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Modification of Macrophages Chemotaxis Caused by *cis*-$Pt(NH_3)_2Cl_2$ (40841)

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It is known that the compound *cis*-$Pt(NH_3)_2Cl_2$ is active against a wide range of tumors (1) in animals (2–4) and in man (5, 6), and it has been shown that its activity is decreased when immunosuppressive drugs are also administered, whereas it is increased when an immunostimulating agent, e.g., Zymosan (7), is given. Platinum compounds have been found to act as immunosuppressive agents. Indeed, CISPLATIN inhibits phytohemagglutinin (PHA)-induced lymphocyte blastogenesis as well as antibody plaque-forming spleen cells in mice. In addition, it has been reported that CISPLATIN inhibits PHA induced lymphocyte blastogenesis in man both *in vitro* and *in vivo;* in mice, it also suppressed graft rejection and graft versus host reactions (8).

On the other hand, it has been emphasized that the immune system can play an important role in the pathogenesis of tumor growth (9). Actually, the importance of cell-mediated reactions in the host defense against tumors is well established (10), and the possible role of macrophages in such a cell-mediated response to, or control of, carcinogenesis has been discussed (11). In this paper we present the results of our studies on the modification of macrophage chemotaxis, one of the vital functions of the monocyte, caused by *cis*-$Pt(NH_3)_2Cl_2$.

Material and methods. Albino Wistar rats weighing 200 ± 15 g were used throughout the experiments. Macrophages were obtained by collecting the peritoneal exudate (about 70% of macrophages) 3 days after a peptone solution was injected ip. The cells were washed three times with sterile isotonic saline solution by centrifugation at 800 rpm and suspended in TC Medium (DIFCO) obtaining 3×10^6 cells cm^{-3}. Chemotaxis was studied by using the Boyden chamber technique (12) as modified by Jungi (13). *cis*-$Pt(NH_3)_2Cl_2$ was prepared according to Kaufmann (14). A 10^{-3} *M cis*-$Pt(NH_3)_2Cl_2$ solution in water was prepared and calculated amounts of this solution were added to the macrophages suspension (overall volume 5 cm^3) in order to obtain Pt concentrations ranging from 1.5 to 63 μg Pt cm^{-3}. The effective concentration was evaluated by atomic absorption spectroscopy. Fresh water solutions were used for each run in order to repress the formation of aquated species.

Forty rats divided in eight groups were used. For *in vitro* tests the exudates of each group of five were collected and mixed, and the pool obtained was divided in several fractions: all of these but one were treated with the drug for the reported time at the given temperature. The untreated fraction was used as control. After the contact time had elapsed, the macrophages were separated from the medium, washed twice in order to eliminate the residual drug, suspended in a new medium and chemotaxis was evaluated as reported above.

In order to exclude that a diminished chemotatic response might be due to a cell damage caused either by the drug or by the manipulation process, viability of the macrophages was controlled by using trypan blue. Only at concentration of Pt higher than 65 ppm did cell damage become evident. The effect of the drug *in vivo* has been investigated by evaluating the chemotactic response of macrophage obtained from the peritoneal exudates of 20 rats treated for 4 days with *cis*-$Pt(NH_3)_2Cl_2$ at a rate of 8 mg kg^{-1} day^{-1} (the overall 4-day dose corresponds to ca. 20 ppm of Pt *in vitro* in our experimental conditions).

Results. Figure 1 gives the depressive influence of the concentration of *cis*-$Pt(NH_3)_2Cl_2$ on the macrophage migration.

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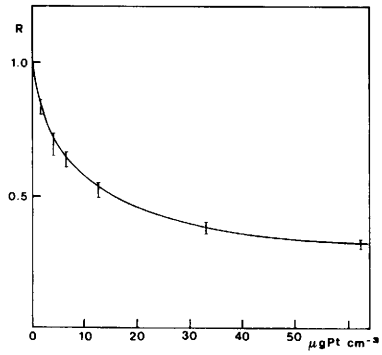

FIG. 1. Ratio of the number of macrophages migrated in the presence of *cis*-$Pt(NH_3)_2Cl_2$ to the number of macrophages migrated in the absence of the drug. Macrophages incubated with the drug for 30 min at 37°C. Standard deviation: $\sigma = 3.9 \times 10^{-2}$.

R represents the ratio of the number of the macrophages migrated in the presence of the drug to the number of macrophages migrated in the absence of the drug (time of contact: 30 min). Standard mean deviations are reported.

The results of the experiments *in vivo* show a defective response of macrophages obtained from treated rats.

Figures 2 and 3 give the effect of contact time and temperature on the reduction of macrophages chemotactic response.

Discussion. The results of our experiments show that the compound *cis*-$Pt(NH_3)_2Cl_2$ depresses the macrophages

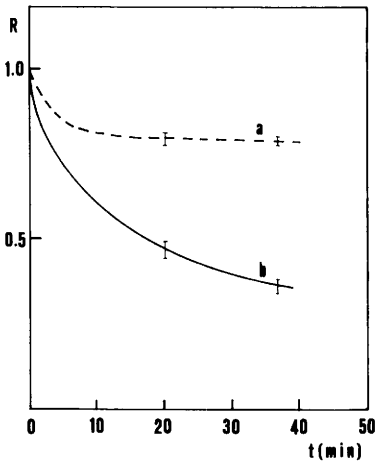

FIG. 2. Effect of time of incubation on the macrophages chemotaxis: – – –, macrophages incubated at 0°C; ——, macrophages incubated at 37°C. σ (a) = 4.8×10^{-4}; σ (b) = 4.4×10^{-2}.

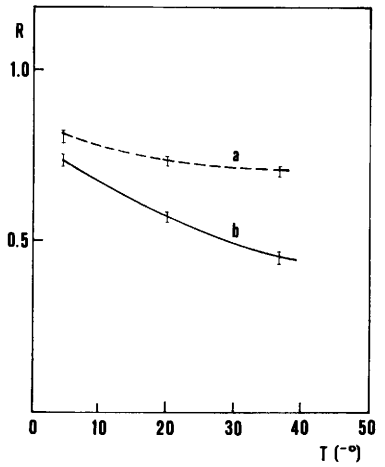

FIG. 3. Effect of temperature of incubation on the macrophages chemotaxis: – – –, macrophages incubated for 5 min; —— macrophages incubated for 30 min. σ (a) = 2.28×10^{-2}; σ (b) = 6.14×10^{-2}.

chemotactic function and such a depression is strongly concentration dependent in the range 0–50 ppm of Pt. It is noteworthy that at concentrations as low as 5 ppm the chemotactic ability is reduced to about 60%. At concentrations higher than 65 ppm, cell damage is evident and the macrophages lose their viability. It is, therefore, not meaningful to evaluate the chemotaxis beyond that concentration. It should also be emphasized that patients with neoplastic diseases (prostate carcinoma, renal and bladder carcinoma, matastatic diseases (15–18)) present a defective macrophage chemotaxis, the defect being related to the poor host defense and to the growth of tumors. It is of relevance, thus, that *cis*-$Pt(NH_3)_2Cl_2$ used in the chemotherapy of several tumors (e.g., testicular and ovarian cancers (19–20), leukemia (21), certain head and neck cancers (22) and other tumors), is itself an agent that can depress the macrophage function.

At the moment it is difficult to assert the mechanism by which *cis*-$Pt(NH_3)_2Cl_2$ or other Pt and Pd complexes inhibit the macrophage chemotaxis. The metal could either act on the cell surface or else interfere with intracellular processes. It is ascertained that CISPLATIN is able to bind the nucleic acid and to proteins (–SH groups?) of the membrane surface (23). At the moment it

seems unlikely that the Pt action is related to an interaction with the macrophage surface constituents.

Considering that the metabolism of the macrophages is strongly temperature dependent (much slower at low temperature than at 37°C) we have studied the temperature and time dependence of the defective chemotactic response. We have used in this study a concentration of 33 ppm of platinum, which was shown to produce a marked chemotactic defect without cell damage. Figures 2 and 3 show that both time of contact and temperature can play an important role on the macrophage response, but the effect of temperature is much more important. These results seem to support the view that Pt does not act on the surface of macrophages (as the temperature variation would not produce such a dramatic change in the Pt–surface constituents interaction), but it does interfere with intracellular processes and, thus, activity is strongly dependent on the concentration level achieved in the cell. As a passive transport can be foreseen for CISPLATIN through the cell membrane (24), it must be concluded that the Pt concentration level in the cell is dependent on the phagocytosis process, which is strongly temperature dependent.

In the experiments *in vivo* a defective response value around 60% with respect to the macrophages obtained from untreated rats was observed (respectively 83.7 ± 5.1 for untreated rats and 49.4 ± 1.9 for treated rats).

Such an *in vivo–in vitro* relationship of the activity of the Pt compound seems to favor an intracellular action of CISPLATIN rather than a surface interaction.

Studies are in progress in order to evaluate the influence of other Pt(II), Pd(II), and Rh(I) complexes on macrophages.

Summary. *cis*-$Pt(NH_3)_2Cl_2$, a drug active against a wide range of tumors in animals and in man, has been found to cause modification of the macrophages chemotaxis. The influence of the temperature and of time of contact with the drug has been investigated and the results seem to support an intracellular action of the drug, rather than a surface interaction.

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