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Cisplatin-induced anemia: a potential interference with iron metabolism at erythroid progenitors level

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To elucidate the potential mechanisms of anemia induced by cisplatin (CDDP) we have evaluated hemolysis, dyserythropoiesis, ferrokinetics and cytotoxicity on erythroid progenitors in 12 patients treated by a CDDP-containing combination chemotherapy and in 6 patients treated by a similar combination but without CDDP. Eight patients, from the CDDP treated group, experienced a pronounced anemia. None had signs of hemolysis. Ferrokinetic study showed a very deep and protracted decrease of ^{59}Fe incorporation during the chemotherapy cycle and the following 2 weeks. These results, along with a normal medullary erythroblastic cellularity, suggest that CDDP induces a deep but transient erythropoiesis alteration leading to anemia in some cases.

Key words: Anemia, cisplatin, erythroid progenitors.

Introduction

Cisplatin (CDDP) is an alkylating agent used alone or in combination chemotherapy for the treatment of various tumors, especially ovarian and testis carcinoma. CDDP toxicity is essentially nephrologic and neurologic and the most constant secondary effects are nausea and vomiting.[1]

Hematological toxicity is mild but the appearance of a deep anemia has been described many times and related in most cases to an immunologic mechanism.[2,3] However, much experimental data and the lack of signs of hemolysis in several cases argued for another mechanism.[3,4] We therefore studied the potential mechanisms of anemia, i.e. hemolysis, dyserythropoiesis and cytotoxicity, on erythroid progenitors in 12 patients treated with a CDDP combination chemotherapy for an ovarian carcinoma. To assess the exact role of CDDP we compared these patients with others treated with a similar combination but without CDDP.

Patients and methods

Patients

Twelve patients (aged between 34 and 73 years; mean 49.7 years) were treated by CAP (CDDP 20 mg/m^2 i.v. over 30 min days 1–5, adriamycin 50 mg/m^2 i.v. day 1, cyclophosphamide 500 mg/m^2 i.v. day 1; one cycle every 3 weeks) for an ovarian carcinoma (stage III or IV according to FIGO classification) and a control group of 6 patients were treated by FAC (5-fluorouracil 500 mg/m^2 i.v. day 1, adriamycin 50 mg/m^2 i.v. day 1, cyclophosphamide 500 mg/m^2 i.v. day 1; one cycle every 3 weeks) for adjuvant chemotherapy of breast cancer.

None of these patients was anemic before the initiation of chemotherapy.

The following parameters were studied for each patient prior to starting the chemotherapy cycle: complete blood and differential count (automatically determined with COULTER ST4), and hemolysis signs—free bilirubin, lacticodehydrogenase (automatically determined with automat

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Hitachi 717), iron level (direct method with automat VP Abbott), haptoglobin level (immunonephelmetric method automatized with BNA Behring), Coombs' test in the presence and absence of CDDP, erythropoietin level (immunoenzymatic technique), research of unstable hemoglobin (Carrel and Kay's test) and electrophoresis of hemoglobin, and reticulocyte count (manual technique).

Bone marrow study

Bone marrow was collected by sternal puncture, at day 1 of the second or subsequent cycle of chemotherapy for cytologic examination and for colony forming unit erythroid (CFU-E) assay. Culture was made in the presence of erythropoietin (1 IU/ml) according to the plasma clot technique described by McLeod *et al.*[5] The final cell concentration was 5×10^5/ml. The score of CFU-E is established after 7 days of culture.

Ferrokinetic study

The ferrokinetics and red blood cell (RBC) survival were studied with a simplified protocol. Briefly, 10 ml of autologous citrated plasma were incubated with 20 μCi of ^{59}ferrous citrate (^{59}Fe) and injected i.v. together with 10 ml of a suspension of autologous RBC labeled with 30 μCi of sodium ^{51}chromate.

Blood samples were obtained 10, 30, 60, 120 min and 8 days after injection.

The ^{59}Fe and ^{51}Cr radioactivity of both plasma and whole blood samples was measured in a well-type gamma scintillation counter, and the plasma iron pool, plasma iron turnover, RBC Fe uptake at day 8, and RBC mean life-span were calculated.[6]

This study was performed during the chemotherapy cycle for 2 patients and after the cycle for 10 patients (for 3 patients during the 2 weeks after the cycle, and for 7 patients during the third week).

Results

Blood examinations

Among the 12 patients treated by CAP, 8 patients presented a pronounced anemia (Hb < 6.5 mmol/l) (CDDP anemia group) and 4 patients did not present anemia (Hb > 7 mmol/l) (CDDP non-

Table 1. Biological characteristics of patients

Patient no.	Hb (mmol/l)	Mean red cell volume (N = 85–95 μ3)	Reticulocytes $\times 10^3$/mm^3	Free bilirubin (N < 15 μmol/l)	Haptoglobin (N = 1–2 g/l)	Iron level (N = 9–27 μmol/l)	Seric ep (N = 29–50 mU/ml)
CDDP group with anemia							
1	6.5	101	120	8	2.1	17.6	nd
2	5.9	92.5	56	6	4	5.8	nd
3	4.6	93	15	9	0.3	28.8	37.5
4	5.7	87	21	5	2.4	8.3	18
5	6.4	87	91	5	3	3.2	150
6	5.4	105	60	5	0.3	16	18
7	5.5	96	26	4	2	14.2	nd
8	6.3	75	20	8	2.8	6.8	29
CDDP group without anemia							
9	7.1	99.4	21	6	2.6	14.4	120
10	7.2	91.7	35	6	1.4	15.7	30
11	7.4	93.9	19	6	2.4	14	nd
12	6.8	80	230	6	1.8	21.2	150
Control group							
13	8.8	89	101	8	1.3	12.6	nd
14	7.9	102	18	5	2.1	10.6	nd
15	7.9	94.5	28	7	2	16.5	nd
16	9	94	112	5	1.4	22.5	nd
17	8.9	86	19	12	1	9.7	nd
18	9.4	100	17	5	1.1	25.9	nd

Abbreviations: Hb, hemoglobin; ep, erythropoietin; nd, not done.

anemia group). No anemia was observed in the control group. Table 1 shows the characteristics of all the ovarian cancer and control groups. None of the patients had signs of hemolysis and there was no erythrocyte CDDP sensitization demonstrable by Coombs' test. Unstable hemoglobin was not detected.

Hemoglobin electrophoresis displayed an increase of HbF in 4 of the 8 patients in the anemia group.

Bone marrow study

Cytological examinations. Cellularity was normal or slightly decreased in 7 of the 8 patients in the CDDP anemia group, in 3 of the 4 patients in the CDDP non-anemia group, and in all patients of the control group (Table 2). When we observed a lowering of cellularity, this existed in all series (erythroblastic, myeloid, megacaryoblastic).

In 3 of the 8 patients in the CDDP anemia group we observed dyserythropoietic changes—large size erythroblasts with nuclear cytoplasmy asynchrony (so-called early megaloblastic changes).

These three cases have no particular relationship to the number of previous chemotherapy cycles and were not associated with a deficit of seric vitamin B_{12} or folic acid. In one case we observed myeloid cell changes (cells without granulation) associated with erythroblastic changes.

We did not observe such modification in the CDDP non-anemia and control groups.

Study of CFU-E. Study of CFU-E displayed insignificant differences between the three groups. The higher number of CFU-E compared to the normal values in some cases (4 of the total of 18 patients) could be explained by the fact that the bone marrow sample was collected shortly after hematological recovery. But in 6 cases we observed a pronounced decrease in CFU-E probably due to the cytotoxic effect of chemotherapy.

Ferrokinetics in CDDP-treated patients

During the chemotherapy cycle, plasmatic iron turnover decreased and the incorporation of ^{59}Fe was undetectable (Table 3).

Table 2. Results of bone marrow study

Patient no.	Bone marrow			Number of CFU-E in presence of ep ($N = 800–1500$)
	Cytology	Cullularity	Percentage of erythroblast	
CDDP group with anemia				
1	Dys	↘	25.6	250
2	N	N	20.6	0
3	Dys	↘	7.2	1040
4	N	N	20	1440
5	N	N	47.2	1250
6	N	N	32	2375
7	Dys	↘↘	44.6	1235
8	N	N	26	100
CDDP group without anemia				
9	N	↘	34.4	2185
10	N	N	25.6	2807
11	N	↘↘	21.2	15
12	N	N	37.2	1082
Control group				
13	N	N	21.7	1213
14	N	↘	30.8	955
15	N	N	14.6	1122
16	N	N	31.2	1925
17	N	N	11.6	275
18	N	N	24.8	455

Abbreviations: N, normal; dys, dyserythropoiesis; ↘, slightly decreased cellularity; ↘↘, pronounced decreased cellularity; ep, erythropoietin.

Table 3. Ferrokinetics study

Case study	Plasmatic iron level (μg/ml) $N = 60–150$	Plasmatic iron pool (mg) $N = 3.5–4.7$	Half life of plasmatic iron (min) $N = 70–110$	Plasmatic iron turnover (mg/day) $N = 35–55$	Percentage of ^{59}Fe erythrocytic incorporation (%) $N = 80$	Movement of iron to hemoglobin (mg/day) $N = 28–44$	Half life of erythrocytes (day) $N = 26–30$
During chemotherapy cycle							
1	95	2.1	187	11.3	0	0	29
2	334	4.6	345	13.4	0	0	7
During the 2 weeks after the cycle							
3	161	3.2	214	15	18	2.7	18
4	222	5	236	21.2	18	1.6	30
5	258	4.7	304	15.3	10.4	3.8	23
During the third week after the cycle							
6	205	5.9	70	83.8	91	76	nd
7	89	1.8	120	15.2	99	15	21
8	138	2.9	58	49.8	57	28.4	20
9	65	2	49	40.9	100	40.9	30
10	54	1.6	56	30.2	81	24.4	nd
11	113	3.2	90	35.6	88	31.3	nd
12	118	4.5	195	23	85	19.5	25

Abbreviation: nd, not done.

During the 2 weeks after the chemotherapy cycle the plasmatic iron turnover was still decreased (about one-third of normal value) and incorporation of ^{59}Fe was very low.

Three weeks after the cycle, plasmatic iron turnover and incorporation of ^{59}Fe returned to normal value.

In all cases, plasmatic iron pool and RBC survival was normal or slightly decreased, except in case 2, where we observed that RBC survival was deeply shortened.

Discussion

The occurrence of anemia during CDDP therapy has been frequently reported since the first clinical trials;[7] this anemia is usually mild but increases with the number of chemotherapy cycles.

In the present study the patients were not treated by CDDP alone. We observed cases of severe anemia in patients treated by a CDDP-containing combination chemotherapy; such a deep anemia was not observed in control patients treated by a similar combination without CDDP. This observation is an argument for the direct implication of CDDP in the genesis of this type of anemia.

Occult hemorrhages are a frequent event in cancer patients but the plasmatic iron level, the lack of microcytosis and of blood spoliation permit the exclusion of this mechanism in the genesis of anemia.

Some authors[2,3] have described several cases of anemia secondary to RBC sensitization induced by CDDP. We did not observe such cases of hemolytic anemia among our patients and it seems that this mechanism is real but infrequent.

As CDDP is eliminated renally and displays an important nephrotoxicity, a defect of erythropoietin synthesis has been advocated as an alternative explanation for the genesis of CDDP-induced anemia.[8] Such a quantitative defect can be excluded in our study as the measure of erythropoietin was normal or very slightly decreased in all patients; also, a qualitative defect of the erythropoietin receptor seems unlikely according to the normal growth of CFU-E, *in vitro*, in the presence of exogenous erythropoietin in most patients.

Our ferrokinetic study showed a very deep and protracted decrease of ^{59}Fe incorporation during the chemotherapy cycle. These results contrast with normal cellularity and the normal CFU-E score in bone marrow; they suggest a direct and early interaction of CDDP with iron supply to erythroblastic precursors, together with a lowering of cell proliferation, and eventually maturation defect as suggested by the morphologic dyserythropoietic changes.

It has been shown, *in vitro*, that cells surviving after exposure to CDDP may retain very durable cytokinetic alteration,[8] and a cytotoxic effect of CDDP on erythroblastic precursors has also been described by Rothmann *et al.*[9,10] So the repetition of CDDP administration could induce, in some patients, accumulative decrease in the erythroblastic pool leading to a deep anemia.

Pollera *et al.*[11] has observed an important increase of ferritin and a pronounced fall in the reticulocyte count during the chemotherapy cycle with CDDP. Sykes *et al.*[12] and Stjernholm *et al.*[13] have shown that CDDP binds transferrin in a sufficient quantity to reach saturation. These data along with our results suggest that the CDDP cytotoxic effect on erythroid cells could result in an interaction between CDDP and the cellular transferrin receptor but further studies with labeled CDDP are necessary to demonstrate this possible mechanism.

Conclusion

The results of this study suggest that:

- CDDP-induced anemia proceeds from a central mechanism.
- This anemia is not due to a quantitative or a qualitative defect of erythropoietin synthesis.
- CDDP may exert a direct and early interference on the iron supply to erythroblasts.

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