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Short Communication

ENHANCEMENT BY HYDROXYUREA OF THE ANTI-HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 POTENCY OF 2′-β-FLUORO-2′,3′-DIDEOXYADENOSINE IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Abstract—Ribonucleotide reductase inhibitors such as hydroxyurea (HU) and related compounds, at low, non-toxic doses, enhance the anti-human immunodeficiency virus type 1 (HIV-1) potency of both purine and pyrimidine 2′, 3′-dideoxynucleosides (ddNs) in human lymphocytes and macrophages. The most marked enhancement of inhibition of HIV-1 replication reported to date has been seen with the purine ddN 2′,3′-dideoxyinosine (ddIno): a low level of HU (0.1 mM) permitted a 4.5-fold reduction in optimal ddIno dosage with no decrease in therapeutic effect or increase in toxicity. We report here even more marked enhancement by HU of the potency of the purine ddN 2′-β-fluoro-2′,3′-dideoxyadenosine (2′-β-F-ddAdo), where the addition of 0.1 mM HU permitted a 7.1-fold reduction in the optimal dose of 2′-β-F-ddAdo in the phytohemagglutinin-activated peripheral blood mononuclear cell HIV-1 test system.

Key words: hydroxyurea; hydroxamates; human immunodeficiency virus type 1 (HIV-1); 2′,3′-dideoxyinosine; 2′-β-fluoro-2′,3′-dideoxyadenosine; peripheral blood mononuclear cells

Several groups have reported recently that HU§ and also hydroxamates increase the potency of purine and pyrimidine ddNs against HIV-1 [1–3]. Of the HU/ddN combinations examined to date, the most effective (in terms of the decrease in concentration of ddN required to produce a standard reduction of viral p24 antigen) has been that with the anti-HIV agent ddIno, and clinical trials of the ddIno/HU combination are currently in progress [2]. With ddIno, a low concentration of HU (0.1 mM) decreased the IC_{90} value of ddIno by 4- to 6-fold in the PHA/PBM system [1, 3]. Despite this increase in potency, there was no increase in the intracellular level of the pharmacologically active 5′-phosphorylated anabolite of ddIno, ddATP‖. The combination of ddIno/HU thus offers the potential advantage of a several-fold reduction of those toxicities of ddIno that arise directly from the parent drug without loss of the anti-HIV-1 effect due to its 5′-triphosphate. The marked potentiation of ddIno by HU relative to that seen with other clinically significant ddNs such as 3′-azido-3′-deoxythymidine and 2′,3′-dideoxycytidine appears to be due to a well-known property of HU (and other ribonucleotide reductase inhibitors), i.e. selective reduction of dATP intracellular pools at concentrations that produce very slight decreases of, or even increases in, the pool sizes of other dNTPs [4, 5]. Since the antiretroviral activity of ddNs as reverse transcriptase inhibitors and viral DNA chain terminators is determined not by the absolute ddNTP level but by the ddNTP/dNTP ratio, it follows that the depletion of dATP would selectively favor the antiviral potency of those purine ddNs, such as ddIno and ddAdo, that give rise to the pharmacologically active metabolite ddATP.

Other anti-HIV purine ddNs, however, can generate active 5′-triphosphate metabolites that compete with dATP. In this short communication, we report the unusually marked potentiation by HU of the ddAdo analogue 2′-β-F-ddAdo, an anti-HIV-1 agent that exerts its antiviral effect through the active metabolite 2′-β-F-ddATP [6, 7].

Materials and Methods

All chemicals used were of reagent grade. HU and PHA were obtained from the Sigma Chemical Co. (St. Louis, MO). ddIno was supplied by Dr. Karl Flora, Developmental Therapeutics Program, National Cancer Institute. 2′-β-F-ddAdo was synthesized in this laboratory [8, 9]. Recombinant interleukin-2 was purchased from R & D Systems (Minneapolis, MN). Radioimmunoassay kits for p24 Gag (group-specific antigen) protein were purchased from DuPont (Boston, MA). PBM were isolated from heparinized venous blood of healthy donors and were incubated for 72 hr with PHA (10 μg/mL) in RPMI 1640 medium supplemented with 15% heat-inactivated fetal bovine serum, 15 units/mL recombinant interleukin-2, 4 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. An HIV-1 strain ($ERS104_{pre}$) was isolated, as described previously, from a patient with advanced HIV-1 infection, before antiviral therapy, at the National Cancer Institute [10]. PHA-stimulated PBM were plated

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§ Abbreviations: ddIno, 2′,3′-dideoxyinosine; ddNs, 2′,3′-dideoxynucleosides; 2′-β-F-ddAdo, 2′-β-fluoro-2′,3′-dideoxyadenosine; HIV-1, human immunodeficiency virus type 1; HU, hydroxyurea; PBM, peripheral blood mononuclear cells; and PHA, phytohemagglutinin.

‖ Gao W-Y, Johns DG and Mitsuya H, unpublished data.

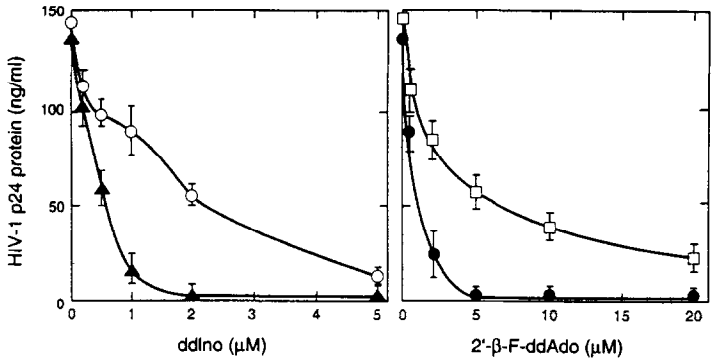

Fig. 1. Enhancement of anti-HIV-1 activities of ddIno (left panel) and 2′-β-F-ddAdo (right panel) by 0.1 mM HU. PHA/PBM were incubated with ddIno alone (○) and ddIno and HU (▲) or with 2′-β-F-ddAdo alone (□) and 2′-β-F-ddAdo and HU (●) for 24 hr before HIV-1 infection. The virus-containing medium was harvested at day 8, and the level of HIV-1 p24 antigen protein was determined by radioimmunoassay. Data represent mean values ± SD for three PBM donors, with quadruplicate determinations in each experiment.

in 24-well tissue culture plates at a density of 1×10^6 cells/well. Drugs (ddIno or 2′-β-F-ddAdo) were added in 2 mL of supplemented RPMI 1640 medium. After incubation for 24 hr, cells were exposed to 2500 HIV-1 50% tissue culture infective doses/well, and half of the culture medium was replaced with fresh culture medium containing the same concentrations of drugs on day 4 after infection. On day 8, the medium was harvested and the amount of p24 protein was determined by radioimmunoassay. All assays were conducted in quadruplicate.

Results and Discussion

The anti-HIV-1 activities of ddIno and 2′-β-F-ddAdo were compared in the presence and absence of 0.1 mM HU, a concentration that we have previously observed to be non-toxic for as long as 10 days in the PHA/PBM system [1]. As seen in Fig. 1, 0.1 mM HU alone showed only slight (<10%) inhibition of HIV-1. However, this concentration of HU reduced the HIV-1 IC_{90} for ddIno 4.5-fold, from 5.0 to 1.1 μM, whereas it reduced the HIV-1 IC_{90} for 2′-β-F-ddAdo by 7.1-fold, from 26.1 to 3.7 μM. Complete inhibition of p24 antigen production by 2′-β-F-ddAdo was seen at 8–10 μM.

2′-β-F-ddAdo, although an analogue of ddAdo and ddIno, differs significantly in its properties from the latter compounds. Unlike ddAdo and ddIno, it is acid-stable, and while a substrate for adenosine deaminase, its rate of deamination by this enzyme is much slower than that of ddAdo [6], thus permitting significant cellular uptake of the drug in its more lipid-soluble non-deaminated form. The deamination product, 2′-β-F-ddIno, unlike ddIno, is not susceptible to cleavage by purine nucleoside phosphorylase. While the greater part of the deaminated drug is readily phosphorylated to the 5′-monophosphate by the same 5′-nucleotidase utilized by ddIno, unlike ddIno, an appreciable fraction of the parent dideoxyadenosine analogue is also 5′-monophosphorylated directly by 2′-deoxycytidine kinase [11]. Although all these factors combine to give a much greater intracellular concentration of the pharmacologically active 5′-triphosphate of 2′-β-F-ddAdo than is obtained with ddIno [12], it is not clear how these properties may relate to the greater susceptibility of 2′-β-F-ddAdo to synergism with HU. Elucidation at the molecular level of the factors responsible for the greater potentiation noted with the 2′-β-fluoro derivative, and the examination of HU effects on other ddAdo and ddIno analogues are currently in progress.

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REFERENCES

1. Gao W-Y, Johns DG and Mitsuya M, Anti-human immunodeficiency virus type 1 activity of hydroxyurea in combination with 2′,3′-dideoxynucleosides. *Mol Pharmacol* **46:** 767–772, 1994.
2. Malley SD, Grange JM, Hamedi-Sangsari F and Vila JR, Synergistic anti-human immunodeficiency virus type 1 effect of hydroxamate compounds with 2′,3′-dideoxyinosine in infected resting human lymphocytes. *Proc Natl Acad Sci USA* **91:** 11017–11021, 1994.
3. Lori F, Malykh A, Cara A, Sun D, Weinstein JN, Lisziewicz J and Gallo RC, Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. *Science* **266:** 801–805, 1994.
4. Bianchi V, Pontis E and Reichard P, Changes of deoxyribonucleoside triphosphate pools induced by hydroxyurea and their relation to DNA synthesis. *J Biol Chem* **261:** 16037–16042, 1986.
5. Slabaugh MB, Howell ML, Wang Y and Mathews CK, Deoxyadenosine reverses hydroxyurea inhibition of vaccinia virus growth. *J Virol* **65:** 2290–2298, 1991.
6. Masood R, Ahluwalia GS, Cooney DA, Fridland A, Marquez VE, Driscoll JS, Hao Z, Mitsuya H, Perno C-F, Broder S and Johns DG, 2′-Fluoro-2′,3′-dideoxyarabinosyladenine: A metabolically stable analogue of the antiretroviral agent 2′,3′-dideoxyadenosine. *Mol Pharmacol* **37:** 590–596, 1990.
7. Hitchcock MJM, Woods K, DeBoeck H and Ho H-T, Biochemical pharmacology of 2′-fluoro-2′,3′-dideoxyarabinosyladenine, an inhibitor of HIV with improved metabolic and chemical stability over 2′,3′-dideoxyadenosine. *Antiviral Chem Chemother* **1:** 319–327, 1990.
8. Marquez VE, Tseng CK-H, Kelley JA, Mitsuya H, Broder S, Roth JS and Driscoll JS, 2′,3′-Dideoxy-2′-fluoro-ara-A: An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). *Biochem Pharmacol* **36:** 2719–2722, 1987.
9. Marquez VE, Tseng CK-H, Mitsuya H, Aoki S, Kelley JA, Ford H, Roth JS, Broder S, Johns DG and Driscoll JS, Acid-stable 2′-fluoro purine dideoxynucleosides as active agents against HIV. *J Med Chem* **33:** 978–985, 1990.

10. Shirasaka T, Yarchoan R, O'Brien MC, Husson RN, Anderson BD, Kojima E, Shimada T, Broder S and Mitsuya H, Changes in drug sensitivity of human immunodeficiency virus type 1 during therapy with azidothymidine, dideoxycytidine, and dideoxyinosine: An *in vitro* comparative study. *Proc Natl Acad Sci USA* **90:** 562–566, 1993.
11. Eriksson S, Kierdaszuk B, Munch-Petersen B, Öberg B and Johansson NG, Comparison of the substrate specificities of human thymidine kinase 1 and 2 and deoxycytidine kinase toward antiviral and cytostatic analogs. *Biochem Biophys Res Commun* **176:** 586–592, 1991.
12. Johns DG, Ahluwalia GS, Cooney DA, Mitsuya H and Driscoll JS, Enhanced stimulation by ribavirin of the 5′-phosphorylation and anti-human immunodeficiency virus activity of purine 2′-β-fluoro-2′,3′-dideoxynucleosides. *Mol Pharmacol* **44:** 519–523, 1993.