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NOTES

Toxicity of 3′-Azido-3′-Deoxythymidine and 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine for Normal Human Hematopoietic Progenitor Cells In Vitro

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The effects of 3′-azido-3′-deoxythymidine (AZT) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine on myeloid and erythroid colony-forming cells were studied by clonogenic assays. Both consistently inhibited granulocyte-macrophage CFU (CFU-GM) and erythroid burst-forming units in a dose-dependent fashion. Concentrations of AZT and 9-(1,3-dihydroxy-2-propoxymethyl)guanine required for 50% inhibition of CFU-GM were, respectively, 0.9 ± 0.1 and 2.7 ± 0.5 μM; those required for 90% inhibition were, respectively, 34.0 ± 2.8 and 35.7 ± 3.6 μM. Erythroid burst-forming units were less sensitive to high concentrations of AZT than were CFU-GM.

3′-Azido-3′-deoxythymidine (AZT), an inhibitor of the human immunodeficiency virus reverse transcriptase, is, at the present time, one of the most promising drugs available for the treatment of acquired immunodeficiency syndrome (AIDS). 9-(1,3-Dihydroxy-2-propoxymethyl)guanine (DHPG), an inhibitor of the cytomegalovirus DNA polymerase (2), has shown encouraging results in the treatment of life-threatening cytomegalovirus infections, a frequent cause of severe morbidity in AIDS patients. The side effects of these agents and especially their myelosuppressive activities have been of some concern in preliminary clinical trials (3, 13). Surprisingly, little attention has been given thus far to the effects of these antiviral drugs on such "host" cells as those of bone marrow. This has led to our study of the effects of AZT and DHPG on the growth of normal human hematopoietic progenitor cells (specifically granulocytes-macrophages and erythrocytes) in vitro, with the results set forth in the present report.

Bone marrow cells obtained by aspiration from the posterior iliac crest of healthy volunteers who had given informed consent were anticoagulated with heparin and layered on a single-step Ficoll-Hypaque discontinuous gradient to remove mature erythroid and myeloid cells. The mononuclear cells, obtained from the interface of the gradient, were collected and washed twice in Hanks balanced salt solution.

The culture assay for granulocyte-macrophage CFU (CFU-GM) was performed by using the modified bilayer soft-agar system described by Metcalf et al. (9). Briefly, the bottom or feeder layer was composed of 0.5% agar (Bacto-Agar; Difco Laboratories, Detroit, Mich.) containing McCoy 5A nutrient medium supplemented with 15% dialyzed fetal bovine serum (heat inactivated at 56°C for 30 min) (GIBCO Laboratories, Grand Island, N.Y.) and 10% (final concentration) giant cell tumor-conditioned medium (GIBCO) as a source of colony-stimulating factor. The desired drug concentrations (or drug diluent for the control) were added to this layer, and 1-ml volumes of this mixture were poured into 35-mm plastic petri dishes. After solidification, a top layer (1 ml) was prepared which contained 0.3% agar, McCoy 5A nutrient medium supplemented with 15% fetal bovine serum, and 10^5 mononuclear cells per ml. The plates were incubated for 14 days in a humidified atmosphere of 5% CO_2–95% air at 37°C. Colonies consisting of 50 or more cells were scored as CFU-GM by using an inverted microscope. Toxicity data were processed by using least-squares linear regression analysis of the logarithm of drug concentration versus CFU-GM survival fraction.

The assay for erythroid progenitors was performed by using the modified technique of Iscove et al. (7). Briefly, mononuclear nonadherent cells (10^5 cells per ml) were cultured in Iscove modified Dulbecco medium–20% fetal bovine serum (heat inactivated at 56°C for 30 min), 0.5 U of sheep erythropoietin (Connaught Labs, Willowdale, Ontario, Canada) per ml–10% T-cell-line-conditioned medium (4) in a final concentration of 1% methylcellulose. Cells were plated in 1-ml portions in 35-mm plastic petri dishes containing 0.1-ml solutions of the investigated drugs. After 14 days of incubation in a humidified atmosphere of 5% CO_2–95% air at 37°C, colonies consisting of 30 or more hemoglobinized cells were counted. Colonies were confirmed as erythroid by using the benzidine technique (6). Toxicity data were processed by using least-squares linear regression analysis of the logarithm of drug concentration versus CFU-GM survival fraction.

Colony formation by CFU-GM was inhibited in a dose-dependent manner by continuous exposure to AZT and DHPG (Fig. 1). The 50% inhibitory dose of AZT was 0.9 ± 0.1 μM, and that of DHPG was 2.7 ± 0.5 μM. Their 90% inhibitory doses were essentially identical: 34.0 ± 2.8 μM for AZT and 35.7 ± 3.6 μM for DHPG. Acyclovir, an antiviral drug extensively used in the treatment of herpesvirus infections, served as a negative control, exhibiting a lack of toxicity for human bone marrow cells. It can be seen in Fig. 1 that more than 80% of the CFU-GM colonies were still

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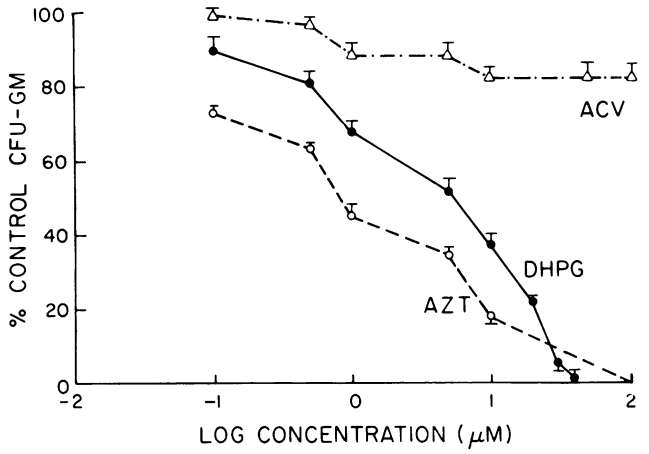

FIG. 1. Effects of continuous exposure (14 days) to increasing concentrations of AZT, DHPG, and acyclovir (ACV) on colony formation of human granulocyte-macrophage precursors grown in soft agar as compared with controls. The plating efficiency was approximately 0.1%. Each point represents the mean ± standard error of at least six experiments with different marrow donors.

viable, as compared with controls, in the presence of 100 μM acyclovir, this result being in agreement with results reported previously (8). AZT and DHPG were also tested for their effects on the growth of normal human bone marrow erythroid burst-forming units (Fig. 2). Although both agents had 50% inhibitory doses against these cells comparable to those against CFU-GM (2.4 ± 0.4 μM for AZT and 1.6 ± 0.2 μM for DHPG), they were much less active at the 90%-inhibitory-dose level, particularly AZT, of which a 208.8 ± 30.2 μM concentration was required to achieve a 90% inhibitory effect.

Chemotherapy represents one of the most potentially important approaches to the treatment of AIDS or AIDS-related infections; among the experimental drugs presently available, AZT and DHPG appear very promising. The activities of both drugs in virus-infected cell lines or virus-cell extract systems or both have been established (2, 10, 12). In contrast, detailed knowledge of their toxicity in normal host cells, particularly in normal human bone marrow, is nonexistent. This is particularly disturbing, because preliminary clinical trials with both drugs have suggested that myelosuppressive effects may be observed. However, this toxicity can be influenced by several factors, including the size of the marrow reserve and cell margination (1, 11), and does not necessarily reflect direct effects of AZT and DHPG on bone marrow cells. The importance of an evaluation of the direct toxicity of these antiviral agents in normal host cells is further emphasized by the facts that prolonged therapy will probably be required with these drugs and that additional depletion of hematopoietic stem cells may lead to increased risks of opportunistic infections in AIDS patients.

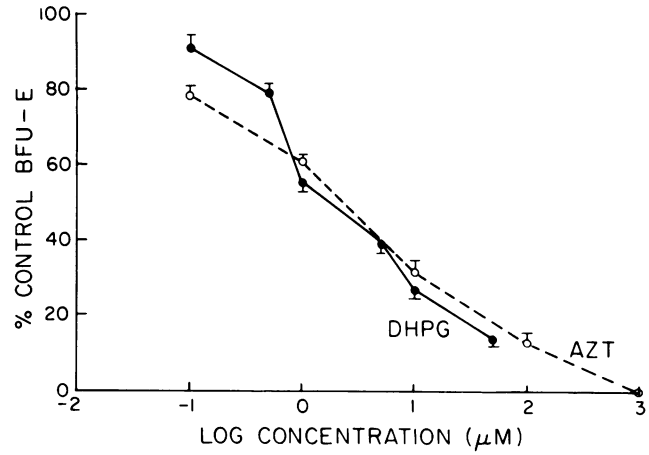

FIG. 2. Effects of continuous exposure (14 days) to increasing concentrations of AZT and DHPG on colony formation of human erythroid precursors grown in methylcellulose as compared with controls. The plating efficiency was approximately 0.05%. Each point represents the mean ± standard error of at least six experiments with different marrow donors. BFU-E, Erythroid burst-forming units.

The results of the present study demonstrate that AZT and DHPG have a direct, dose-dependent inhibitory effect on myeloid and erythroid human progenitor cells in vitro. Consistent inhibition of CFU-GM occurred with 0.9 ± 0.1 μM AZT and 2.7 ± 0.5 μM DHPG, and 90% inhibition occurred with 34.0 ± 2.8 μM AZT and 35.7 ± 3.6 μM DHPG. In AIDS patients with normal hepatic and renal functions, peak levels in plasma of approximately 4 to 6 μM have been reported after the administration of 2.5 mg of AZT per kg (13), and maximum levels in plasma of 15 to 30 μM have been detected after the administration of 5 mg of DHPG per kg (J.-P. Sommadossi, unpublished data). Although direct extrapolation of our in vitro studies to the clinical situation should be made with caution, we feel that 0.7 to 1 μM AZT and 2 to 3 μM DHPG in plasma may represent approximate thresholds for exerting cytotoxic effects on normal human myeloid cells. Erythroid precursors were slightly less sensitive than were myeloid precursors to the cytotoxic effects of both drugs. Of note was the significant increased toxicity of AZT in these human progenitor cells as compared with other cell lines (5, 10). This increased toxicity probably results from an increased conversion of AZT to its active triphosphate or from the presence of a larger fraction of rapidly dividing cells (S-phase) in human bone marrow progenitor cells as compared with the other cell lines (or both) (5, 10). The lack of toxicity of acyclovir in hematopoietic stem cells, consistent also with the absence of myelosuppression in vivo, further suggests that this study is of great interest as a predictive test of the toxicity of future anti-human immunodeficiency virus agents in normal tissues.

The present study provides the first analysis of the effects of AZT and DHPG on normal human hematopoietic cell proliferation in vitro. The data demonstrate the need to elucidate the mechanism of toxicity of these drugs as an important element in developing strategies for the therapy of AIDS or AIDS-related infections.

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