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Some Effects of Chemotherapeutic Drugs

III. Short- and Long-Term Effects of *cis*-Platinum on Various Hematopoietic Compartments and on the Kidney of the Mouse

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Summary. *cis-Platinum is a relatively new active anticancer drug. In the study described in this paper, its toxicity was tested in the hematopoietic and renal systems of mice after six injections of 3 mg per kg body weight at 10-day intervals.*

Acute hematopoietic toxicity was studied by determining the survival of pluripotent (CFU-S) and granulo-macrophagic unipotent (GM-CFC) stem cells. The number of nucleated cells in the bone marrow and in the spleen and the number of granulocytes in the blood were determined.

Renal toxicity was studied by histological examination of kidneys from treated mice compared with control animals.

The number of stem cells in the bone marrow and in the spleen decreased during the treatment. One year after treatment, the autorepopulating ability of CFU-S was still diminished in spite of normal numbers of these cells.

No renal damage could be demonstrated by light microscopy when the protocol described was used.

Introduction

cis-Diamminedichloroplatinum(II) is one of the most important of the modern antitumor drugs, active mainly in cancer of the testis and ovary and, to a lesser degree, in carcinomas of the respiratory tract, cervix, and bladder, as shown by Rosencweig et al. [12].

Its main toxicity is renal, as reviewed by Madias and Harrington [10]. Clinically, renal failure is prevented by inducing diuresis with mannitol or furosemide [2].

Even though the drug has no apparent hematological effect, it seemed it would be interesting to determine the possibility of qualitative disturbances and late effect.

Few studies have so far been carried out on the hematopoietic tissue. A recent paper of Jenkins et al. [8] reported a marked reduction in the number of pluripotent stem cells per femur 24 h after two or four doses of *cis*-platinum; Ogawa et al. [11] demonstrated a greater toxicity of platinum in murine GM-CFC than in human GM-CFC; and Zak et al. [16] reported little change in the more mature bone marrow compartments.

In this study, the doses usually used for human patients were adapted for normal mice (clinical protocol: 3 mg/kg six times at 6-month intervals; adapted protocol for mice: 3 mg/kg six times at 10-day intervals) and the effects of the drug on various hematopoietic compartments (CFU-S, GM-CFC, bone marrow nucleated cells, and white blood cells) and the renal system of the mouse were studied 10 days after each injection and 1 year after the last of the six injections of 3 mg *cis*-platinum per kg body weight.

Materials and Methods

This study was performed in female or male CBA/01a SPF mice between 2 and 3 months of age, weighing about 20 g.

Each treated group or control group consisted of 30 or 40 mice. Each irradiated recipient group for each point in Fig. 1 and Tables 1–3 consisted of 10 mice. In all, 430 mice were used for these experiments.

cis-Platinum 0.06 mg (3 mg per kg body weight) was injected IP into each mouse six times at 10-day intervals. This dose is

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Abbreviations used in this paper: CFU-S, pluripotent hemopoietic stem cells assessed by the spleen colony technique; GM-CFC, granulo-macrophagic progenitor cells; BFU-E, erythroid progenitor cells; E/G ratio, ratio of erythroid and granulocytic colonies in the recipient spleen and assessed by histological examination; Ara-C, cytosine arabinoside

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equivalent to a dose of about 60 mg/m^2 in man, based on the conversion factor of Freireich et al. [5].

A diuretic (furosemide) was administered IP 30 min before each injection of *cis*-platinum (0.25 mg furosemide per mouse, 12.5 mg/kg).

Ten days after each injection, at the time of the next injection, the numbers of CFU-S, GM-CFC, and nucleated cells in the bone marrow of one leg and the spleen, and the number of white blood cells (WBC) per cubic millimeter of blood were assessed.

The number of CFU-S was determined by the technique of Till et al. [14]. Bone marrow nucleated cells (8×10^4) or spleen cells (10^6) were injected (IV) into mice previously given 9 Gy of whole-body ^{137}Cs irradiation. After a further 9 days, spleens of the recipient mice were harvested and fixed in Bouin's solution. The number of macroscopic colonies was determined. To determine CFU-S differentiation pathways, the spleens were analysed for the histological types of colonies and the ratio of erythroid colonies to granulocytic colonies (E/G ratio) was calculated.

The number of GM-CFC was determined by the in vitro technique of Worton et al. [15] with methyl-cellulose.

The autorepopulating capacity of the CFU-S compartment was determined by the technique of Hellman [1, 7]. This capacity can be expressed in terms of Rs, a measure of the self-renewal potential of the stem cell compartment. Rs is the ratio of the number of CFU-S recovered (after 14 days) in the hind limb bone marrow to the number seeding the hind limb prior to transplantation. Rs was determined 1, 4, 6, 12, and 18 months after the end of the treatment.

Evaluation of the drug effect on kidneys was assessed by testing for proteinuria and by histological examination. Kidneys obtained from three mice of each group were examined by light microscopy after fixation in Dubosq Brasil liquid. The kidneys were embedded in paraffin and cut at 2 μm. Masson's trichrome with light green, PAS, and silver impregnation according to Jones' method were always performed.

Results

Effects on the Hematopoietic Tissue

Ten days after each injection the number of medullary and splenic CFU-S fluctuated around 50% of control values (Fig. 1) after which the number of CFU-S increased slowly to reach normal values 18 months after the end of the treatment.

At 1, 4, 6, 12, and 18 months after the end of the treatment the Rs ratio was lower than the Rs ratio of untreated mice of the same age (Table 1).

No significant modification was observed in the E/G ratio of the spleen colonies generated by bone marrow from platinum-treated mice as compared with the E/G ratio of the spleen colonies generated by normal bone marrow (Table 2).

The number of GM-CFC 10 days after each injection was between 22% and 70% of control values; 1 year after the treatment the number of GM-CFC was about 85% of control values (Fig. 1).

During the treatment the number of WBC varied around the numbers found in controls (Table 3). A total recovery was observed 1 year after the end of the treatment.

No significant change in the number of bone marrow nucleated cells was observed during the treatment, whereas the number of splenic nucleated cells was decreased (Table 3).

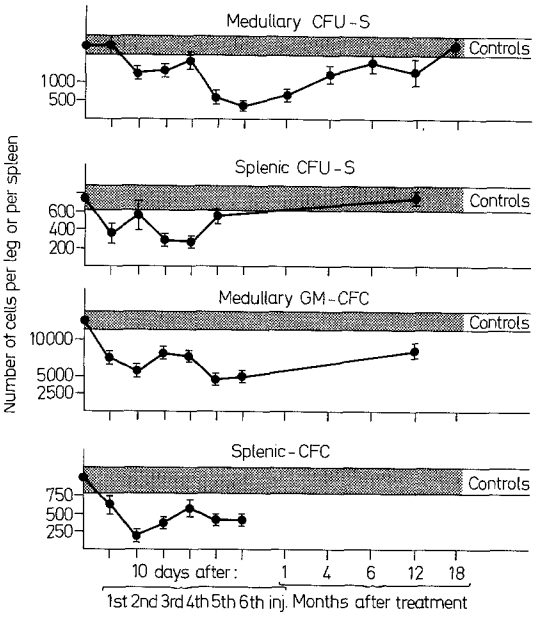

Fig. 1. Evolution of the number of medullar and splenic CFU-S and GM-CFC during the six injections of *cis*-platinum 1, 4, 6, 12, and 18 months after the sixth injection. (Means of two or three separate experiments ± SEM)

Table 1. Determination of the Rs ratio (number of CFU-S at the end of the transfer to number of CFU-S initially seeding the hind limb) at 1, 4, 6, 12, and 18 months after treatemnt

$$R^2 = \frac{\text{No. of CFU-S per hind limb (14 days)}}{K \times \text{no. of CFU-S injected}}$$

Time after six injections	Controls	Platinum-treated
1 month	28.6 ± 5.1	4.8 ± 0.7
4 months	31.7 ± 2.7	16.4 ± 0.3
6 months	36.0 ± 2.1	9.0 ± 0.3
12 months	24.4 ± 2.2	8.4 ± 1.3
18 months	38.5 ± 7.3	15.3 ± 0.2

Effects on Kidneys

There was no proteinuria in treated mice.

No significant morphological change was observed in kidneys from treated mice as compared with control animals. The glomerular mesangial axes were

Table 2. Determination of E/G ratios of spleen colonies generated by bone marrow from control and platinum-treated mice

	No. of nodules	No. of spleens		Eryth.	Granul.	Mega.	Undiff.	E/G ratio
Controls	75	8	Absolute number	43	17	11	4	2.5
			Percentage of total colonies	57.3	22.7	14.7	5.3	2.5
Platinum-treated, 10 Days after 1st injection	85	9	Absolute number	56	17	5	7	3.3
			Percentage of total colinies	66.0	20.0	5.9	8.2	3.3

Table 3. Evolution of the number[a] of medullar and splenic nucleated cells and the number[a] of white blood cells per millimeter of blood during and 1 year after treatment

	Nucleated cells				White blood cells/mm^3 blood	
	No. of nucleated cells in the leg × 10^6		No. of nucleated cells in the spleen × 10^8			
	Controls	Treated	Controls	Treated	Controls	Treated
10 Days after 1st injection	14.0	20.8 148%	1.5	1.4 93%	3,350 ± 649	4,033 ± 466 120%
10 Days after 2nd injection	17.9	17.7 99%	1.1	1.1 100%	8,766 ± 2,629	7,033 ± 896 80%
10 Days after 3rd injection	18.0	20.0 111%	1.1	1.0 87%	23,4000 ± 559	12,150 ± 849 52%
10 Days after 4th injection	18.0	12.6 70%	1.3	0.7 54%	5,866 ± 1,571	26,500 ± 4,290 167%
10 Days after 5th injection	18.0	16.3 90%	1.3	0.8 62%	6,016 ± 764	4,200 ± 635 70%
10 Days after 6th injection	14.0	16.1 115%	0.9	0.6 67%	16,400 ± 556	15,267 ± 930 93%
1 Year after 6th injection	20.3	17.0 85%	1.4	1.2 86%	8,500 ± 2,662	11,200 ± 1,228 132%

[a] Means of two at three separate experiments ± SEM

increased in both the normal and the treated mice examined 1 year after the last injection. In all groups, the tubules and the interstitial tissue looked normal in the cortex as well as in the medulla. The blood vessels were normal.

Discussion

The study of the effects of drugs on the hematopoietic stem cell compartments is of great importance, because it seems that hematologic complications, such as aplasia, residual injury, or secondary leukemia occur after cancer chemotherapy. The clinical studies with platinum [9, 13] have shown that blood toxicity is low: leuco- and thrombopenia reach a maximum of 30%, and the patients do not require supportive care. However, better understanding of the damage to the stem cell compartment may help to prevent acute and late effects.

The first observation to point out is that 1 year after treatment, even though the number of CFU-S is back to normal, the proliferative capacity of CFU-S in treated mice is lower than that of normal CFU-S. This fact is very important, because patients receiving a certain chemotherapy regimen, even if sufficient capacity for self-renewal is retained, may be extremely sensitive to later cytotoxic therapy.

Preliminary data seem to indicate that *cis*-platinum, in contrast to other drugs [6], has no effect on the determination of the CFU-S differentiation pathway, as 10 days after the first injection the E/G ratio of spleen colonies is the same as the ratio in spleen colonies generated by normal CFU-S.

There is a striking correlation between the response of the stem cell compartment in the bone marrow and in the spleen; the spleen does not seem to compensate for the modifications observed in the bone marrow. On the contrary, splenic nucleated cells seem to be more sensitive than bone marrow nucleated cells.

Another point to note is the parallel decrease in the number of pluripotent stem cells and the number of stem cells committed towards granulopoiesis while the number of WBC does not significantly change. Thus, WBC counts seem to give little indication of the effect of drugs on the stem cell compartments, as we have already shown [3, 4].

During this protocol, the *cis*-platinum administration did not produce appreciable renal damage according to light microscopy examination. This is probably due to the massive diuresis induced before the treatment. However, reversible acute renal failure related to acute tubular necrosis was observed in patients receiving *cis*-platinum. In these cases the renal toxicity depended upon the hydration conditions and could be prevented by hyperdiuresis.

In conclusion, *cis*-platinum administered with furosemide has no apparent effect on the kidney as judged by histological analysis. *cis*-Platinum has an effect on CFU-S kinetics 10 days after each injection. The number of CFU-S returns to normal by 1 year after the end of the treatment, but the qualitative characteristics of these cells are modified. One year after the end of the treatment the repopulating capacity of CFU-S is decreased compared with that in controls of the same age.

There does not appear to be any leukemia, but this should be confirmed as the number of animals tested 1 and 2 years after treatment is not sufficient to allow a definite conclusion.

In view of these results it seems necessary to investigate the stem cell compartment of patients to assess the complete integrity of the hematopoietic tissue after *cis*-platinum treatment, especially for the late effects and subsequent therapy.

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