Cisplatin Toxicity: Effects on Phagocyte Functions*

RUGGERO FUMARULO
Institute of General Pathology, University of Bari, Italy
and MICHELE ARESTA
Department of Chemistry, University of Bari, Italy

Abstract

The *in vitro* effects of cisplatin on human polymorphonuclear functions were studied in order to establish if the drug is able, during chemotherapy, to induce an immune depression of host response and an increased susceptibility to infections. All the phagocytic functions tested were negatively influenced, but only chemotaxis was really depressed by cisplatin used in therapeutic concentrations (from 10^{-5} to 10^{-6} M). This finding was confirmed in chemotactic tests performed on leukocytes elicited from cancer patients after cisplatin intravenous administration. The binding to the tubulin sulfhydryl groups and the inhibition of microtubule polymerization have been proposed to explain how the drug affects cytoskeleton activities.

Introduction

During recent years *cis*-diamminedichloroplatinum(II) (cisplatin) has become a potent chemotherapeutic agent for the treatment of a variety of human tumors. The primary mechanism by which it prevents tumor growth appears to be binding to DNA and inhibition of nucleotide synthesis. However other mechanisms have been reported, such as: (i) enhancement of spontaneous monocyte-mediated cytotoxicity; (ii) activation of NK cells effector function; (iii) increase of the antigenicity of tumor cells, which suggests some cisplatin host stimulatory properties [1]. On the other hand, cisplatin, when given in high doses, can induce a suppression of both humoral and cell-mediated immune response and depress the growth of T and B lymphocytes [2]. To extend the field of the cisplatin immune influence, we have investigated its effects on phagocytosis, the quick reaction of the so-called professional phagocytes (granulocytes and monocytes) against bacteria. This investigation was suggested by the observation that cisplatin chemotherapy, among the other toxic side effects, may be complicated by a myelosuppression. This is noteworthy because the increased incidence of clinical infections during cytostatic therapy may be caused by a leukopenia subsequent to the myelosuppression. Our studies have attempted to verify if cisplatin is able to induce also a functional modification of the phagocytes besides leukopenia (non-immediate cytotoxic effect). The object of our investigation was the polymorphonuclear leukocyte (PMN): this cell is highly specialized for the performance of its primary function phagocytosis and destruction of microorganisms. All the functional stages of activated PMN (chemotaxis, engulfment, degranulation, respiratory burst, bacterial killing) were studied on human blood polymorphonuclear leukocytes exposed to cisplatin *in vitro* or elicited from cisplatin-treated cancer patients.

Experimental

Chemicals

Cisplatin, prepared according to Kauffmann and Cowan [3], was dissolved in Krebs-Ringer phosphate solution (KRP), pH 7.4, to a concentration of 1 mg/ml. Further dilutions were effected in KRP to obtain cisplatin concentrations ranging from 10^{-5} to 10^{-6} M, corresponding to plasma concentrations (half-life of ~0.4–0.5 h) obtained after administration of 100 mg/m^2 of cisplatin by rapid intravenous injection [4]. Higher, but not therapeutic, drug concentrations (up to 10^{-3} M) were used *in vitro* to investigate the different drug sensitivities of the various PMN functions tested. *N*-Formylmethionylleucylphenylalanine (FMLP), phorbol myristate acetate (PMA), zymosan, phenolphthalein glucuronate and cytochalasin B were purchased from Sigma Chemicals.

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Methods

Cell suspensions: leukocytes containing 90–93% neutrophils were prepared from fresh blood of healthy donors by dextran sedimentation and centrifuging through Ficoll/sodium metrizoate. The cells were freed from erythrocytes by hypotonic lysis, washed twice and resuspended in calcium-free, 5 mM glucose KRP. All these procedures were carried out at 4 °C. Cell viability was determined by monitoring lactate dehydrogenase release and by Trypan-blue exclusion test.

Chemotaxis was evaluated by a modified Boyden chamber technique [5]. Endotoxin-activated serum was used as chemoattractant. Endocytosis was tested by microscopic examination of leukocyte suspension challenged with opsonized zymosan particles (0.5 mg/10^7 PMNs) after 15 min of incubation at 37 °C. Phagocytosis frequency (PhF) and index (PhI) were calculated.

Degranulation was measured by the release of β-glucuronidase (enzyme marker of azurophil granules) from cells untreated or pretreated with cytochalasin B (5 μg/ml) by monitoring the nmoles of phenolphthalein released after 5 h of incubation with phenolphthalein glucuronate at pH 5.2 [6].

Respiratory burst was evaluated as oxygen consumption determined by polarography using a Clark oxygen electrode (Yellow Spring Inst.) attached to a thermostated (37 °C) plastic vessel in which 10^7 PMNs in 2 ml KRP containing 1 mM KCN and 0.5 mM $CaCl_2$ were magnetically stirred [7]. PMA (20 ng/ml) and FMLP (1 μM) were the stimulatory agents.

Bacterial killing was performed by challenging 2×10^7 PMNs with 3×10^6 opsonized *Escherichia coli* in 2 ml KRP for 60 min at 37 °C. After incubation the cells were washed twice, lysed and inseminated in nutrient agar on Petri plates: the colony growth observed after 24 h was the expression of killed bacteria.

In order to study the *in vitro* effects of the drug, PMNs were suspended in cisplatin concentrations ranging from 10^{-6} to 10^{-3} M (corresponding to 0.2–195 μg Pt/ml, as evaluated by atomic absorption spectroscopy). After 15 min of incubation at 37 °C with the drug, the cells were centrifuged at 800 g, washed twice and resuspended in KRP. An untreated cell fraction was used as control. This method of incubation was followed for the evaluation of all the functions tested but chemotactic tests were performed after cisplatin incubations ranging from 5 to 30 min, at 4 °C and 37 °C.

Patients

Six patients affected by ovarian carcinoma were selected for cisplatin chemotherapy. No patient showed any clinical or laboratory evidence of bacterial infection that could alter the phagocytic activities. We evaluated for each patient the chemotactic activity of blood PMNs elicited immediately before and 24 h after i.v. cisplatin administration (100 mg/m^2).

Results

The results reported express the mean of five determinations for each experimental condition. Figure 1 gives the chemotactic responsiveness of PMNs exposed to increasing cisplatin concentrations (preincubation for 15 min at 37 °C). An evident decrease in chemotactic activity is already observed at 10^{-6} M cisplatin. The effects of this concentration for different times and temperatures of preincubation are depicted in Fig. 2: the inhibition is more pronounced at 37 °C than at 4 °C and is dependent on preincubation time. Figure 3 depicts the chemotactic activity of PMNs elicited from venous blood of patients just before and 24 h after i.v. injection of

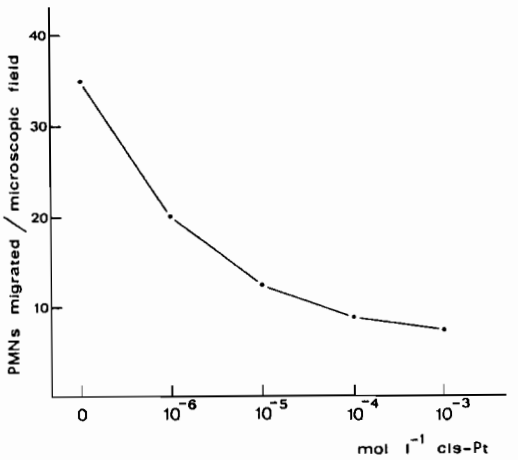

Fig. 1. The effect of cisplatin on chemotaxis: dose dependence.

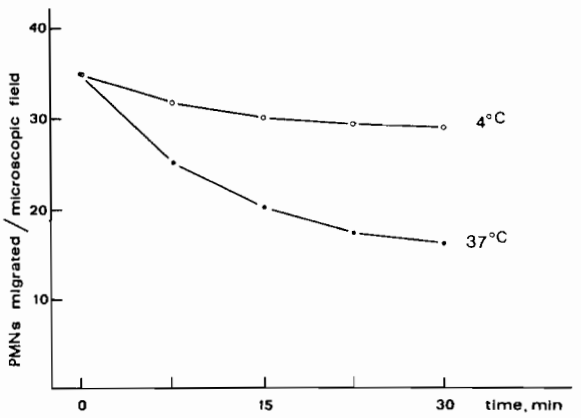

Fig. 2. The effect of 10^{-6} mol cisplatin on chemotaxis: time and temperature dependence.

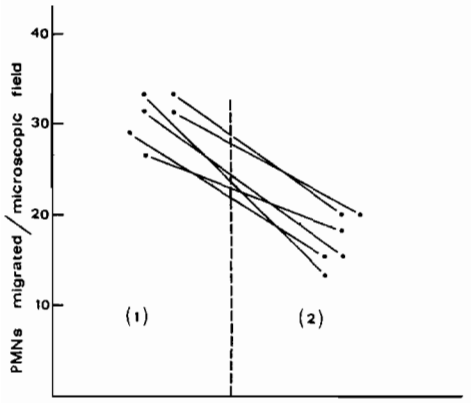

Fig. 3. Chemotaxis of PMNs elicited from patients just before (1) and 24 h after (2) an i.v. injection of cisplatin (100 mg/m^2).

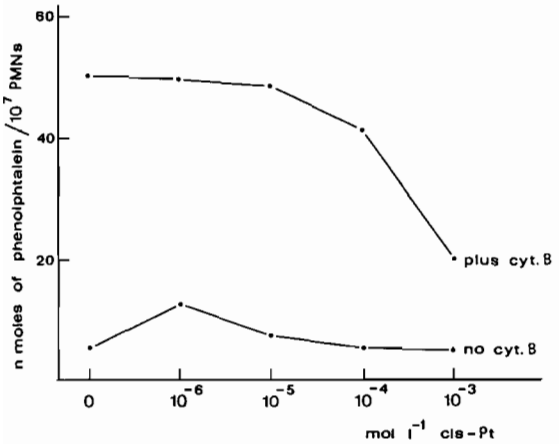

Fig. 4. The effect of cisplatin on β-glucuronidase degranulation: Influence of cytochalasin B.

TABLE I. The Effect of Cisplatin on Frequency (PhF) and Index (PhI) of Phagocytosis

Cisplatin	PhF	PhI
0	90	4
10^{-6} mol	90	4
10^{-5} mol	90	4
10^{-4} mol	87	4
10^{-3} mol	73	2

cisplatin, 100 mg/m^2. The 'preincubation' *in vivo* of PMNs with the drug re-exhibits the same effect as *in vitro*.

Table I presents the variations of frequency and index of engulfment of opsonized zymosan particles. Only PMNs preincubated with 10^{-3} M cisplatin show a clear decrease with respect to controls.

Figure 4 shows the spontaneous β-glucuronidase exocytosis after the incubation with cisplatin alone

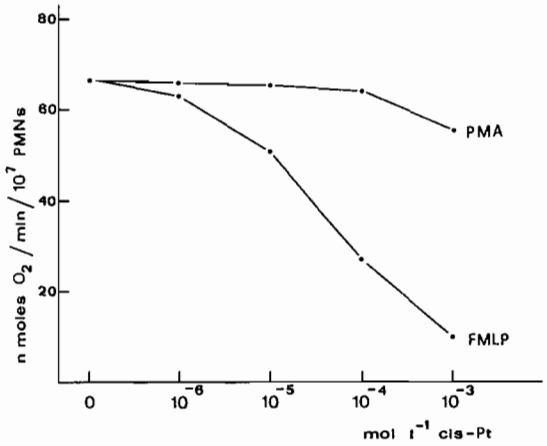

Fig. 5. the effect of cisplatin on oxygen consumption by PMA and FMLP.

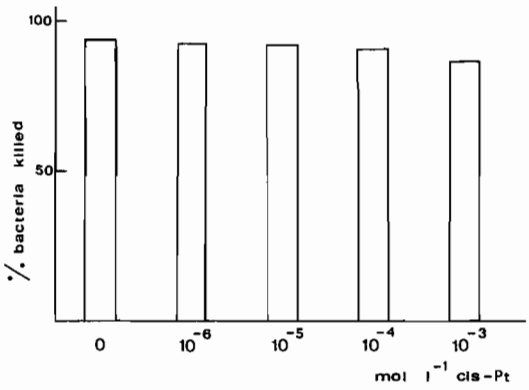

Fig. 6. The effect of cisplatin on bacterial killing.

or with cisplatin plus cytochalasin B, a fungine metabolite which enhances spontaneous enzyme release by interference with the cytoskeleton. A clear inhibition is observed only for 10^{-4} M cisplatin preincubation in cytochalasin B treated cells while, in the absence of cytochalasine B, a low drug concentration even stimulates the enzyme release.

Figure 5 presents the oxygen consumption of PMNs preincubated with the drug and then stimulated with PMA or FMLP. The respiratory burst is less inhibited than chemotaxis by the drug, but the depressive effect is more evident when the formylpeptide is used as stimulatory agent: the same concentration of cisplatin (10^{-4} M) inhibits (about 60%) the effect of FMLP, while it does not affect the stimulation by PMA, which is clearly depressed only for a very high drug dose.

The effect of the drug on bacterial killings is depicted in Fig. 6: this activity is hardly depressed and only by high cisplatin concentrations. Figure 7 shows a synoptic picture of the effects produced on phagocytes preincubated for 15 min at 37 °C with the highest cisplatin plasma levels attainable

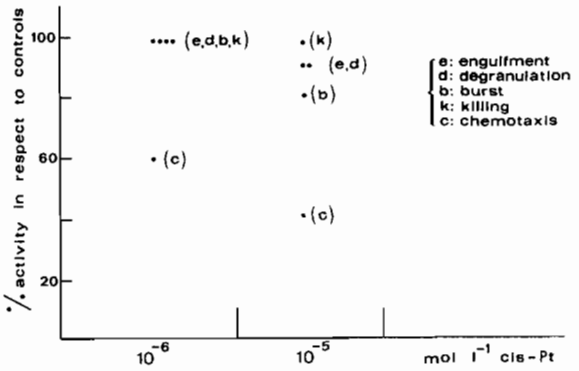

Fig. 7. Comparative effects of plasma therapeutic cisplatin levels on human phagocyte functions '*in vitro*'.

during chemotherapy (from 10^{-5} to 10^{-6} M). Only chemotaxis is depressed, while the other PMN activities are practically unaffected.

All these results are expressed in terms of viable cells and none of these inhibitions can be ascribed to cell necrosis. In fact, the Trypan-blue exclusion test indicates cell viability as high as 90–95% after incubation with the drug. Moreover, the release of lactate dehydrogenase in the extracellular medium was constantly less than 7% of the total enzymatic activity for controls and cisplatin-treated cells.

Discussion

It is our opinion that cisplatin inhibition of phagocyte function recognizes different mechanisms with respect to its prevention of tumor growth. We gained information by a study of chemotactic activity observed under various experimental conditions. First of all, cisplatin inhibits PMN functions only if the cells have been preincubated with the drug: no significant change was observed on adding cisplatin to cell suspensions just before the assay. The inhibition shows a clear dependence on time and temperature of preincubation with the drug. This finding suggests an intracellular point of action, which is supported by a report [8] on a temperature-dependent transport across the cell membrane of a platinum compound [*cis*-dichloro(dipyridine)platinum-(II)^{3}H] similar to cisplatin. But, it is also necessary to hypothesize some binding of cisplatin to cell membrane because a light inhibition was observed after preincubation at 4 °C, when the transport across the membrane is very small. Moreover the inhibition is not short-lived: washing the cells did not reverse the effects of the drug. Similar results have been reported in peritoneal rat macrophages [9] and in human monocytes [10].

With regard to the site of action of the drug, we think that it is possible to explain most effects of cisplatin by suggesting that the cytoskeleton is the main target of the drug. The cytoskeleton is a dynamic structure (formed by a system of microfilaments and microtubules) responsible for the movement of the cell *in toto* and for the arrangement of the intracellular organules. This microfilament network, by the reversible contractility of an actomyosin gel arranged just beneath the cell membrane, induces all the shape changes (lamellipodium, uropode, pseudopodes) and is the real machinery of the cell locomotion and phagocytosis. The microtubules are polymers of a protein, tubulin, assembled by associated microtubule proteins. In PMN stimulated by a chemotactic factor, the microtubules are seen to radiate from the centriole towards the microfilament-enriched poles, thus leading the direction of the movement. Recently the ability of cisplatin to inhibit *in vitro* the microtubule polymerization has been shown [11] according to contact time and temperature, although this reaction was still possible at 4 °C: in our experiments the chemotaxis shows a slight decrease after drug incubation at low temperatures. This mechanism of inhibition can be due to the ability of cisplatin to strongly bind to tubulin by an irreversible reaction through covalent binding to sulfhydryl group sites [12]: after 1 h contact time at 27 °C with 2.5×10^{-4} M cisplatin, no microtubules were detected and a reduction was found in the number of sulfhydryl groups for the tubulin dimer. It is well known that the process of microtubule assembly needs free sulfhydryl groups. The effect of the microtubule system can explain the specific inhibition of the directional movement of the PMN: in fact, only chemotaxis, but not chemokinesis (non-directional movement in the absence of a chemical concentration gradient of the stimulus), is affected by cisplatin preincubation (data not shown). It is difficult to explain the inhibition of zymosan engulfment; a mechanism dependent on the contractility of the microfilament system has been reported even with alteration by microtubular binding agents [13]. A cisplatin effect on the microtubular system has also been suggested for the inhibition of degranulation. The drug could act in the same fashion as colchicine and vinblastine by a microtubular disruption. However, in order to point out the inhibition of β-glucuronidase release, it was necessary to pretreat the cells with cytochalasine B which disrupts the microfilaments by preventing the actin/ABP (actin binding protein) bond [14]. In the absence of this fungine metabolite and at low concentrations, cisplatin even shows a stimulation of enzyme release. This finding parallels again the behavior of colchicine and vinblastine [15] which are able to induce, at low doses, a linear organization of microtubules, while at higher concentrations they cause depolymerization. The microtubule system seems to play a lesser role in the influence of cisplatin on the respiratory burst: this non-mito-

chondrial respiration of the phagocytes is due to the activation of a membrane oxidase characterized by electron transport from NADPH to oxygen along flavins and a cytochrome *b* which, during stimulation, translocate from specific granules to plasma-membrane. Even if the biochemical purification and characterization of this enzyme are not yet clear, it can be inhibited by drugs able, as cisplatin is, to bind to –SH groups [16, 17]. The burst by FMLP is inhibited more quickly than that by PMA, probably because FMLP acts on the external part of the plasma-membrane while PMA acts on its hydrophobic nucleus [18, 19]. A cisplatin binding to –SH groups of the cell membrane can depress more efficiently the stimulation of the formyl peptide. The drug did not show any noteworthy effect on bacterial killing. This is not surprising because, besides the dependence on the respiratory burst (hardly depressed by cisplatin), the killing also occurs by a non-oxidative mechanism not influenced by the drug.

In conclusion, we can affirm that cisplatin can affect in a different fashion all the phagocyte functions but, when used in a concentration comparable to that of plasma levels observed during cancer chemotherapy, only chemotaxis is really depressed. This result parallels the polymorphonuclear chemotactic inhibition in patients after cisplatin administration. Chemotaxis inhibition is, however, noteworthy because the directional movement is the first step of the functional chain which leads the leukocyte to the sites of infection or inflammation where it carries out all the other functions. Therefore we think that our data, without undermining the great usefulness of cisplatin as an antineoplastic drug, may contribute in explaining a possible increase in infection susceptibility of the treated patients and suggest a more accurate prophylaxis from infectious agents during administration of the drug.

Acknowledgements

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