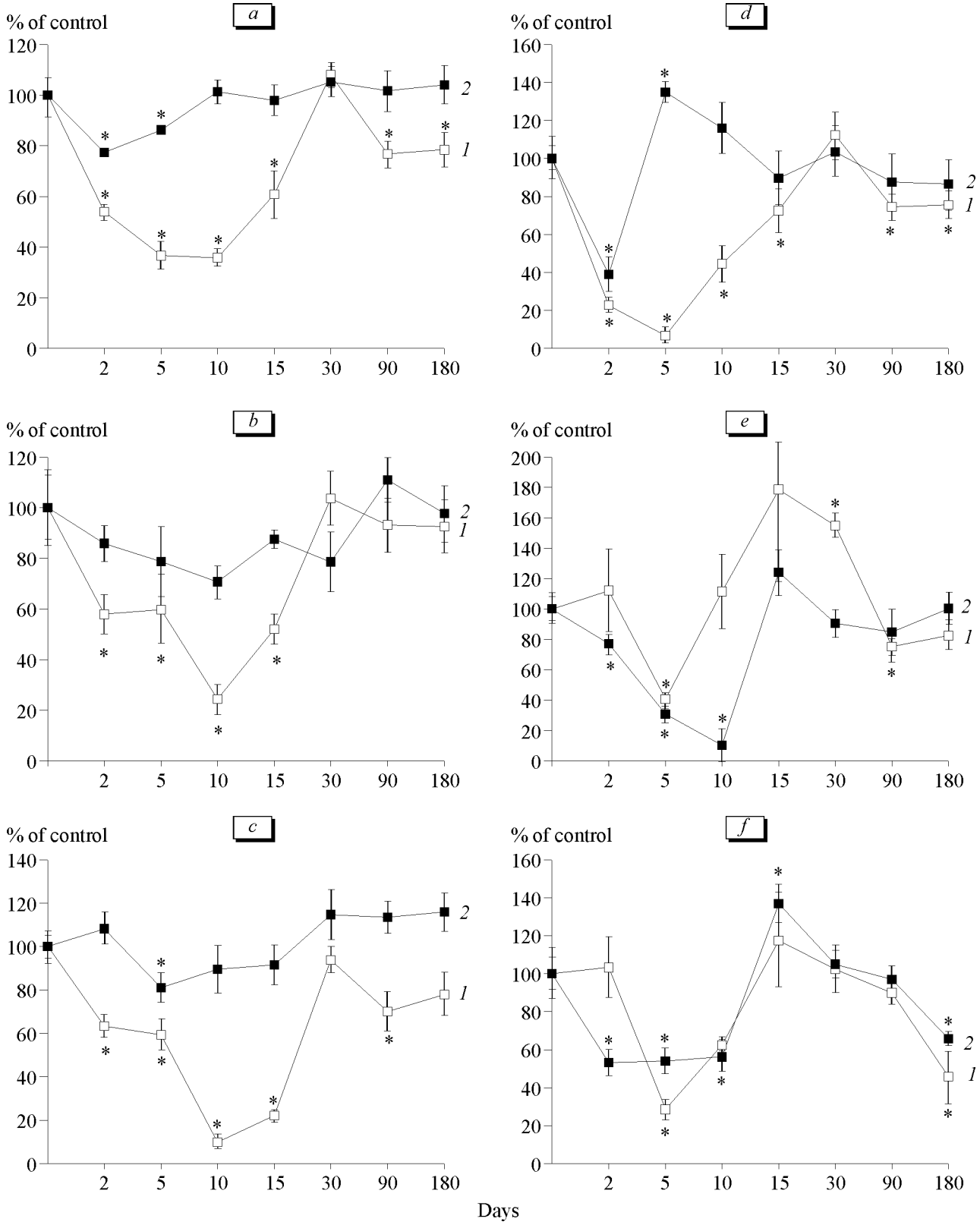

Fig. 2. Total count of bone marrow myelokaryocytes (*a*), immature (*b*) and mature neutrophils (*c*), erythroid cells (*d*), and lymphocytes (*e*) and weights of the thymus and spleen (*f*) in rats treated with carboplatin (*1*) and platidiam (*2*). *f*: weights of the thymus (*1*) and spleen (*2*) after carboplatin treatment.

cells and number of cells with deletions were estimated. We counted peripheral blood erythrocytes with micronuclei, which are hypothesized to develop from acentric chromosome fragments [3,8,12].

The results were analyzed by method of variation statistic [4].

RESULTS

We compared parameters of the peripheral blood and bone marrow in rats receiving carboplatin and cisplatin in MPD (platidiam, Lachema, 4 mg/kg intravenously, Figs. 1 and 2) [2].

In the early period after carboplatin injection (days 5-15) we observed a decrease in peripheral hematocrit (to 30% of the baseline level, day 10) and pronounced short-term reticulocytopenia (day 5, Fig. 1). Cisplatin moderately decreased hematocrit on days 2, 5, and 15, which was accompanied by an increase in the reticulocyte count. Recovery of erythropoiesis in both groups was accompanied by reticulocytosis (days 10-30). Leukopenia (leukocyte count 41.9% of the baseline) observed 5 days after carboplatin injection was associated with neutro- and lymphocytopenia. By contrast, cisplatin increased the count of leukocytes at this term. Platinoids produced similar effects on the bone marrow: total cellularity decreased due to low contents of immature and mature neutrophils, erythronormoblasts, and lymphocytes. In rats receiving carboplatin these changes were more pronounced and persisted for a longer time (Fig. 2). In the follow-up period (days 90 and 180), parameters of the peripheral blood in rats treated with carboplatin remained practically unchanged. The count of mature neutrophils tended to decrease. Regenerative anemia in animals receiving cisplatin was probably related to irreversible microcirculatory changes in the kidneys [2]. The decrease in bone marrow cellularity in carboplatin-treated rats was associated with low counts of mature neutrophils, erythronormoblasts, and lymphocytes. Cisplatin normalized parameters of the bone marrow.

Carboplatin decreased the weights of lymphoid organs in rats (Fig. 2, *f*). Cytological assay of the thymus revealed a moderate increase in the count of apoptotic cells and bodies 2 days after treatment. Histological examination of the thymus showed a decrease in the count of cells in the cortical and medullar zones, karyopyknosis and karyorrhexis of lymphocytes, and phagocytosis of dead cells by macrophages. Severe hypoplasia of the thymus (28% of the initial thymus weight, Fig. 3, *b*) was related to disappearance of small lymphocytes on day 5 after treatment. Depletion of the cortical zone was most pronounced, which caused zone inversion. Hassall's bodies were enlarged and contained cell detritus. The weight and cellularity of the thymus increased 10 days after carboplatin injection and did not differ from the control on days 30 and 90. The weight of the thymus in carboplatin-treated rats was below the control 180 days after treatment. Histological examination showed that thymus lobules decrease in size. The connective tissue developed between lobules and penetrated them, which impaired normal composition of the thymus (Fig. 3, *c*). The weight of the spleen decreased by 2 times on days 2-10. Histological examination revealed depletion of thymus-dependent zones, including the marginal zone of lymphoid follicles and periarteriolar lymphoid sheaths. Splenomegaly due to the formation of hemopoiesis foci was noted during the recovery period (day 15). On days 30 and 90 after treatment, the structure of the spleen did not differ from normal. On day 180 the weight of the spleen was lower than in the control due to lymphoid depletion of the red and white pulps.

Cytogenetic assay of the bone marrow in mice showed that the count of abnormal metaphases 24 h

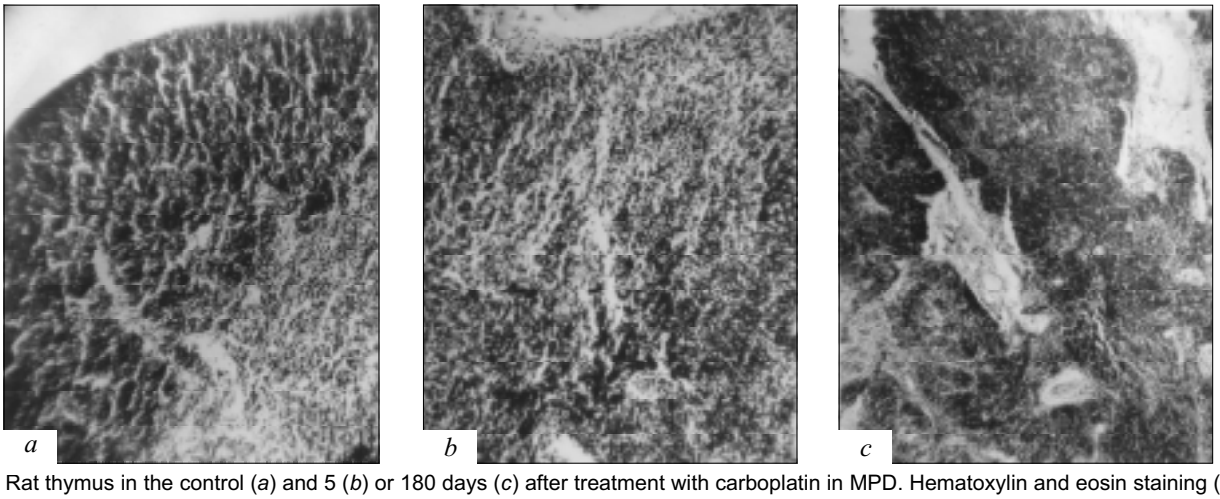

Fig. 3. Rat thymus in the control (*a*) and 5 (*b*) or 180 days (*c*) after treatment with carboplatin in MPD. Hematoxylin and eosin staining (×150). Cells depletion (*b*) and sclerotic and atrophic changes (*c*).

after carboplatin injection increased to 16.5±1.5% (*vs*. 2.3±0.9% in the control, $p<0.001$) primarily due to accumulation of chromosomal aberrations, while after 48 h this parameter did not differ from normal. The number of peripheral blood erythrocytes with micronuclei in mice increased 6-72 h after carboplatin administration and peaked 48 h after treatment (7.6±0.5 *vs*. 1.7±0.2‰ in the control, $p<0.01$).

Thus, single intravenous injection of carboplatin in MPD to BALB/c mice induces chromatid aberrations in the bone marrow and increases the count of peripheral blood erythrocytes with micronuclei. The preparation possesses higher myelotoxic activity than cisplatin. Intravenous injection of carboplatin in MPD to rats induced early reversible changes (2-30 days), including hyporegenerative anemia, leukopenia (neutro- and lymphocytopenia), thrombocytopenia, inhibition of granulocyto- and erythropoiesis, and hypoplasia of lymphoid organs, and produced a moderate apoptosis-inducing effect. Delayed effects of carboplatin (90 and 180 days) included moderate hypoplasia of erythro- and granulocytopoiesis and promoted involution of lymphoid organs.

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