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Adverse effect of *cis*-diamminedichloroplatinum II (CDDP) on porphyrin metabolism in man

C. G. Alexopoulos, G. Chalevelakis, C. Katsoulis, and G. Pallikaris

Second Department of Internal Medicine, Athens University, Evangelismos Medical Center, 45, Ipsilandou Street, 10676 Athens, Greece

Summary. The possible effect of cisplatin on porphyrin metabolism was studied in 25 patients with various malignancies treated with high-dose *cis*-diamminedichloroplatinum. Haematocrit, red blood cells, haemoglobin, white blood cells, platelets and reticulocytes together with coproporphyrin and protoporphyrin in red blood cells were determined before each course of chemotherapy in all patients. In addition, coproporphyrin, uroporphyrin, δ-aminolevulinic acid, and porphobilinogen were determined in the urine just before and 24 h after each course of treatment.

Cisplatin administration was followed by a significant suppression of coproporphyrin and protoporphyrin in red blood cells and coproporphyrin, uroporphyrin, δ-aminolevulinic acid and porphobilinogen in urine. The changes observed paralleled similar changes in haematocrit, red blood cells and haemoglobin, strongly suggesting that cisplatin-induced anaemia may be due to a blocking effect of the drug affecting one or more enzymatic steps in the biosynthesis of porphyrins and haem. A moderate fall in the white blood cell count and a mild fall in platelets together with a steady increase of reticulocytes were also observed during treatment.

Introduction

cis-diamminedichloroplatinum II (CDDP) was the first heavy-metal-containing complex found to have significant antitumour activity in man [13]. It is now considered the chemotherapeutic agent of choice in the treatment of testicular tumours and ovarian adenocarcinomas [2]. Despite its considerable nephrotoxicity and neurotoxicity, CDDP is a moderately myelotoxic agent. Its myelosuppression concerns mainly the erythroid series [17], although no satisfactory explanation for this relative selectivity has so far appeared in the literature. Indeed, there is a striking lack of information on the possible effect of CDDP on the haemoglobin metabolism.

We therefore decided to study the metabolism of porphyrins in patients treated with high-dose CDDP, and our results are presented and discussed below.

Methods

Patients. Twenty-five patients with various malignancies were included in the study. There were six men and nineteen women with a mean age of 51 years (16–68 years). Sixteen patients had ovarian carcinoma (stage III and IV, FIGO); two, testicular cancer; four, lung cancer; one, uterine cervical carcinoma; one, pleural mesothelioma; and one, laryngeal carcinoma.

Chemotherapy. The only chemotherapeutic agent used throughout the study was CDDP, given according to a uniform schedule of 100 mg/m^2 IV every 4 weeks. Eight patients received a total of two courses each; ten patients, three courses; five patients, four courses; one patient, five courses; and one patient, six courses.

Parameters studied. (a) Haematocrit, red blood cells, haemoglobin, white blood cells, platelets, and reticulocytes were estimated in the peripheral blood of the patients, and coproporphyrin and protoporphyrin in the red blood cells. For these purposes 15 ml venous blood was drawn from each patient into a heparinized tube just before each course of chemotherapy.

(b) Coproporphyrin, uroporphyrin, δ-aminolevulinic acid (δ-ALA), and porphobilinogen (PBG) were determined in the urine of the patients. For this purpose a urine sample was collected in a dark bottle just before and 24 h after each course of chemotherapy.

Methods. (a) The method described in 1956 by Mauzerall and Granick [10] was used for quantitative determination of PBG and δ-ALA in the urine, and the results are expressed in international (SI) units.

(b) Coproporphyrin and uroporphyrin in the urine were quantitated according to the method of Rimington [12]. The results are again expressed in international (SI) units.

(c) For the quantitative determination of coproporphyrin and protoporphyrin in red blood cells we used the method described by Rimington et al. in 1963 [11], and the results are expressed in SI units.

Statistics. For the statistical analysis the following tests were used: (a), *t*-test; (b) x^2-test; (c) parametric correlation test.

Results

Haematological parameters

A steady decrease in the values for haematocrit, haemoglobin and number of red blood cells with increasing num-

Offprint requests to: C. G. Alexopoulos

Table 1. Observed changes in haematological parameters, during treatment with cisplatin, in 25 patients who received more than one courses

Parameter studied	Increased	Decreased	Stable	X^2	*p* value	End result
	(Observed N°/Total N°)					
Haemocrit	1/25	23/25	1/25	38,7	$p<0.001$	Decrease
Red cells	2/25	22/25	1/25	33,7	$p<0.001$	Decrease
Haemoglobin	2/25	23/25	0/25	39,0	$p<0.001$	Decrease
Platelets	11/25	7/25	7/25	1,3	$p<0.3$	No significant change
White cells	4/25	18/25	3/25	16,9	$p<0.001$	Decrease
Reticulocytes (absolute N°)	21/25	3/25	1/25	35,7	$p<0.001$	Increase

ber of chemotherapy courses was observed in 23 of 25 patients (Table 1), and this decrease was statistically highly significant ($P<0.001$). The observed decrease was calculated at approximately 8% per course of treatment.

A statistically highly significant ($P<0.001$) decrease in the white blood count was also demonstrated (Table 1), with a diminution of more than $2\times10^9/l$ in seven cases (28%) and a diminution of between $0.5\times10^9/l$ and $2\times10^9/l$ in further ten cases (40%).

Platelets (Plts) demonstrated no significant changes ($P<0.3$) during chemotherapy (Table 1).

It is of interest that the absolute number of reticulocytes demonstrated a significant ($P<0.001$) increase during chemotherapy in 21 of the 25 (84%) patients (Table 1).

In Fig. 1 the mean values for haematocrit, haemoglobin, red blood cells and reticulocytes are plotted against the number of chemotherapy courses given. There is a negative correlation between haematocrit, haemoglobin, red blood cells, and number of courses, while reticulocytes demonstrate a positive correlation with the number of treatments.

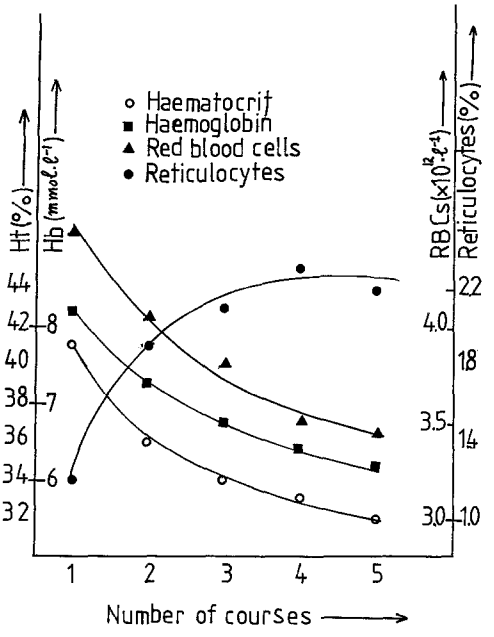

Fig. 1. Mean values for haemoglobin (Hb), haematocrit (Ht), red blood cells (RBGs) and reticulocytes, plotted against the number of chemotherapy courses in 25 patients who each received more than one course of chemotherapy

Porphyrins and their precursors

a) Porphyrins in the blood. Protoporphyrin levels fell significantly during treatment with CDDP in 23 of the 25 patients (92%) while it increased in the other 2 patients (Table 2). Coproporphyrin also decreased significantly in 15 of the 25 patients (60%), while it increased gradually in 8 patients. It remained stable throughout treatment in 1 patient, and in another 1 it increased before the sixth course, having initially decreased (Table 2). Statistical analysis showed that the decrease was significant for both protoporphyrin and coproporphyrin ($P<0.001$ and $P<0.005$, respectively).

b) Porphyrins and their precursors in urine. Urinary samples for porphyrins and their precursors were analysed both before and after each course of chemotherapy. We were therefore able to observe changes due first to the direct effect of a single drug administration and secondly to the additive effect of consecutive courses of chemotherapy.

Table 2. Observed changes in porphyrins and their precursors, during cisplatin treatment, in 25 patients who received more than one courses

Parameter studied	Increased	Decreased	Stable	X^2	*p* value	End result
	(Observed N°/Total N°)					
Urinary δ-ALA	4/25	20/25	I/25	25,0	$p<0.001$	Decrease
Urinary PBG	2/25	23/25	0/25	39,0	$p<0.001$	Decrease
Urinary CP	0/25	25/25	0/25	50,0	$p<0.001$	Decrease
Urinary UP	0/25	25/25	0/25	44,2	$p<0.001$	Decrease
Blood CP	9/25	15/25	1/25	11,8	$.002<p<.005$	Decrease
Blood PP	2/25	23/25	0/25	39,0	$p<0.001$	Decrease

Table 3. Observed changes in urinary porphyrins and their precursors, before and after each course, in 25 patients who received a total number of 77 courses

Parameter studied	Increased	Decreased	Stable	X^2	*p* value	End result
	(Observed N°/Total N°)					
Urinary δ-ALA	6/77	68/77	3/77	104,9	$p<0.001$	Decrease
Urinary PBG	6/77	59/77	12/77	65,6	$p<0.001$	Decrease
Urinary CP	1/77	76/77	0/77	148,1	$p<0.001$	Decrease
Urinary UP	3/77	71/77	3/77	120,1	$p<0.001$	Decrease

δ-Aminolevulinic acid. A total of 77 courses of chemotherapy were administered during the study, and in 68 the values for δ-ALA were found to be significantly lower (an average of 30%) 24 h after injection of CDDP than before administration. In six courses the values were higher after the injection, while they remained unchanged in three other courses (Table 3). The incidence of decrease is highly significant ($P<0.001$).

Consecutive administration of CDDP produced a cumulative effect on urinary δ-ALA, which fell progressively in 20 of the 25 (80%) patients. It increased in 4 patients, while it remained practically stable in 1 (Table 2). The incidence of decrease proved to be highly significant at $P<0.001$ (Fig. 2A).

Porphobilinogen. Urinary PBG was found to be significantly (an average of 42%) lower 24 h after CDDP injection than before administration in 59 of the total of 77 courses administered. In 6 courses PBG was increased after the injection, while it remained unchanged in 12 courses (Table 3).

Consecutive administration of CDDP also produced a cumulative effect on PBG, which demonstrated a progressive decrease in 23 of the 25 (92%) patients (Table 2, Fig. 2A).

Coproporphyrin and uroporphyrin. The values for coproporphyrin were found to be significantly (an average of 28%) lower 24 h after CDDP injection in 76 of the 77 courses. It was higher in only 1 course of treatment (Table 3). Similarly, the uroporphyrin level was lower (an average of 29%) after CDDP injection in 71 of the 77 courses administered. It increased in 3 courses and remained unchanged in another 3 (Table 3). The incidence of decrease was highly significant ($P<0.001$) for both porphyrins. Consecutive courses of treatment produced a cumulative effect on the

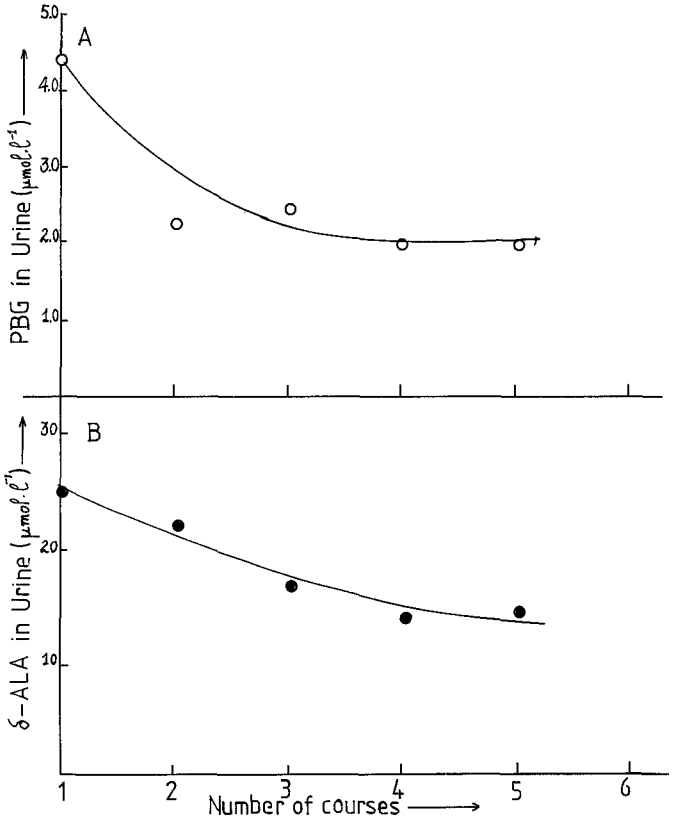

Fig. 2A, B. Observed changes in mean values of urinary PBG **(A)** and δ-ALA **(B)** during CDDP treatment in 25 patients who each received more than one course

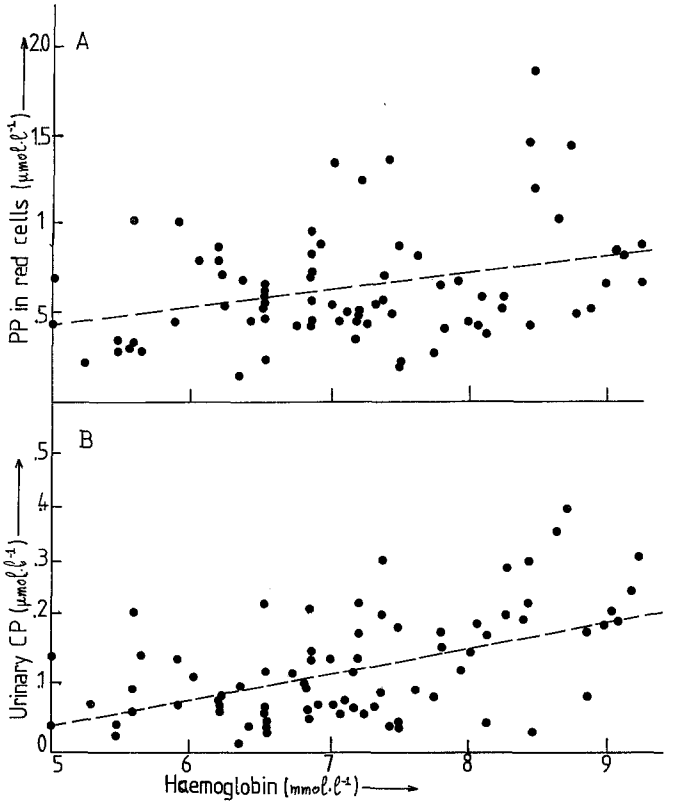

Fig. 3. **A** Red cell protoporphyrin/(PP) and **B** urinary coproporphyrin (CP) plotted against haemoglobin level during 77 courses of CDDP treatment

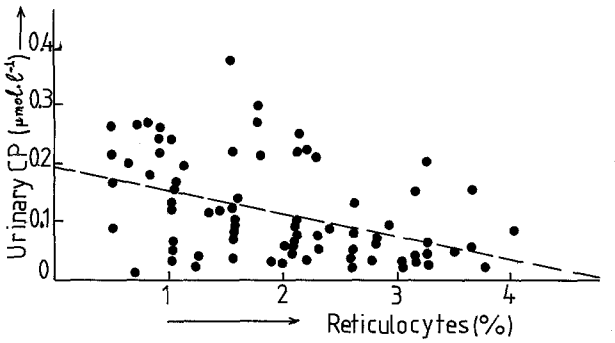

Fig. 4. Urinary Coproporphyrin (CP) versus reticulocyte number during 77 courses of CDDP treatment

urinary excretion of both porphyrins, since both protoporphyrin and uroporphyrin decreased progressively during chemotherapy in all 25 patients (Table 2).

The individual values for red blood cell protoporphyrin and urinary coproporphyrin in all 77 courses of chemotherapy were plotted against the corresponding values for haemoglobin and reticulocytes (Fig. 3 and 4). It was demonstrated that as the treatment progressed fall in haemoglobin was associated with a decrease in red cell protoporphyrin and urinary coproporphyrin. These correlations were shown to be statistically significant ($r=0.305$, $P<0.01$) and highly significant ($r=0.529$, $P<0.01$), respectively (Table 4). In contrast reticulocytes showed an inverse relation to the urinary coproporphyrin (Fig. 4), and this proved to be highly significant ($r=0.430$, $P<0.001$; Table 4).

Discussion

Progressive anaemia, not infrequently requiring blood transfusion, in the absence of severe leukopenia and thrombocytopenia is a well-established side effect of CDDP treatment [3, 17]. This relative selectivity for the erythroid series in toxicity suggests that anaemia following CDDP chemotherapy must be due at least to an additive mechanism, apart from the nonspecific myelosuppression seen with most antineoplastic agents. Nevertheless, very little work has been directed at clarification of the mechanism responsible for the CDDP-induced anaemia in humans. Rossof et al. hypothesized that nephrotoxicity might be responsible for a reduced production of erythropoietin [14]. The observation that vigorous hydration ameliorates kidney toxicity [6] without reducing myelotoxicity [4] suggests that this hypothesis is probably not the true explanation.

Haemolytic anaemia has been reported during CDDP treatment [7]. Nevertheless, haemolysis must be a very rare phenomenon with CDDP treatment, while our findings on reticulocytic changes during treatment – as discussed below – suggest that haemolysis cannot be in any way an important factor in the pathogenesis of anaemia.

Most recently Rothman and Weick [15], using in vitro cultures of bone marrow from a patient who developed severe nonhaemolytic anaemia during CDDP treatment, demonstrated very poor growth of stem cells, mainly affecting the erythroid series. Those findings show a selectively suppressive effect of CDDP on erythroid precursors, but do not offer any explanation for the mechanism involved. We believe that our present findings concerning the effects of CDDP on the metabolism of porphyrins are quite relevant from this point of view.

CDDP administration was followed by a significant suppression of coproporphyrin and protoporphyrin in red blood cells, and of coproporphyrin, δ-ALA, and PBG in urine. More specifically, coproporphyrin demonstrated a steady decrease from course to course in 92% of patients, while protoporphyrin decreased in 60% of cases.

In urine where coproporphyrin, uroporphyrin, δ-ALA and PBG were estimated before and 24 h after CDDP administration, a 30%–40% reduction in their values was found in 78%–97% of total courses.

A cumulative effect of the drug was also demonstrated, since δ-ALA showed a steady decrease from course to course in 80% of patients and PBG in 92% of patients, while coproporphyrin and uroporphyrin decreased progressively in 100% of cases. A statistically significant inverse relation was found between all the above parameters and the number of chemotherapy courses, and their decline had a tendency to plateau after the third course of treatment.

The observed changes in the values of red blood cell coproporphyrin and protoporphyrin and urinary coproporphyrin, uroporphyrin, δ-ALA and PBG paralleled similar changes in haematocrit, haemoglobin and red blood cells. Thus, CDDP administration was followed by progressive anaemia in 92% of patients, with an average haemoglobin reduction of 8% per course of treatment. This contrasts with the changes in white blood cell count, which demonstrated a significant decline in only 32% of patients, and platelet number, which showed only a mild decrease in 28% of cases.

The positive correlation found between the changes in the various parameters of porphyrin metabolism and the changes in haemoglobin, haematocrit and red blood cells strongly suggests that CDDP-induced anaemia may be due to a blocking effect of the drug affecting one or more enzymatic steps in the biosynthesis of porphyrins and haem.

CDDP exerts its antineoplastic effect mainly by way of a selective and permanent inhibition of DNA synthesis [5] and of interchain and intrachain cross-linking of the compound with DNA molecules [13].

Table 4. Comparison between various parameters studied during cisplatin treatment, in a total of 77 patient-courses

Compared parameters x–y	a	b	r	*p* value
Retics-Urinary CP	.251	–.050	–.4305	$p<0.001$
RBCs-Urinary CP	–.229	9.8×10^{-14}	.547	$p<0.001$
Hb-Urinary CP	–.191	.030	.529	$p<0.001$
Hb-Blood PP	–.029	.095	.305	$0.001<p<0.01$

It is therefore very likely that a similar effect on the DNA of bone marrow cells results in reduced synthesis of mRNA molecules responsible for the synthesis of various enzymes involved in the biosynthesis of haem, namely δ-ALA synthetase (δ-ALAS), δ-ALA dehydratase (δ-ALAD), uroporphyrinogen synthetase (UROS) and ferrochelatase.

Progenitors of erythroid cells and proerythroblasts are cells with a high capacity for synthesis of DNA-mRNA molecules specialized in haemoglobin biosynthesis [1], and they could be primary cellular targets among the bone marrow series.

On the other hand, CDDP exists in the body in a dynamic equilibrium with cationic species formed through a series of aquation reactions, exemplified in the following equation [8]:

$$cis-\left[Pt(NH_3)_2Cl_2\right]^0 \underset{}{\overset{+H_2O}{\rightleftharpoons}} cis-\left[Pt(NH_3)_2(H_2O)Cl\right]^+$$

$$+Cl^- \overset{+H_2O}{\rightleftharpoons} cis-\left[Pt(NH_3)_2(H_2O)_2\right]^{2+}+Cl^-$$

Experimental work in mice has demonstrated that effective blockade of δ-ALAD, UROS and ferrochelatase activity takes place in the presence of PT^{2+} and Pt^{4+} ions [9]. It is therefore possible that, apart from the DNA-mRNA mediated inhibition in porphyrin synthesis, haem biosynthesis can be further inhibited through a direct effect of the cationic species of cisplatin on several enzymes.

Either of these mechanisms would eventually lead to inhibition of the proliferation and differentiation of erythropoietic cells and a decrease in the haemoglobin concentration in red cells. Furthermore, the direct effect of cationic species on the enzymatic synthesis of haem would provide a reasonable explanation for the relative selectivity of cisplatin myelotoxicity towards the erythroid series. The observed cumulative toxicity of the drug during the course of treatment can be easily explained by the well-known fact that cisplatin remains in human body for many days [16].

Caution must nevertheless be exercised when our results concerning porphyrins and their precursors in urine are interpreted, since it is possible that some of the changes observed are due to an effect of cisplatin on haem metabolism in the liver. Finally, the steady increase in reticulocytes during treatment might either represent an effort of the bone marrow to compensate for the induced anaemia or be the result of a subclinical haemolysis. The degree of the observed increase was nevertheless disproportionately small compared with the degree of anaemia, which makes the second explanation most unlikely.

On the basis of our findings, we believe that the following conclusions are reasonable:

1. Cisplatin-II administration is followed by a moderate degree of myelosuppression because of a damaging effect at the stem cell level. This type of myelosuppression affects the erythroid myeloid and megakaryocytic series.

2. Erythropoietic cells must suffer additive selective damage related to their highly specialized function of haemoglobinogenesis. This selective damage explains our constant finding of a significant decrease in all parameters involved in the various stages of porphyrin and haem biosynthesis.

3. Cisplatin may induce a cumulative effect on porphyrin/haem biosynthesis during the course of treatment.

4. Haemolysis during cisplatin chemotherapy cannot be an important factor in anaemia.

Although it seems very likely that the effect of cisplatin on porphyrin metabolism is due to a blockade of various enzymatic steps, the precise enzyme(s) blocked and the precise nature of the damage can be only determined by studying the enzymatic activity of the various enzymes involved in porphyrin/haem biosynthesis in the erythropoietic cells of patients treated with cisplatin in vitro. Such a study is already under way in our laboratory.

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