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NOTES

Lack of Toxicity of Acyclovir to Granulocyte Progenitor Cells In Vitro

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Granulocyte progenitor cells were grown with acyclovir to study potential marrow toxicity. Concentrations of up to 220 μM had little effect on progenitor cell growth.

Treatment of viral infections with the presently available antiviral agents is often complicated by hematological toxicity. Both 5-iodo-2'-deoxyuridine (2) and cytosine arabinoside (1, 15) have been associated with marrow toxicity, and, in addition, these two agents have failed to show efficacy in human studies. Adenine arabinoside has shown promise in the treatment of herpes simplex virus encephalitis (17), hepatitis B (10), and perhaps herpes zoster infection of the immunosuppressed host (16). However, some of these trials have also been complicated by marrow suppression (10, 11, 16). Acyclovir {9-[(2-hydroxyethoxy)methyl]guanine} is a new antiviral agent under investigation for the treatment of human herpesvirus infections. Because this agent requires virus-specified thymidine kinase for activation and because normal cellular thymidine kinase does not convert acyclovir to its active triphosphate form, toxicity to normal human cellular metabolism should be lower than with these other antiviral compounds (6, 12). However, the hematological sensitivity of patients receiving or recovering from cytotoxic chemotherapy may be greater than that of otherwise normal persons. Hematopoietic toxicity is of particular concern in patients receiving marrow transplants, many of whom develop severe herpesvirus infections (7-9) and could potentially benefit from an effective and nontoxic antiviral agent. To investigate the possible marrow toxicity of acyclovir, we studied its effect on the growth of granulocyte-monocyte colony-forming cells in vitro.

Bone marrow samples from 10 normal individuals were obtained by needle aspiration from the posterior iliac crest. Mononuclear cells were collected by Ficoll-Hypaque density separation and used as a source of granulocyte-monocyte precursor cells. Toxicity studies were carried out by using a modification of a previously described semisolid tissue culture technique (14). Mononuclear cells at a concentration of 10^5 cells per ml were plated in tissue culture dishes by using a 0.3% agar, 20% fetal calf serum, and alpha medium. Lymphocyte-conditioned medium was added at 10% concentration as a stimulator for colony growth. The same lot of stimulator was used throughout the study. Acyclovir was added at the time of plating to quadruplicate plates in concentrations ranging from 1×10^{-2} to 5×10^2 μg/ml. Control plates contained no acyclovir, but were otherwise identical. Colonies were grown in a humidified incubator with an atmosphere of 5% CO_2 in air at 37°C. At 14 days, colonies of 40 or more cells were scored by using an inverted microscope. The mean colony count of the quadruplicate samples at each drug concentration was expressed as a percentage of the colony count observed for the control (no drug) cultures. The results for each of the 10 marrows were then averaged to generate the toxicity curve.

The mean (± one standard deviation) of progenitor cell growth at each concentration is shown in Fig. 1. Acyclovir had no effect on the growth of granulocyte-monocyte colony-forming cells at concentrations of up to 44 μM (10 μg/ml), and even at a concentreation of 220 μM, progenitor cell growth averaged 78% of the control. Growth was progressively suppressed at higher concentrations, however.

These results are consistent with the study of Crumpacker et al. (4), who found that thymidine incorporation by a human fibroblast cell line (350 Q) was unaffected at a concentration of 200 μM, although thymidine incorporation by Vero cells was decreased at 50 μM. Collins and Bauer also reported inhibition of growth of Vero cells at concentrations higher than 50 μM (3). The

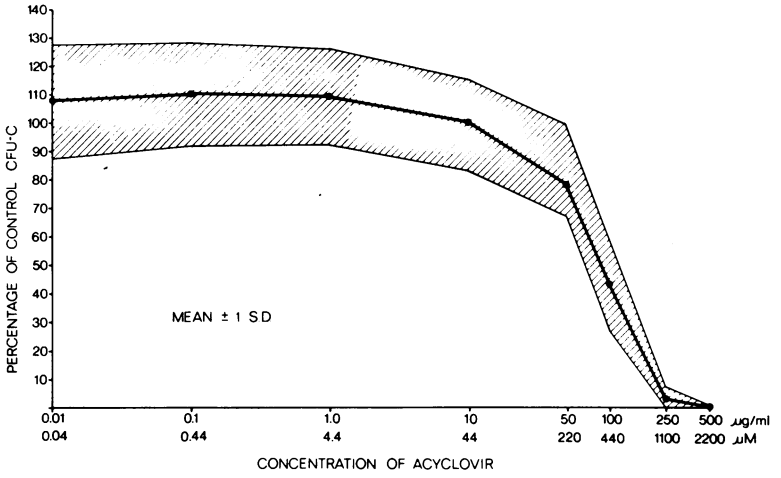

FIG. 1. *Effect of acyclovir on the growth of granulocyte-monocyte colony-forming cells (CFU-C) in vitro. Each point represents the mean of 10 determinations. The crosshatched area shows one standard deviation (S.D.). The data are expressed as the percentage of granulocyte progenitor cell growth compared with cultures that were not exposed to acyclovir.*

peak plasma level in patients with normal renal function being treated for herpes simplex virus or varicella-zoster virus infection is expected to be lower than 40 to 60 μM at a projected dose range of 5 to 10 mg/kg given every 8 h (5). This is 1/5 to 1/4 the concentration of acyclovir that caused even minimal in vitro suppression of granulocyte progenitor cells in this study. Crumpacker et al. (4) found the mean 50% infective dose for herpes simplex virus types 1 and 2 and varicella-zoster virus to be 0.15 μM, 1.62 μM, and 3.75 μM, respectively. Thus, the toxic-to-therapeutic ratios for the treatment of these three herpesviruses would be 1,450, 140, and 60, respectively.

Based on these data with granulocyte progenitor cells from normal persons, it is hoped that acyclovir will be safe for the treatment of herpes simplex virus and varicella-zoster virus infections even in patients expected to have unusually sensitive marrows. This is suggested by the results of preliminary uncontrolled trials in which even marrow transplant patients tolerated acyclovir administration (13). An exception may be patients with impaired renal function in whom higher plasma levels may occur (13) or patients treated with higher doses of acyclovir, for example, doses which might be required for human cytomegalovirus infection (4). Indeed, both the relative resistance of human cytomegalovirus to acyclovir and the possiblity of marrow suppression at higher plasma levels may make the treatment of human cytomegalovirus infection with acyclovir more difficult than treatment of herpes simplex virus and varicella-zoster virus infections.

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LITERATURE CITED

1. **Betts, R. F., D. A. Zaky, R. G. Douglas, Jr., and G. Royer.** 1975. Ineffectiveness of subcutaneous cytosine arabinoside in localized herpes zoster. Ann. Intern. Med. **82**:778–783.
2. **Boston Interhospital Virus Study Group and the National Institute of Allergy and Infectious Diseases-Sponsored Cooperative Antiviral Clinical Study.** 1975. Failure of high dose 5-iodo-2'-deoxyuridine in the therapy of herpes simplex virus encephalitis. Evidence of unacceptable toxicity. N. Engl. J. Med. **292**:599–603.
3. **Collins, P., and D. J. Bauer.** 1979. The activity *in vitro* against herpes virus of 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine) a new antiviral agent. J. Antimicrob. Chemother. **5**:431–436.
4. **Crumpacker, C. S., L. E. Schnipper, J. A. Zaia, and M. J. Levin.** 1979. Growth inhibition by acycloguanosine of herpesviruses isolated from human infections. Antimicrob. Agents Chemother. **15**:642–645.
5. **deMiranda, P., R. J. Whitley, M. R. Blum, R. E. Keeney, N. Barton, D. M. Cocchetto, S. Good, G. P. Hemstreet III, L. E. Kirk, D. A. Page, and G. B. Elion.** 1979. Acyclovir kinetics after intravenous infusion. Clin. Pharmacol. Ther. **26**:718–728.
6. **Elion, G. B., P. A. Furman, J. A. Fyfe, P. deMiranda, L. Beauchamp, and H. J. Schaeffer.** 1977. Selectivity of action of an anti-herpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A. **74**: 5716–5720.
7. **Meyers, J. D., H. C. Spencer, Jr., J. C. Watts, M. B. Gregg, J. A. Stewart, R. H. Troupin, and E. D. Thomas.** 1975. Cytomegalovirus pneumonia after human marrow transplantation. Ann. Intern. Med. **82**: 181–188.
8. **Neiman, P., P. B. Wasserman, B. B. Wentworth, G. F. Kao, K. G. Lerner, R. Storb, C. D. Buckner, R.**

A. Clift, A. Fefer, L. Fass, H. Glucksberg, and E. D. Thomas. 1973. Interstitial pneumonia and cytomegalovirus infection as complications of human marrow transplantation. Transplantation **15**:478–485.

9. **Neiman, P. E., W. Reeves, G. Ray, N. Flournoy, K. G. Lerner, G. E. Sale, and E. D. Thomas.** 1977. A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. J. Infect. Dis. **136**:754–767.
10. **Pollard, R. B., J. L. Smith, E. A. Neal, P. B. Gregory, T. C. Merigan, and W. S. Robinson.** 1978. Effect of vidarabine on chronic hepatitis B virus infection. J. Am. Med. Assoc. **239**:1648–1650.
11. **Rytel, M. W., and H. M. Kauffman.** 1976. Clinical efficacy of adenine arabinoside in therapy of cytomegalovirus infections in renal allograft recipients. J. Infect. Dis. **133**:202–205.
12. **Schaeffer, H. J., L. Beauchamp, P. deMiranda, G. B. Elion, D. J. Bauer, and P. Collins.** 1978. 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature (London) **272**:583–585.
13. **Selby, P. J., R. L. Powles, B. Jameson, H. E. M. Kay, J. G. Watson, R. Thornton, G. Morgenstern, H. M. Clink, T. J. McElwain, H. G. Prentice, R. Corringham, M. G. Ross, A. V. Hoffbrand, and D. Brigden.** 1979. Parenteral acyclovir therapy for herpesvirus infections in man. Lancet **ii**:1267–1270.
14. **Singer, J. W., P. J. Fialkow, L. Steinmann, V. Najfeld, S. J. Stein, and W. A. Robinson.** 1979. Chronic myelocytic leukemia (CML): failure to detect residual normal committed stem cells in vitro. Blood **53**:264–268.
15. **Stevens, D. A., G. W. Jordan, T. F. Waddell, and T. C. Merigan.** 1973. Adverse effect of cytosine arabinoside on disseminated zoster in a controlled trial. N. Engl. J. Med. **289**:873–878.
16. **Whitley, R. J., L. T. Ch'ien, R. Dolin, G. J. Galasso, C. A. Alford, Jr., Editors, and the Collaborative Study Group.** 1976. Adenine arabinoside therapy of herpes zoster in the immunosuppressed. NIAID Collaborative Antiviral Study. N. Engl. J. Med. **294**:1193–1199.
17. **Whitley, R.J., S.-J. Soong, R. Dolin, G. J. Galasso, L. T. Ch'ien, C. A. Alford, and the Collaborative Study Group.** 1977. Adenine arabinoside therapy of biopsy-proven herpes simplex encephalitis. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study. N. Engl. J. Med. **297**:289–294.