Synergistic Antiproliferative Effect of Interferons and Azidothymidine on HL60 Cells

Hiroko NAKAE* and Shozo ASADA

Kobe Women's College of Pharmacy, Motoyama Kitamachi, Kobe 658, Japan. *Received August 5, 1991*

Proliferation of human leukemia cells, HL60, was synergistically inhibited by a combination of human interferons and azidothymidine *in vitro*. Combination of interferon-γ (3000 U/ml) and azidothymidine (30 μM) inhibited cell growth by 76%, whereas interferon-γ alone suppressed growth by 23% and azidothymidine alone by 33%. Interferon-α-2a and interferon-β also exerted synergistic effects with azidothymidine, but the potentiation was weaker than that by the combination of interferon-γ and aziodthymidine.

Keywords interferon; azidothymidine; HL60; synergistic inhibition

Interferons are reported to exert synergistic antineoplastic effects when combined with cytotoxic drugs.[1] Although dideoxynucleosides are well-known antiviral agents, they are also reported to be cytotoxic.[2-4] We have investigated effects of the combination of human interferons and azidothymidine, a member of a family of dideoxynucleosides and found that cell growth of human leukemia cells, HL60, was synergistically inhibited by the combination.

Experimental

Materials Azidothymidine was purchased from Sigma Chemical Co. Recombinant human interferon-α-2a (IFN-α) was obtained from Takeda Chemical Industries, Ltd., human interferon-β-(IFN-β) from Toray Industries, Inc. and recombinant human interferon-γ(IFN-γ) from Shionogi & Co., Ltd.

Cells HL60 cells were supplied from Riken Gene Bank, Tsukuba, Japan. The cell line was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere containing 5% CO_2.

Growth Experiments One milliliter of cell suspension (6—8 × 10^4 cells/ml) and test compounds were added to wells of 24-well plates in triplicate. Cells were grown for 72 h and cell number was counted by a Coulter counter ZM. Results are the mean ± S.D. of three different experiments. Analysis of drug interactions was done according to previous studies.[5,6]

TABLE I. Effect of Human Interferons and Azidothymidine on Proliferation of HL60 Cells

Drug concentration		Inhibition of cell growth (%)
Interferon (U/ml)	Azidothymidine (μM)	
IFN-α		
3000	0	23 ± 5
10000	0	33 ± 6
0	100	46 ± 8
3000	100	65 ± 7
10000	100	75 ± 4
IFN-β		
3000	0	24 ± 5
0	30	31 ± 2
0	100	43 ± 11
3000	30	63 ± 7
3000	100	76 ± 10
IFN-γ		
3000	0	23 ± 8
0	30	33 ± 7
0	100	45 ± 9
3000	30	76 ± 5
3000	100	87 ± 4

Cell suspension (6—8 × 10^4/ml) and test compounds of 1 ml were incubated in 24-well plates in triplicate. Cell number was counted after 72 h by a Coulter counter. Results are the mean ± S.D. of three experiments.

Results

Proliferation of HL60 cells was inhibited by adding human interferons and/or azidothymidine as shown in Table I. IFN-α (10000 U/ml) suppressed cell growth by 33% after 72 h incubation, and azidothymidine (100 μM) by 46%. Combination of IFN-α and azidothymidine reduced cell growth by 75% which is greater than that which would be anticipated from an additive effect (64%). IFN-β (3000 U/ml) decreased cell growth by 24% and azidothymidine (30 μM) by 31%. Inhibition by a combination of IFN-β and azidothymidine was 63% which was much greater than that from an additive effect. IFN-γ showed stronger combined effect. IFN-γ (3000 U/ml) alone depressed cell growth by 23% and azidothymidine (30 μM) by 33%. Combination of the two drugs inhibited cell growth by 76% whereas 49% is anticipated from an additive effect. Experiments with other concentrations of drugs showed similar effects. From these results it is concluded that the combination of the interferons and azidothymidine produced synergistic antiproliferative effects. Among the three interferons, combination with IFN-γ exhibited the most prominent effect.

Discussion

It was reported that recombinant interferon-α exhibited enhanced antiviral effect on human immunodeficiency virus when combined with azidothymidine or dideoxycytidine.[7,8] Antivital effects of dideoxynucleosides are assumed to be caused by the metabolites, 5'-triphosphates, which act as a terminator of DNA (deoxyribonucleic acid) synthesis. The mechanism for the cytotoxicity, however, is less clear, though the inhibition of thymidylate kinase might contribute to the cytotoxicity as proposed by Furman *et al.*[9] Therefore, it is obscure what mechanisms operate in the potentiated antiproliferative effect by combinations of the drugs shown in this study. Many modes of interaction can be envisioned. A specific biochemical interaction might modulate the activity of interferons or azidothymidine and act on cell growth, replication or differentiation. The present results showed that the combination with interferon-γ produced a more dominant effect than other interferons, which might give a clue to an understanding of the interactions.

References

1) S. Wadler and E. L. Schwartz, *Cancer Res.*, **50**, 3473 (1990).
2) J. Balzarini, R. Pauwels, M. Baba, P. Herdwijn, E. de Clercq, S. Broder and D. G. Johns, *Biochem. Pharmacol.*, **37**, 897 (1988).

3) H. W. Cook and M. W. Spence, *Neurochem. Res.*, **14**, 279 (1989).
4) A. Fridland, M. C. Connelly and R. Ashmun, *Mol. Pharmacol.*, **37**, 665 (1990).
5) J. H. Schiller, D. G. Groveman, S. M. Schmid, J. K. V. Willson, K. B. Cummings and E. C. Borden, *Cancer Res.*, **46**, 483 (1986).
6) L. Elias and H. Crissman, *Cancer Res.*, **48**, 4868 (1988).
7) K. L. Hartshorn, M. W. Vogt, T-C. Chou, R. S. Blumberg, R. Byington, R. T. Schooley and M. S. Hirsh, *Antimicrob. Agents Chemother.*, **31**, 168 (1987).
8) M. W. Vogt, A. G. Durgo, T-C. Chou, L. A. Coleman, T. J. Paradis, R. T. Schooley, J. C. Kaplan and M. S. Hirsh, *J. Infect. Dis.*, **158**, 378 (1988).
9) P. A. Furman, J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. N. Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya and D. W. Barry, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8333 (1986).