Suppression of Lymphocyte Blastogenesis in Man Following *cis*-Platinous Diamminodichloride Administration[1] (37015)

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cis-Platinous diamminodichloride (PDD) has been shown to induce regression of animal tumors (1–3). It has also been found to be effective in human malignancies (4–6). Immune responses of the host play an important role in the control of malignant process (7). PDD has been shown to suppress antibody plaque forming spleen cells and graft versus host reaction in mice (8, 9). It prolonged the survival of skin grafts against H_2 histocompatibility in mice (10). PDD also suppressed phytohemagglutinin (PHA)-induced lymphocyte blastogenesis *in vitro* (11). The present study was undertaken to determine the *in vivo* effect of this compound on lymphocytes. PHA-induced lymphocyte blastogenesis was utilized to monitor the effect of PDD.

Materials and Methods. Five patients receiving PDD for solid tumors were included in this investigation. Standard techniques were utilized to set up lymphocyte cultures. To describe briefly, 15 ml of heparinized blood were drawn from the patients at various intervals. The blood was allowed to sediment for 1 hr at 37° and white cell-rich plasma was obtained. The granulocytes were allowed to phagocytose fine iron powder at 37° for 15 min under constant shaking and sedimented with a magnet. The supernatant contained approximately 95% lymphocytes and was harvested. Over 99% cells were viable by trypan blue exclusion test. The final cell count was adjusted to 1.5×10^6 cells/ml in TC 199 (Difco) containing penicillin and streptomycin. The lymphocytes were cultured in the presence or absence of 0.1 ml of PHA (Difco P) 1:100 dilution in triplicate. The cultures were kept at 37° for 96 hr in an incubator with 5% CO_2 atmosphere. At the end of the incubation period, smears were prepared and stained with Wright's stain. The percentage of transformed cells was determined microscopically. Blastogenesis after treatment was expressed as percentage of the pretreatment level. The pretreatment blastogenesis was taken as 100%. The blastogenic response was studied before the administration of drug and after treatment at various intervals.

PDD was purified at our Institute and found to be pure by infrared and ultraviolet spectroscopy and thin layer chromatography (Dr. R. J. Speer). It was dissolved in normal saline at pH of 2.9 and administered intravenously. PDD was given at a rate of 1 mg/kg dose/hr except for one case in this study (Table I, D). The results were analyzed statistically using Duncan's multiple range and multiple *F* tests (12).

Results. The pretreatment blastogenic transformation varied from 23 to 35% in these patients. Patient A in Table I received the smallest dose in this study, that is 1 mg/kg body weight. There was significant inhibition of blastogenesis at 15 min, 2 hr and 18 hr ($p < .01$). The response returned to 80% of the normal in 24 hr. At 2 mg/kg dose (B) the inhibition was more pronounced at 15 min and 2 hr as compared with the 1 mg/kg dose. There was complete inhibition of blastogenesis at 15 min. The response returned to normal at 24 hr. Patient C received 2.5 mg/kg dose intravenously. There was marked inhibition of blastogenesis at 15 min and 2 hr. The inhibition was less pronounced at 18 hr and again returned to normal levels at 24 hr. The effects of PDD given as a slow infusion over 24 hr were observed in patient D. The dose in this case was 2.5 mg/kg body

[1] Presented in part at the American Association for Cancer Research Meeting, Boston, MA, May 3–6, 1972. Supported by a grant from Medical Research, Inc. of Dallas.

TABLE I. Effect of PDD Administration on Lymphocyte Blastogenesis.

Patient	PDD dose mg/kg	Blastogenesis index[a]; time after PDD treatment: 15 min	2 hr	18 hr	24 hr	48 hr
A	1	23[b]	23[b]	57[b]	80	—
B	2	1[b]	12[b]	—	100	—
C	2.5	9[b]	3[b]	27[b]	110	—
D	2.5 (in 24 hr)	7[b]	3[b]	31[b]	69[b]	120

[a] Expressed as percentage of the pretreatment level.
[b] Inhibitions were significant ($p < .01$).

weight. There was marked inhibition at 15 min and at 2 hr. The inhibition was less pronounced at 18 and 24 hr after PDD. The blastogenic response returned to normal level at 48 hr. Figure 1 shows the effect of PDD given in divided dosage. The total dose of 4 mg/kg was given as 0.5 mg/kg twice a day for 4 days. Each dose was administered over a period of 30 min. Blastogenesis was studied 3 hr after the morning dose on each day. Three hours after the first dose of 0.5 mg/kg the blastogenic response was reduced to 72%. It declined to 44% on the second day, and 32% on the fourth day. Following the cessation of treatment the response was 36% at 48 hr and 72% at 72 hr.

Discussion. The intact cellular immunity is of great importance in the host defense against malignant disease. The lymphocyte blastogenesis was utilized in the present study to investigate the extent and the length of suppressive effect in patients being treated with PDD. An immediate effect on the lymphocytes was noticed as evidenced by inhibition of blastogenesis 15 min following the administration of the drug. The dosage employed in this study is therapeutically effective in certain human tumors (4–6).

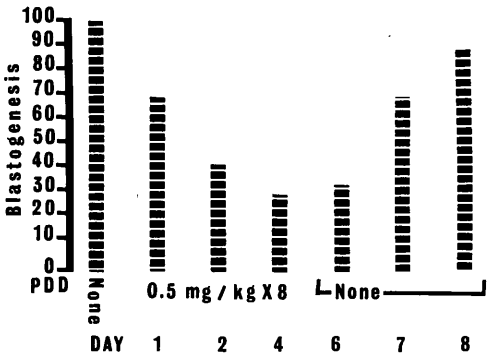

FIG. 1. Effect of PDD given in divided doses (0.5 mg/kg every 12 hr × 8 doses). The inhibition of blastogenesis was less pronounced but lasted for 72 hr after the cessation of PDD treatment.

The immunosuppressive effect lasted for 18 to 72 hr after the treatment which is relatively short. In 2 patients receiving 2.5 mg/kg dose an actual rebound phenomenon was observed where blastogenic response after recovery was 10 to 20% higher than the pretreatment level. Such a rebound phenomenon has been reported with other immunosuppressive drugs. The short-lived immunosuppressive effect of PDD may prove to be advantageous. A rapid recovery of the immune system may help in the control of the malignant disease.

The mechanism of inhibition of blastogenesis may be explained on the basis of inhibition of deoxyribonucleic acid by PDD (13). The inhibition of blastogenesis was prolonged when 4 mg/kg dose was divided into 8 doses and given over 4 days. Although the effect lasted longer with this schedule the inhibition was less pronounced at any given point compared with single and smaller injections. This observation may be taken advantage of to minimize immunosuppression in patients, provided the doses prove to be effective in inducing tumor regression.

Summary. The lymphocyte blastogenic response to PHA was studied in patients receiving *cis*-platinous diamminodichloride for various tumors. This compound produced significant inhibition of blastogenesis ($p < .01$) in doses ranging from 1 to 4 mg/kg body weight given in different schedules. The

inhibition lasted from 18 to 72 hr. A single dose administration produced higher degree of inhibition than divided doses.

We thank Mr. Harry Clifford for his technical assistance in carrying out lymphocyte blastogenesis procedure.

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Received Sept. 13, 1972. P.S.E.B.M., 1973, Vol. 142.