# Effect of *cis*-Platinous Diamminodichloride on Graft Rejection: Prolonged Survival of Skin Grafts Against $H_2$ Histocompatibility[1] (36705)

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*cis*-Platinous diamminodichloride (PDD) belongs to a recently discovered new class of antitumor compounds. It has been reported to be effective both in animal tumors and in human neoplasms (1–5). It is also immmunosuppressive. The following immune responses have been shown to be suppressed by this agent: (a) phytohemagglutinin induced lymphocyte blastogenesis, (b) antibody plaque forming spleen cells, and (c) graft versus host reaction (6–10). The present study was undertaken to investigate the effect of this agent on graft rejection. The survival of skin grafts against $H_2$ histocompatibility barrier was studied in mice treated with PDD.

*Materials and Methods. Mice.* Female C57BL/10 mice served as donors and congenic resistant line, B10 D2-new, which differ at $H_2$ locus, served as recipients. The mice weighed between 18–20 g and were obtained from Jackson Laboratory, Bar Harbor, ME.

*PDD.* This compound was purified in our laboratory and found to be pure by infrared and ultraviolet spectroscopy and thin layer chromatography (Dr. R. J. Speer). It was dissolved in *N* saline at pH 2.9 and administered to the animals ip without delay. The dosage and schedule of PDD treatment are given in Tables I and II.

*Skin Grafts.* Split thickness skin grafts obtained from the tail of the donors were placed on the side of the trunk of the recipients (11). The animals were anesthetized with ketamine (Ketalar, Parke Davis, Detroit, MI) 2 mg/10 g body weight, given ip. The grafting procedure was done under aseptic conditions. Vaseline gauze dressing was placed over the graft which was secured in place with a thin plaster cast. The cast was removed on day 9 and the grafts were inspected daily for signs of rejection (12). The animals were given food and water *ad libitum*.

*Statistical analysis.* The results were analyzed statistically through computer using Duncan's multiple range and multiple *F* tests (13).

*Results.* The results of different doses of PDD given on day 0 (the day of skin grafting) are depicted in Table I. The controls receiving isografts showed no rejection during the period of observation of 1 month. Untreated B10 D2-new mice receiving grafts from C57BL/10 mice showed a mean survival of 11 days. The dose of 8 mg/kg was not exceeded because this dose has been found to give maximum immunosuppression in other systems (9, 10). An increase in this dose also leads to deaths in animals. The $LD_{50}$ is 12 mg/kg body weight. Maximum prolongation of 127% over the controls, which was statistically significant, was seen in mice receiving PDD 8 mg/kg on the day of transplantation ($p < .01$). A reduction in dose to 4 and 2 mg/kg diminished the graft survival. The slight prolongation observed with 2 and 4 mg/kg dose was not significant. Fractionation of the total dose of 8 mg/kg into 4 doses, given daily starting with day 0, resulted in significant prolongation ($p < .01$) of the skin grafts but it was less than the group receiving total dose in 1 injection. When the total dose of 8 mg/kg was divided into 4 doses and individual injections spread further apart, that is, given on every other day, no prolongation of the graft survival was observed.

PDD was also administered at different time periods from the day of transplantation (Table II). The prolongation of skin graft

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TABLE I. Effect of Different Dosage of PDD on Skin Graft Survival.

| No. of animals | Dosage (mg/kg) | Day of treatment | Survival days (mean ± SE) | Percentage prolongation |
|---|---|---|---|---|
| 6 | — | 0 | 11 ± 0.4 | 0 |
| 5 | 8 | 0 | 24 ± 3.4 | 127 |
| 6 | 4 | 0 | 14 ± 1.5 | 27 |
| 5 | 2 | 0 | 14 ± 0.7 | 27 |
| 5 | 2 × 4 | 0, 1, 2, 3 | 18 ± 1.7 | 64 |
| 5 | 2 × 4 | 0, 2, 4, 6 | 11 ± 0.4 | 20 |

was maximum when the drug was administered on the day of transplantation. The effect declined if the drug was given either before or after skin grafts.

*Discussion.* Various antitumor agents have been shown to be immunosuppressive. PDD, which is a new compound, also seems to fall in the same category. The most effective antitumor dose in mice is 8 mg/kg body weight. Same dose is effective in producing immunosuppression. It has been shown that PDD suppresses lymphocyte blastogenesis as well as graft versus host reaction, suggesting the effect of this compound on cellular immune responses (6, 7, 10). The present work provides further evidence in this direction. The drug prolonged the survival of skin grafts against $H_2$ histocompatibility barrier. It appears that the drug is most effective during the initial phases of graft rejection, since maximum prolongation was seen when the drug was given at the time of transplantation. A delay in the administration of the drug led to abolition of the effect. The drug was also effective when given prior to transplantation and significant suppression of graft rejection was seen by treatment up to 3 days before skin graft. The total dose given in a single injection seems to be the best mode of administering this drug for achieving prolonged survival of skin grafts. Fractionation of the dose was also effective, but the prolongation was less marked.

*Summary.* The new antitumor agent *cis*-platinous diamminodichloride suppressed graft rejection against $H_2$ histocompatibility barrier in mice. The prolongation of graft survival was maximum (127%) at a dose of 8 mg/kg body weight given as a single injection on the day of transplantation. This effect declined at a lower dosage level. The total dose of 8 mg/kg fractionated into 2 mg/kg/day and given for 4 days also produced significant prolongation of skin grafts but was less marked as compared with the single dose. The drug was less effective if given before or after the transplantation.

TABLE II. Survival of Skin Graft Against $H_2$ Histocompatibility in Mice Receiving PDD in Various Schedules.

| No. of animals | Dosage (mg/kg) | Day of treatment | Survival days (mean ± SE) | Percentage prolongation |
|---|---|---|---|---|
| 6 | — | — | 11 ± 0.4 | 0 |
| 5 | 8 | −7 | 14 ± 1.6 | 27 |
| 5 | 8 | −3 | 18 ± 2. | 64 |
| 5 | 8 | −1 | 18 ± 1.3 | 64 |
| 5 | 8 | 0 | 24 ± 3.4 | 127 |
| 5 | 8 | +1 | 17 ± 1.9 | 55 |
| 5 | 8 | +3 | 16 ± 1. | 45 |
| 5 | 8 | +7 | 11 ± 0.7 | 0 |

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