PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 146 333-336 (1974)

Effect of *cis*-Platinum(II)Diamminedichloride and Gallium Nitrate on the Ability of Spleen Cells to Induce a Graft-Versus-Host Reaction[1] (38099)

ANDREW M. MUNSTER, PHILLIP GREENBERG, STUART GREENBERG, ANNE G. LEARY, AND GLEN R. GALE

Veterans Administration Hospital; Department of Surgery and Department of Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29403

Cis-platinum(II)diamminedichloride (NSC-119875) (platinum) and gallium nitrate·$9H_2O$ (gallium) are two new antitumor agents under current investigation. The immunological effects of such compounds are of obvious importance. In the case of platinum, inhibition of phytohemagglutinin-induced blast transformation, antibody formation, and suppression of the graft-versus-host (GvH) reaction, when the compound was administered to the recipient, have all been reported (1–4). The effects of gallium have been investigated less extensively.

The rat popliteal node assay method of the GvH reaction, recently developed, is a reliable method of quantitating thymic-dependent lymphocyte function (5). It not only distinguishes between strong and weak histocompatibility differences, but is capable of measuring the suppressive effects of radiation, thermal and mechanical trauma, and uremia (6–8). The principle of the assay consists of the injection of parental rat spleen cells into the hind footpad of the F_1 hybrid. The cells migrate to the draining popliteal lymph node; there, since the donor cells are recognized as "self" by the recipient, rejection does not occur. Rather, the donor cells recognize the other parent component of the hybrid recipient as "nonself," and mount a reaction against it in the node. Blastogenesis and cell division ensue, resulting in node enlargement. When such nodes are excised and weighed 1 wk after spleen cell injection, the resulting weight is a reflection of the immunological competence of the thymic-dependent spleen cells of the donor. If the donor is manipulated in various ways or given drugs, subsequent splenectomy and the performance of the assay give an indication of the immunological effects of such treatment on the donor.

We decided therefore to investigate the effects of platinum and gallium in this model, using doses sufficient to induce toxicity as evinced by morbidity and weight loss.

Materials and Methods. Adult Lewis (P) and (Lewis × BN)F_1 rats, weighing about 200 g, were used. These rats differ at the strong H-1 histocompatibility locus. Donor P rats received a single ip injection of platinum at 1 mg/kg, or gallium at 40 mg/kg given daily for 10 days. Donor rats were weighed daily and sacrificed at various intervals for removal of spleen cells. At the time of sacrifice the P rats were heart punctured and peripheral blood and differential counts were performed. Spleens were excised aseptically from two to three donors, minced finely with scissors, pressed through a 100-gauge stainless steel wire mesh, and suspended in medium TC-199 (Difco). Dilution was calculated to obtain a final dose for injection of 4×10^7 spleen cells in 0.5 ml medium, which was injected into the right hind footpad of F recipients in groups of five. The left hind footpad was injected with 0.5 ml of medium only as internal control. Control experiments were also carried out in which syngeneic cells (F to F) were injected using the same dose; in other experiments either the donor P rats or the re-

[1] Supported in part by Research Grants CA-11811 and CA-12791 (to G.R.G.). The authors also acknowledge the generous donation of K_2PtCl_4 from Matthey Bishop, Inc., from which the NSC-119875 was synthesized.

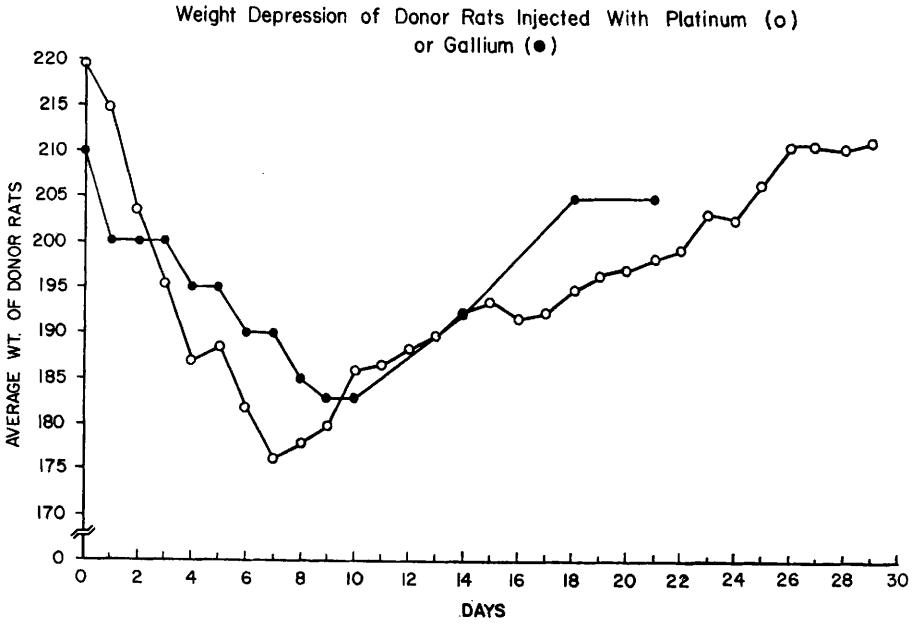

FIG. 1. The effect of platinum and gallium injections on donor rat weights.

cipient F rats were subjected to 450 R of total body radiation 10 days prior to splenectomy. All nodes were excised and weighed 1 wk after injection.

Results. Platinum. The single injection of the donors caused an average weight loss of 45 g by 7 days, after which recovery followed (Fig. 1). The total peripheral white count was depressed immediately after injection, then underwent a rebound increase by the fifteenth day, following which it returned to normal. The differential lymphocyte count remained

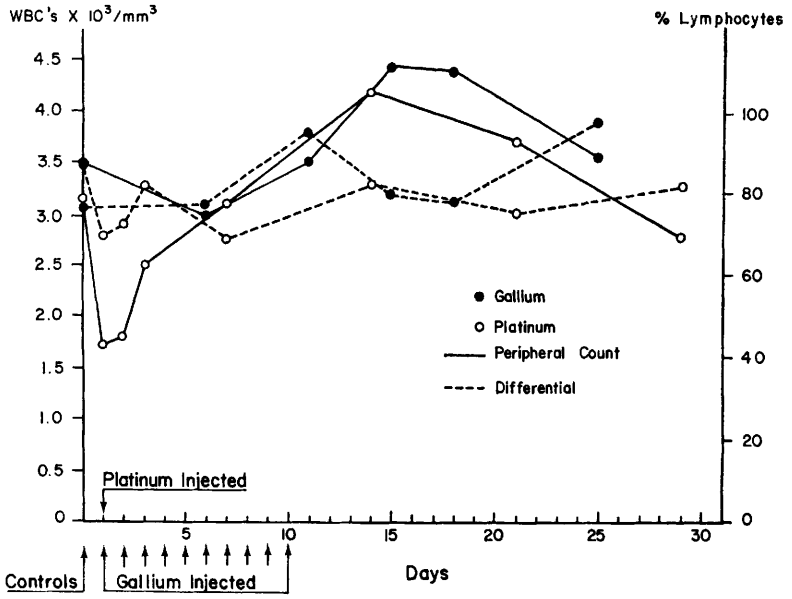

FIG. 2. The effect of platinum and gallium injections on donor blood count.

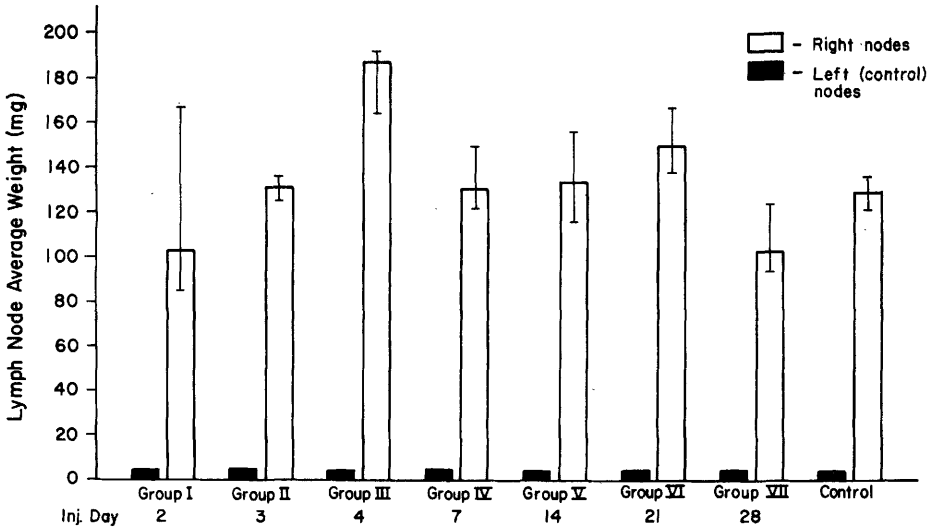

FIG. 3. The effect of platinum treatment of donor Lew rats on the popliteal node of (Lew × BN)F_1 recipients.

within normal range (Fig. 2). The results of the graft-versus-host assay are shown in Fig. 3. Using Schaffe's method of analysis of variance, there was no statistically significant suppression of the reaction at any time, even during the time of maximal weight loss in the donors.

Gallium. Donor rats again lost weight, although not as dramatically as with platinum, averaging 20 g, and began to regain weight immediately upon cessation of the injections at 10 days (Fig. 1). The only notable effect on the peripheral blood picture was a slight lymphocytosis at 15 days (Fig. 2). The results of the graft-versus-host assay are shown in Fig. 4, which also includes the syngeneic controls and the radiated controls. As expected, there was no significant reaction in the syn-

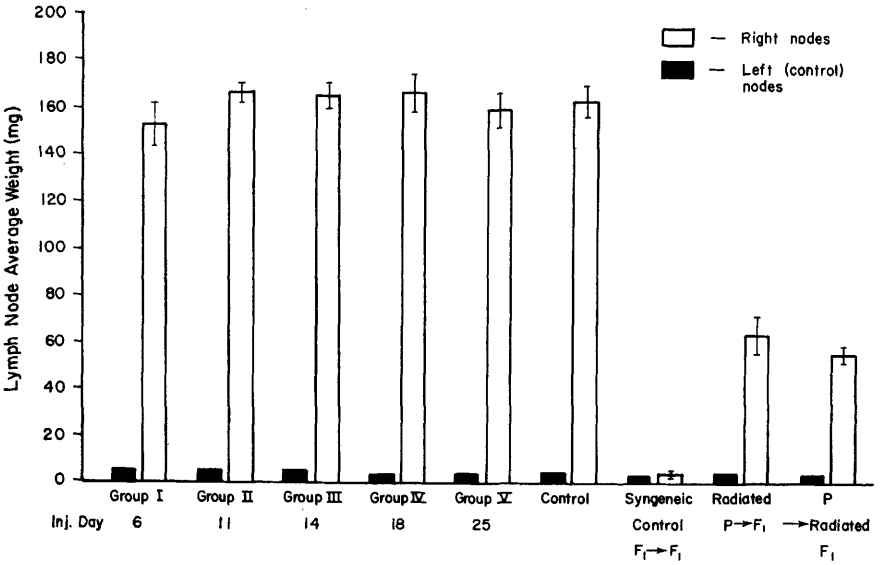

FIG. 4. The effect of gallium treatment of donor Lew rats on the popliteal node of (Lew × BN)F_1 recipients, with syngenic and radiated controls.

geneic controls, but there was a severe suppression of the reaction when either donors or recipients were radiated. However, the administration of gallium had no statistically significant effect in any group.

Discussion. Our failure to demonstrate a suppression of the GvH reaction following platinum administration has to be reconciled with prior reports of immunological suppression in the literature, particularly with the reported depression of the GvH reaction using the spleen index assay by Khan and Hill (3). Those authors studied the effect of platinum on recipients rather than on donors. The contribution of the recipient to the GvH reaction is uncertain, since it cannot be a specific reaction mounted against the histocompatibility antigens of the donor. Because it has been documented that healthy lymphocytes in the recipient are necessary for the reaction, confirmed in our experiments by the results of recipient radiation, these cells probably act as a target organ for the invading splenic lymphocytes. Any alteration in the membranes of the recipient lymphocytes, not necessarily immunologically specific, might therefore be expected to diminish the reaction. The attack by the donor spleen cells is, on the other hand, immunologically specific and directed at the histocompatibility locus of the other parent's (in this case, BN) component in the recipient. In our experiments, therefore, platinum did not abrogate the ability of the thymic-dependent lymphocytes of treated rats to respond to a foreign histocompatibility antigen, a fact which may be of importance in terms of current concepts of immunological surveillance. Certainly, the retention of the capability of thymic lymphocytes to recognize foreign antigens would seem to be one of the desiderata of successful antitumor agents when given in doses sufficient to cause systemic toxicity and tumor regression.

Previous reports of depression of plaque-forming ability by platinum treated cells (2) is not contradictory to our findings, since plaque-forming cells are generally accepted to be of the bursal cell line while here we have measured the response of thymic-dependent cells to histocompatibility antigen. The reported suppression of phytohemagglutinin responsiveness of platinum-treated cells (1) is more difficult to explain, since this reaction is supposedly a property of thymic-dependent cells. However, there is increasing evidence that the inductive mechanisms of phytohemagglutinin and natural antigens on the thymic lymphocyte are different; e.g., the effect of radiation affects the responses to varying antigens differently (9). In addition, the phytohemagglutinin studies with regard to platinum were *in vitro* rather than *in vivo* studies.

In conclusion, it would seem that the measurement of thymic lymphocyte function by an *in vivo* assay system, such as the one presented here, is an important adjunct in the investigation of proposed new antitumor agents within their therapeutic dose range.

Summary. We used the recently described rat popliteal node assay, a sensitive *in vivo* test of thymic-dependent lymphocyte function, to measure the effects of *cis*-platinum(II) diamminedichloride and gallium nitrate on the ability of spleen cells from treated parental rats to induce a graft-versus-host reaction in hybrid recipients. In doses sufficient to cause morbidity and weight loss of the donor rats, no significant suppression of the reaction could be demonstrated.

1. Howle, J.A., Thompson, H.S., Stone, A.E., and Gale, G.R., Proc. Soc. Exp. Biol. Med. **137**, 820 (1971).

2. Khan, A., and Hill, J.M., J. Surg. Oncol. **3**, 565 (1971).

3. Khan, A., and Hill, J.M., Transplantation **13** 55 (1972).

4. Khan, A., and Hill, J.M., Infect. Immun. **4**, 320 (1971).

5. Ford, W.L., Burr, W., and Simonsen, M., Transplantation **10**, 258 (1970).

6. Munster, A.M., Eurenius, K., Mortensen, R.F., and Mason, A.D., Jr., Transplantation **14**, 106 (1973).

7. Munster, A.M., and Gressitt, S.E., Proc. Soc. Exp. Biol. Med. **143**, 106 (1973).

8. Munster, A.M., Wilson, R.E., and Greenberg, S., Unpublished data.

9. Coifman, R.E., Good, R.A., and Meuwissen, H.J., Proc. Soc. Exp. Biol. Med. **137**, 155 (1971).

Received June 19, 1973. P.S.E.B.M. 1974, Vol. 146,