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CIS-DICHLORODIAMMINEPLATINUM (II)

PERSISTENT AND SELECTIVE INHIBITION OF DEOXYRIBONUCLEIC ACID SYNTHESIS *IN VIVO**

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Abstract—*Cis*-dichlorodiammineplatinum(II) (*cis*-$[Pt(NH_3)_2Cl_2]^0$) at 10^{-4} M inhibits the incorporation *in vitro* of thymidine-methyl-^3H, uridine-5-^3H, and L-leucine-^{14}C into the acid-insoluble fraction of Ehrlich ascites tumor cells from untreated mice only after a period of preincubation. Similarly, when mice bearing well developed tumors are given this compound intraperitoneally, subsequent assessment *in vitro* of incorporation of these isotopic precursors into tumor cells from injected mice shows marked impairment of incorporation of all three; the greatest inhibition occurs 6–12 hr after injection. After this depression, the rates of incorporation of uridine and L-leucine return to control values or somewhat greater, while a striking suppression of the rate of incorporation of thymidine persists for at least 96 hr. These data were interpreted as indicative of a possible metabolic transformation by the cells of the inorganic moiety from an inactive to an active form, although alternate tentative explanations were also proposed. It was suggested that if the persistent inhibitory action on DNA synthesis is directly related to the chemotherapeutic efficacy of this agent or a metabolic product thereof, a less frequent treatment regimen may be as effective as daily injections while evoking fewer toxic reactions.

IN AN investigation of possible effects of an electrical field on growth processes in bacteria, Rosenberg *et al.*[1] noted an unexpected response in that individual cells of *Escherichia coli* attained lengths up to 300 times the normal length under the influence of a current of 2 amp at 1000 cps delivered between platinum electrodes. Exhaustive investigations revealed that one or more long-lived new chemical species was created by the electrical current and was responsible for the unbalanced growth. Several platinum salts as well as other group VIII b transition metal compounds were subsequently shown to induce cell elongation when added to the medium at 1–10 ppm. One of the most active compounds, *cis*-dichlorodiammineplatinum(II) (*cis*-$[Pt(NH_3)_2Cl_2]^0$), has now been shown to retard the growth of the sarcoma 180 and to enhance markedly the survival times of mice bearing the L1210 leukemia.[2] Studies of the distribution of platinum ion within *E. coli* after induction of filaments with *cis*-$[Pt(NH_3)_2Cl_2]^0$ showed that the metal was associated with metabolic intermediates, nucleic acids, and cytoplasmic proteins.[3] In their most recent communication,

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Rosenberg *et al.*[2] noted that there is no knowledge of the fate of the compounds injected into the animals or of the mechanism of action against the tumor cells.

The present report describes our initial experiments concerning the biochemical effects of *cis*-$[Pt(NH_3)_2Cl_2]^0$ in another transplantable tumor.

MATERIALS AND METHODS

Cis-dichlorodiammineplatinum(II) was synthesized as described by Kauffman and Cowan[4] from potassium tetrachloroplatinate(II) (Alfa Inorganics). Elemental analysis (Galbraith Laboratories) showed, in per cent: Pt, 65·27; N, 9·46; H, 1·97; Cl, 23·41. Calculated for $[Pt(NH_3)_2Cl_2]^0$: Pt, 65·03; N, 9·33; H, 2·01; Cl, 23·62. A strain of Ehrlich ascites tumor which has been used in this laboratory for several years was maintained in BALB/c mice (Flow Laboratories). Radioisotopes were from New England Nuclear Corp., and Eagle's minimum essential medium with Hanks' balanced salt solution and 0·002 M glutamine was from Microbiological Associates. Assessments of the rates of synthesis of DNA, RNA, and protein were done by measuring the incorporation of thymidine-methyl-^3H, uridine-5-^3H, and L-leucine-uniformly labeled-^{14}C, respectively, into the cold 5% trichloroacetic acid (TCA)-insoluble fraction of the cells. The acid-insoluble material was solubilized in hydroxide of Hyamine, added to a toluene-based phosphor (PPO-POPOP), and radioactivity was measured with a liquid scintillation spectrometer (Mark I, Nuclear-Chicago Corp.).

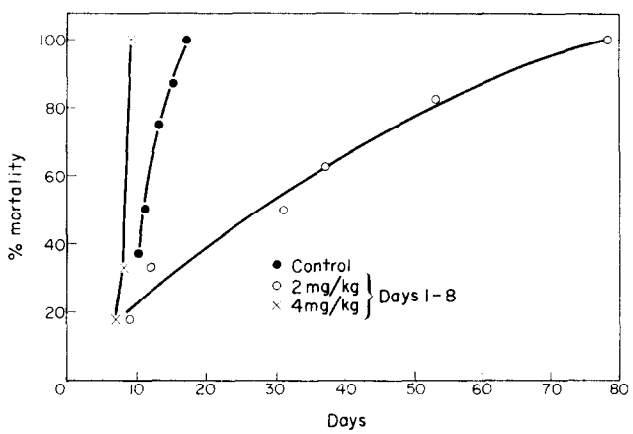

FIG. 1. Survival of mice bearing the Ehrlich ascites tumor when treated with *cis*-$[Pt(NH_3)_2Cl_2]^0$ by intraperitoneal injections.

RESULTS

Initial experiments were directed toward verifying the chemotherapeutic action of *cis*-$[Pt(NH_3)_2Cl_2]^0$ against the Ehrlich ascites tumor. The results of therapy at 2·0 and 4·0 mg/kg on days 1 through 8 are shown in Fig. 1. The higher dose was unequivocally toxic, reducing the average survival time to 8·5 days as compared with 12·4 days for the saline-injected controls. With the lower dose, however, the average survival time was 36·6 days, 295 per cent that of the control group. It is believed that some of the

earlier deaths in the lower dose groups were due to toxicity of the injected material, since gross examination did not reveal detectable tumors.

Consequently, a similar experiment was carried out at 1·5 and 2·5 mg/kg on days 1 through 7; the results are shown in Fig. 2. On these regimens there were no deaths in the treated groups attributable to platinum toxicity; i.e. all animals displayed well developed tumors prior to death. Some of the mice which survived the longest had solid tumors originating at the inoculation site in the abdominal wall, while others showed no gross evidence of tumor. Mice on the lower dosage lost about 20 per cent of their initial weight during the 7 days of treatment; those at the higher dosage lost about 25 per cent. Resumption of weight gain began about 4 days after injections were

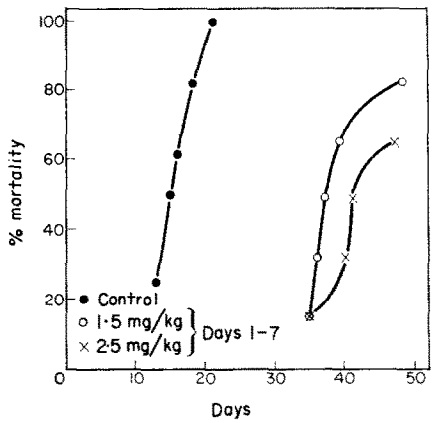

FIG. 2. Same as Fig. 1 except different regimen.

discontinued. The Ehrlich ascites tumor thus appears to be approximately as sensitive to *cis*-$[Pt(NH_3)_2Cl_2]^0$ as the sarcoma 180 and leukemia L1210.

To determine if a suppression of the rate of synthesis of DNA, RNA, or protein is effected by *cis*-$[Pt(NH_3)_2Cl_2]^0$, aliquots of a 1% (v/v) ascites tumor cell suspension in medium were incubated with the compound for up to 6 hr at a concentration of 10^{-4} M (= 29·8 μg/ml). After this, thymidine-methyl-^{3}H (1·0 μc/ml), uridine-5-^{3}H (1·0 μc/ml), or L-leucine-^{14}C (0·2 μc/ml) was added and the reaction was terminated with an equal volume of 10% TCA after a 20-min pulse labeling period. Figure 3 shows that DNA, RNA, and protein synthesis were markedly inhibited, but only after a period of preincubation.

To determine if a similar pattern of inhibition occurs *in vivo*, mice bearing well developed ascitic tumors were randomized into groups of 3 or 4; half of the groups were given *cis*-$[Pt(NH_3)_2Cl_2]^0$ as a single intraperitoneal injection at 10 mg/kg, and the other half received only the appropriate volume of saline. At intervals up to 96 hr after injection, cells were removed, pooled and washed once by sedimentation and resuspension in medium. Cell suspensions from treated and control groups were adjusted to the same concentration in terms of cells per milliliter by use of a Coulter counter (model B), and in all experiments varied only slightly from 10^6 cells/ml. (It is of interest that when cell suspensions from treated and control groups were prepared so as to be identical in terms of number of cells per milliliter, centrifugation of

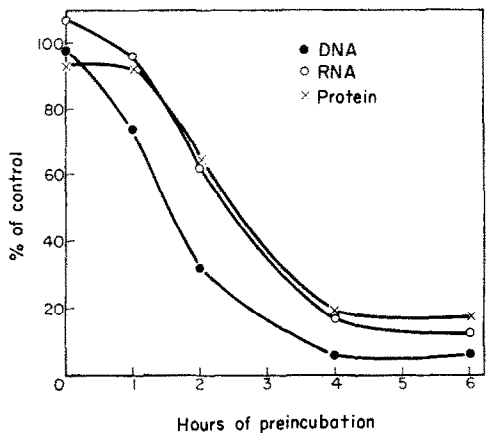

FIG. 3. Effects of *cis*-$[Pt(NH_3)_2Cl_2]^0$ on synthesis of DNA, RNA and protein by Ehrlich ascites tumor cells from untreated mice as a function of period of exposure *in vitro* to the inhibitor.

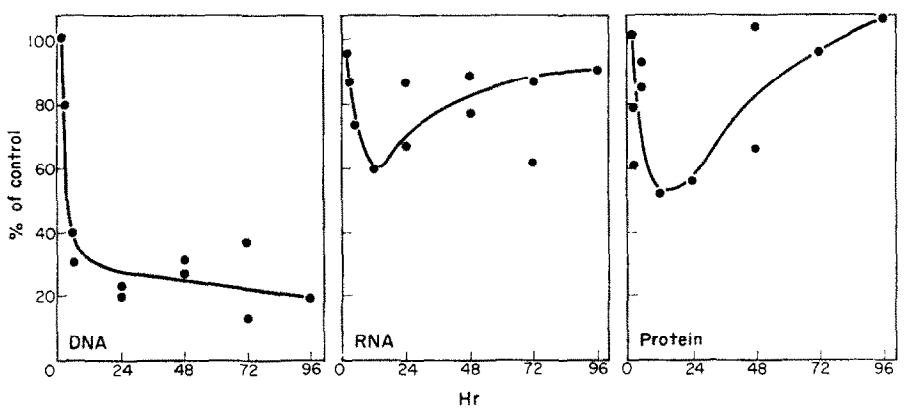

FIG. 4. Effects of a single intraperitoneal injection of *cis*-$[Pt(NH_3)_2Cl_2]^0$ at 10 mg/kg in mice on the subsequent synthesis of DNA, RNA and protein by Ehrlich ascites tumor cells when measured *in vitro* at various intervals after injection.

aliquots of equal volumes of each suspension showed the packed cell volume of the treated cells to be up to 40 per cent greater than that of control cells.) Isotopically labeled thymidine, uridine, and L-leucine were added as above, and the reactions were terminated with 10% TCA after 20 min. The extent of incorporation of each precursor was expressed as per cent of incorporation into control cells and the data are shown in Fig. 4. Cells which were removed from mice 1 hr after treatment were unimpaired in their capability to incorporate each precursor. However, beginning at about 2 hr after treatment, there was a detectable reduction in the extent of incorporation of each, and these reductions were more pronounced after 4–6 hr. At 12–24 hr after injection, there was a tendency toward reversal of the effects on incorporation of uridine and

L-leucine, and this restoration to substantially normal activity was virtually complete after 72–96 hr. The most impressive feature of these experiments, however, was the profound and extended suppression of the rate of incorporation of thymidine into DNA after a single injection of *cis*-$[Pt(NH_3)_2Cl_2]^0$ at the relatively low dose of 10 mg/kg. The relationship of this reduction of rate of DNA synthesis to the chemotherapeutic action of this inorganic complex is by no means unequivocal, but is under investigation.

DISCUSSION

A number of tentative suggestions are evoked by the foregoing preliminary data. The first of these concerns the actual chemical species responsible for the selective inhibition of DNA synthesis, which is effected several days after injection of *cis*-$[Pt(NH_3)_2Cl_2]^0$. The initial insensitivity *in vitro* of ascites tumor cells from treated and untreated mice raises the implication that this compound may not be the active agent *per se*, but may be converted to an active material. Further, the data suggest that more than one metabolic step could be involved. In the simplest concept, A→B→C, where A is *cis*-$[Pt(NH_3)_2Cl_2]^0$, and B and C are as yet unidentified metabolites. Compound A is conceived as being substantially devoid of activity against the test system; compound B is a derivative which is active against all three of the parameters investigated; and compound C is highly selective for DNA synthesis and is devoid of activity against RNA and protein synthesis. Partial support for at least one metabolic alteration has been obtained (H. S. Thompson, this laboratory, unpublished data) in the finding that only about half of the platinum injected as *cis*-$[Pt(NH_3)_2Cl_2]^0$ into rats is recovered in the first 24–48 hr urine collection periods. After this interval, virtually no platinum is found in urine up to 6 days after injection, and none is found in feces. An alternative explanation of the data could just as well propose only a single step transformation in which A is converted to B. In this proposal, however, it would have to be postulated that B reversibly and transiently inhibits RNA and protein synthesis but virtually irreversibly inhibits DNA synthesis.

It is apparent that other tentative explanations could just as well be offered to explain the delayed onset of inhibitory actions. Among these would be: (1) a slower rate of diffusion into the cell of this lipid-insoluble complex (through membrane pores?), or (2) a platinum-membrane interaction which is time dependent and which confers a greater rate of diffusion of the compound into the cell. The point to be emphasized, however, is that, if a metabolic transformation is indeed involved, the desirability of identifying the actual chemical species responsible for the extended action on DNA synthesis is obvious.

In the unpublished experiments mentioned above, rats have displayed considerable renal toxicity (glucosuria, proteinuria) after several daily injections of relatively low doses of *cis*-$[Pt(NH_3)_2Cl_2]^0$. If the extended and selective action on DNA synthesis after a single injection into mice is directly related to the chemotherapeutic action of this agent, the data strongly suggest that daily injections are not essential, and that toxicity may be reduced by injections on each second, third or fourth day without compromising therapeutic efficacy.

Experiments are currently underway to define the enzyme or enzymes inhibited *in vivo*, to assess the mammalian toxicity at the electron microscope level, to characterize the possible metabolic transformation(s) of the injected material, to detect

embryopathic or teratogenic actions or both, in chicken embryos, and to determine the types of ultrastructural changes extant in filamentous forms of gram-negative bacilli induced by this agent.

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