NOTES

Antitherpesviral Activity and Inhibitory Action on Cell Growth of 5-Alkenyl Derivatives of 1-β-d-Arabinofuranosyluracil

HARUHIKO MACHIDA,1* AKIRA KUNINAKA,1 HIROSHI YOSHINO,1 KAZUYOSHI IKEDA,2 AND YOSHIHISA MIZUNO2

Research Laboratory, Yamasa Shoyu Company Limited, Choshi, 288,1 and Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060,2 Japan

Antitherpesviral activity of 5-vinyl-1-β-d-arabinofuranosyluracil was as high as that of 1-β-d-arabinofuranosylthymine, whereas the former was less inhibitory to cell growth than the latter. 5-Propenyl- and 5-butenyl-1-β-d-arabinofuranosyluracil were less active than 5-vinyl-1-β-d-arabinofuranosyluracil.

Several thymidine analogs have been developed which inhibit selectively herpes simplex virus (HSV) replication without any significant action against cell growth (3–8, 11, 12). The selectivity is reported to be attributed to the fact that these compounds can be phosphorylated by the virus-induced deoxyribomycin nucleoside kinase but not by cellular thymidine kinase (1, 2, 13). In a previous paper (11), it was reported that among 5-alkyl derivatives of 1-β-d-arabinofuranosyluracil (ara-U), 5-ethyl-ara-U, as well as 1-β-d-arabinofuranosylthymine (ara-T), displayed a wide margin of safety. We have also synthesized 5-alkenyl derivatives of ara-U from 5-hydroxymethyl-ara-U (Ikeda et al., submitted for publication). In this communication, their inhibitory activities against both HSV replication and cell growth are described.

Human embryonic lung fibroblast (HEL-F) cells, kindly supplied by T. Kuwata, Chiba University, were used in this study. HSV type 1 (HSV-1) strain VR-3 and HSV type 2 (HSV-2) strain MS, kindly supplied by S. Yamazaki, National Institute of Health of Japan, and two isolates of HSV from patients with herpes keratitis (HSV WT-20 and HSV WT-34) (9), kindly supplied by T. Kurimura, Tottori University School of Medicine, were employed for antiviral experiments. Methods for cultivation of the cells and propagation of the viruses were described previously (10, 11). Antitherpesviral activity of the compounds was determined by a modified virus-rating method as described previously (11).

The antiviral activity was also expressed as the minimal discernible virus inhibitory concentration (MIC) of the compound at which HSV-induced cytopathogenic effect was depressed more than 50%. The inhibitory action on cell growth was determined after 4 days of incubation of exponentially growing HEL-F cells with various concentrations of the compound, and was expressed in terms of the 50% cell growth-inhibitory dose (ID₅₀) obtained graphically.

The antitherpesviral activity of 5-vinyl-ara-U was almost the same as that of ara-T (Table 1). Thus, 5-vinyl-ara-U is considered to be more effective than 5-ethyl-ara-U, 5-iododeoxyuridine, and arabinosyladenine which has been shown to be less active than ara-T (11). 5-Vinyl-ara-U was highly active against HSV-2 as well as against HSV-1. On the other hand, 5-propenyl-ara-U exhibited moderate activity and 5-butyl-ara-U showed relatively low activity against HSV-1. Their activities against HSV-2 were much lower than those against HSV-1. Thus, the antitherpesviral activity of 5-alkenyl derivatives of ara-U is concluded to decrease with increasing chain length in the substituent just like the activity of 5-alkyl derivatives of ara-U (11). Activity of 5-vinyl-ara-U against two isolates of HSV was compared with that of ara-T (Table 1). 5-Vinyl-ara-U was somewhat more active against HSV WT-34 than was ara-T, although it exhibited relatively low activity against HSV WT-20 which was resistant to 5-iododeoxyuridine (9) as well as to ara-T (11).

Influence of 5-alkenyl derivatives of ara-U on the growth of HEL-F cells was also tested (Table 1). The cell growth was inhibited by both ara-T and 5-vinyl-ara-U at high concentrations only. Although the ID₅₀ of ara-T was reported to be 150 μg/ml in a previous paper (11), it was shown to be 140, 75, and 85 μg/ml (average, 100 μg/ml) in three separate experiments. The ID₅₀ of 5-vinyl-ara-U was about sixfold that of ara-T. 5-Propenyl- and 5-butyl-ara-U scarcely inhibited the cell growth even at a concentration as high as 1,000 μg/ml.

The antiviral indexes (the ID₅₀ against HEL-F cells divided by the MIC against HSV-1) of ara-T, 5-vinyl-ara-U, 5-propenyl-ara-U, and 5-butyl-ara-U were 100, 620, more than 100, and
more than 31, respectively. In terms of the margin of safety, therefore, 5-vinyl-ara-U seems to be superior to ara-T.

Cheng (2) and Cheng et al. (3) reported that although 5-vinyl-deoxyuridine markedly inhibited replication of HSV-1 and HSV-2, it also inhibited the growth of WI-38 cells, a human diploid cell line derived from embryonic lungs, and of other cell lines (CV-1 and L1210 cells) at a concentration of 5 μM. On the other hand, 5-vinyl-ara-U did not inhibit at all the growth of HEL-F cells even at a concentration of 50 μg/ml (185 μM). Probably 5-vinyl-ara-U may be much more resistant to cellular thymidine kinase than the corresponding derivative of deoxyuridine. Alternatively, 5-vinyl-ara-U 5′-triphosphate, if 5-vinyl-ara-U would be phosphorylated intracellularly, could be much less inhibitory to cellular deoxyribonucleic acid polymerase than 5-vinyl-deoxyuridine 5′-triphosphate. Similarly, 5-propyl-ara-U (11) was also recognized to be less active than 5-propyl-deoxyuridine (6). Müller et al. (14, 15) reported that ara-T was phosphorylated not only by HSV-infected cells but also by noninfected cells, and it was a potent inhibitor of the growth of mouse leukemia cells L5178Y. However, as presented here and reported by others (1, 5, 8, 11, 13), inhibitory action of ara-T on cell growth is rather low in general. In addition, 5-vinyl-ara-U was less inhibitory to growth of HEL-F cells than ara-T, which was highly effective against HSV-induced encephalitis and had an extremely low toxicity for mice (10). Satisfactory chemotherapy with 5-vinyl-ara-U against HSV infection may be expected.

LITERATURE CITED

1. Aswell, J. F., G. P. Allen, A. T. Jamieson, D. E. Campbell, and G. A. Gentry. 1977. Antiviral activity of arabinosylthymine in herpesvirus replication: mechanism of action in vivo and in vitro. Antimicrob. Agents Chemother. 12:243-254.

2. Cheng, Y.-C. 1977. A rational approach to the development of antiviral chemotherapy: alternative substrates of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) thymidine kinase (TK). Ann. N.Y. Acad. Sci. 284:594-598.

3. Cheng, Y. C., B. A. Domin, R. A. Sharma, and M. Bobek. 1976. Antiviral action and cellular toxicity of four thymidine analogues: 5-ethyl-, 5-vinyl-, 5-propyl-, and 5-allyl-2′-deoxyuridine. Antimicrob. Agents Chemother. 10:119-122.

5. De Clercq, E., J. Descamps, P. De Somer, P. J. Barr, A. S. Jones, and R. T. Walker. 1979. (E)-5-(2-bromovinyl)-2′-deoxyuridine: a potent and selective antiviral agent. Proc. Natl. Acad. Sci. U.S.A. 76:2947-2951.

7. De Clercq, E., and D. Shugar. 1975. Antiviral activity of 5-ethyl pyrimidine deoxynucleosides. Biochem. Pharmacol. 24:1073-1078.

8. Gentry, G. A., and J. F. Aswell. 1975. Inhibition of herpes simplex virus replication by ara-T. Virology 65: 294-296.

12. Meldrum, J. B., V. S. Gupta, and J. R. Saunders. 1974. Cell culture studies on the antiviral activity of ether derivatives of 5-hydroxymethyldeoxyuridine. Antimicrob. Agents Chemother. 6:393-396.

15. Müller, W. E. G., R. K. Zahn, J. Arendes, and D. Falke. 1979. Phosphorylation of arabinofuranosylthymine in non-infected and herpes virus (TK- and TK+) infected cells. J. Gen. Virol. 43:261-271.