Toxicity Trial of Prophylactic 9-[2-Hydroxy-1-(Hydroxymethyl)Ethoxymethyl]Guanine (Ganciclovir) after Marrow Transplantation in Dogs

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The effects of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine, or ganciclovir, administered during the immediate posttransplant period to dogs given total-body irradiation and autologous marrow transplants were studied. Doses of ganciclovir of 3.0 mg/kg (body weight) per day were well tolerated without detectable delay in hematopoietic recovery, whereas doses of 5.0 mg/kg per day were associated with delayed platelet recovery.

Cytomegalovirus (CMV) infection, particularly CMV pneumonia, is a serious complication of marrow transplantation. The incidence of CMV pneumonia among patients undergoing allogeneic transplantation for hematologic malignancies is approximately 15%, and 85% of those patients who develop CMV pneumonia die (13). CMV pneumonia, in fact, represents the single most common cause of death among patients undergoing allogeneic marrow transplantation for acute myelogenous leukemia in first remission or chronic myelogenous leukemia in chronic phase.

Patients who are seronegative for CMV can be protected from infection by using only CMV-seronegative blood products during the transplant period (2). Unfortunately, 50% of patients undergoing transplantation are CMV seropositive, and for this group of patients, there is no proven means to either prevent CMV infection or treat infection once it occurs. Acyclovir, vidarabine, and various interferon preparations used alone or in combination have not been shown to prevent CMV infection or to improve survival following CMV pneumonia (14). The acyclic nucleoside analog 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine, or ganciclovir, has substantially greater activity against CMV in vitro than previously available agents do (1, 8, 10, 11). Initial studies in marrow transplant patients and in patients with acquired immune deficiency syndrome (AIDS) indicate substantial antiviral activity in vivo as well. Clinical improvement has been observed among AIDS patients treated for CMV retinitis, enteritis, or pneumonia in uncontrolled studies (3, 7, 9, 12). Although a similar antiviral effect has been observed in marrow transplant patients treated for CMV pneumonia, with both inhibition of virus excretion and a marked decrease in the titer of CMV recovered from lung tissue after treatment, survival from CMV pneumonia was not improved in initial trials (5, 15, 16). Moreover, ganciclovir treatment has been associated with transient marrow suppression in 25 to 50% of patients treated (3, 6, 7, 9, 12, 15, 16).

In view of the demonstrated antiviral activity of ganciclovir against CMV in vivo, it is possible that it may be effective

if used in a prophylactic manner, that is, before CMV-seropositive patients develop virus reactivation and clinically apparent infection. Unfortunately, the hematopoietic suppression associated with ganciclovir treatment might preclude its prophylactic use in the early postgrafting period. Because of this concern, the effects of ganciclovir on hematopoietic recovery were studied in dogs given total-body irradiation and autologous marrow transplants.

Beagles or harriers raised at the Fred Hutchinson Cancer Research Center or bought from commercial kennels were dewormed, vaccinated against distemper, hepatitis, leptospirosis, and parvovirus, and observed for disease for at least 2 months before the transplant. The dogs weighed 7 to 18 kg and were 7 to 18 months of age at the time of marrow grafting. Research was conducted according to principles included in the "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23, revised 1985) prepared by the National Research Council. The research protocol was approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center.

Autologous marrow was harvested from femurs and humeri by needle aspiration as previously described (17). The marrow was stored at 4°C for no more than 6 h while the animals were being irradiated. The number of marrow cells harvested and subsequently reinfused ranged from 2.0×10^8 to 5.0×10^8 marrow cells per kg (body weight). The day of marrow infusion was designated day 0.

Directly after marrow harvest, dogs were given 9.2 Gy of total-body irradiation at a rate of 7 cGy/min, delivered by two opposing cobalt-60 sources. This corresponded to 1,200 R measured in midair at a rate of 9.3 R/min. Details of the radiation dosimetry have been described elsewhere (18). Shortly after irradiation, the dogs were given back the stored autologous marrow by intravenous infusion.

To mimic the posttransplant situation in humans, in which ganciclovir would be used prophylactically, animals were given posttransplant immunosuppression (5). Cyclosporine was given at a dose of 7.5 mg/kg twice a day intramuscularly on days 0 to 7 and at the same dose twice a day orally on days 8 to 25. In addition, animals received methotrexate at a dose of 0.4 mg/kg intravenously on days 1, 3, 6, and 11 posttransplant.

Ganciclovir was given intravenously twice a day (every 12

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TABLE 1. Hematopoietic recovery in dogs given total-body irradiation, autologous marrow grafts, and posttransplant ganciclovir

Dog no.	Ganciclovir dose ^a (mg/kg per day)	Day of ANC ^b >500/mm ³	Day of platelet count		
			>20,000/mm ³	>50,000/mm ³	>100,000/mm ³
Control ^c	0	10 (7–12)	15 (11–23)	23 (18–35)	28 (20–38)
1 (BC258)	1.0	11	13	20	24
2 (C307)	3.0	9	13	15	26
3 (C345)	3.0	13	15	28	36
4 (C152)	5.0	11	36	38	44
5 (C328)	5.0	11	17	30	38
6 (C316)	5.0	11	21	34	40

^a Administered as divided doses every 12 h.^b ANC, Absolute neutrophil count.^c Data obtained from control dogs are expressed as median (range) values.

h), starting on the day of marrow infusion. The dose of ganciclovir per day was 1.0 mg/kg (for one dog), 3.0 mg/kg (for two dogs), or 5.0 mg/kg (for three dogs). Postgrafting care, including parenteral fluids, electrolytes, antibiotics, and in vitro-irradiated platelet infusions, was administered as described elsewhere (17). Complete blood counts, including hematocrit, leukocyte count, and differential and platelet counts, were obtained on the day of marrow infusion, on day 5 after marrow grafting, and daily thereafter. Marrow engraftment was assessed as previously described (4). The day of absolute neutrophil count increase was defined as the day of sustained recovery of the peripheral neutrophil count to greater than 500/mm³ after the posttransplant nadir, and the day of platelet increase was defined as the first day of a sustained platelet count of greater than 20,000/mm³ without transfusion support. Control data were obtained for 16 dogs receiving the same radiation dose and postgrafting care and have been previously published (4). Two dogs died of bacterial infection within 7 days of the transplant and were excluded from the analysis.

The results are shown in Table 1. The one dog receiving a dose of 1.0 mg/kg per day and the two dogs receiving a dose of 3.0 mg/kg per day had normal hematopoietic recovery, with kinetics similar to those seen when no ganciclovir was used. Although the three dogs that received a dose of 5.0 mg/kg per day recovered their absolute neutrophil counts with normal kinetics, there was an appreciable delay in platelet recovery. On average, these animals recovered platelet counts of 20,000/mm³ 10 days later than controls and platelet counts of 50,000 and 100,000/mm³ 12 days later than controls.

The data suggest that doses of up to 3.0 mg/kg per day of ganciclovir given prophylactically after autologous marrow transplantation are well tolerated but that higher doses may be associated with delayed recovery of platelet counts. The results of this study provide only a very general estimate. With only a few animals at each dose level, a dose-response curve could not be accurately drawn, and although the sensitivities of canine and human marrow to most agents are quite similar, it is possible that there are differences in the way the two species respond to ganciclovir. Furthermore, the marrow used here was autologous, unmanipulated marrow which had never been exposed to chemotherapy or radiation therapy before the transplant, a situation which may be quite different from that expected in the clinical setting of marrow transplantation in which ganciclovir would be used. Nonetheless, these studies suggest that doses of ganciclovir in the therapeutic range can be used in the dog during the immediate posttransplant period and thus provide support for beginning similar toxicity studies in humans.

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