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Selective protection of toxicity of 2′,3′-dideoxypyrimidine nucleoside analogs by β-D-uridine in human granulocyte-macrophage progenitor cells

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Abstract

β-D-Uridine protected human granulocyte-macrophage lineage cells in both semi-solid (granulocyte-macrophage colony-forming units, CFU-GM) and liquid cultures against the toxic effects of 3′-azido-3′-deoxythymidine (AZT), 3′-fluoro-3′-deoxythymidine (FLT) and a combination of AZT and FLT, without impairment of the activities of these respective drugs against human immunodeficiency virus (HIV) replication. In addition, β-D-uridine also protected human CFU-GM against toxicity of the in vivo AZT metabolite, 3′-amino-3′-deoxythymidine (AMT). β-L-uridine and α-D-uridine, two stereoisomers of the natural form, and the base uracil, were unable to protect cells against either AZT or FLT toxicity, whereas β-D-uridine-5′-bis(SATE)phosphotriester, a prodrug of β-D-uridine-5′-monophosphate, successfully protected cells against AZT toxic effects, suggesting that β-D-uridine needs to be metabolized to its nucleotides to exert a pharmacological effect. These data suggest in addition that AZT, FLT and AMT share a common target site(s) of toxicity involved in myelosuppression.

Keywords: β-D-uridine; CFU-GM; AZT; AMT; FLT

3′-Azido-3′-deoxythymidine (zidovudine, AZT) was the first drug to demonstrate clinical benefits against human immunodeficiency virus (HIV) infections in controlled long-term clinical trials (Fischl et al., 1987; Yarchoan et al., 1986). However, AZT treatment is limited on account of its toxic effects on bone marrow cells, manifested by anemia and neutropenia (Gil et al., 1987; Richman et al., 1987). Although decreased AZT dosage has

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been recommended, these toxicities remain substantial, resulting in the need for transfusion, additional dose reduction or interruption of treatment (Fischl et al., 1990; Collier et al., 1990). 2′,3′-Dideoxycytidine (zalcitabine, ddC), 2′,3′-dideoxyinosine (didanosine, ddI) and recently 2′,3′-didehydro-3′-deoxythymidine (stavudine, D4T) have been approved for second-line monotherapy treatment of HIV infections in patients who are intolerant of AZT. Clinical trials of 3′-fluoro-3′-deoxythymidine (FLT) have been discontinued due to severe hematotoxicity (Flexner et al., 1994) which was consistent with our in vitro studies demonstrating that FLT was a most toxic compound in human CFU-GM liquid cultures (Faraj et al., 1994). In the same context, our group demonstrated that AZT had a direct inhibitory effect on the growth of human CFU-GM and BFU-E in soft-agar clonogenic assays at concentrations approximating 1 μM (Sommadossi and Carlisle, 1987), as subsequently confirmed by several other groups (Dainiak et al., 1988; Du et al., 1990; Ganser et al., 1989; Johnson et al., 1988). Our laboratory has also demonstrated that an endogenous natural nucleoside, β-D-uridine, was able to reverse and to protect the AZT toxicity in human CFU-GM cells without impairment of the anti-HIV activity of the drug (Sommadossi et al., 1988). This β-D-uridine protection was also confirmed in human lymphoid cells (Cox, 1991; Szebeni et al., 1991). Other approaches including use of cytokines such as IL-1, IL-3 and GM-CSF have been shown to overcome the toxicity of AZT by stimulating the growth of bone marrow cells (Gallicchio et al., 1991). In contrast, no enhancement of colony number was observed in β-D-uridine treated cultures (Sommadossi et al., 1988), suggesting that β-D-uridine may directly affect a major toxic target site of AZT. We have recently developed a liquid culture system of human granulocyte-macrophage lineage cells using pluripotent progenitor CD34$^+$ cells purified from bone marrow cells (Faraj et al., 1994). These CD34$^+$ cells undergo extensive proliferation for 14 days and the differentiated cells exhibit normal morphological features in response to specific hematopoietic growth factors. The ranking of several 2′,3′-dideoxynucleosides (ddNs) for toxic effects assessed in these liquid cultures were in agreement with data obtained by using semi-solid cultures (Faraj et al., 1994).

The present report describes the protection of the toxic effects of AZT, FLT and 3′-amino-3′-deoxythymidine (AMT) by β-D-uridine, using both semi-solid agar and liquid cultures. Bone marrow cells were obtained by aspiration from the posterior iliac crest of healthy volunteers. The methodologies for both assays have previously been described in detail (Faraj et al., 1994; Sommadossi and Carlisle, 1987). α-D-Uridine was synthesized as previously described by Debart et al. (1992). β-L-Uridine was synthesized from 1-*O*-acetyl-tri-*O*-benzoyl-L-ribofuranose through coupling with silylated uracil, followed by deacylation with methanolic ammonia and crystallization: m.p. 163–165°C; $[\alpha]^{20} + 12.1$ (0.9, DMSO). Its chemical and physical characteristics were in accordance with reported values (Hóly, 1973; Wu and Chargaff, 1969). β-D-uridine-5′-bis(*S*-acetyl-2-thioethyl)phosphotriester (UMP-S) (Fig. 1) was synthesized by condensing the corresponding diisopropyl phosphoramidite diester with 2′,3′-*O*-methoxymethylidene-β-D-uridine, followed by subsequent in situ oxidation and deprotection as recently reported with other nucleosides (Lefebvre et al., 1995). UMP-S was fully characterized by ^{1}H and ^{31}P NMR, UV and mass spectrometry and its purity was confirmed by analytical HPLC. β-D-Uridine, UMP-S and the test drugs were added at day 1 of all experiments. In the absence of tested drugs, viability of control cells cultured for 14 days was greater than 95% as measured by

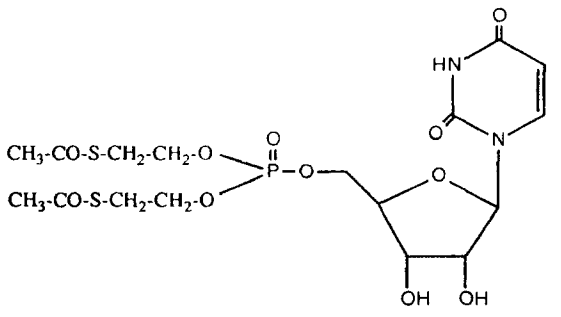

Fig. 1. Chemical structure of Uridine 5′-bis(SATE)phosphotriester or UMP-S.

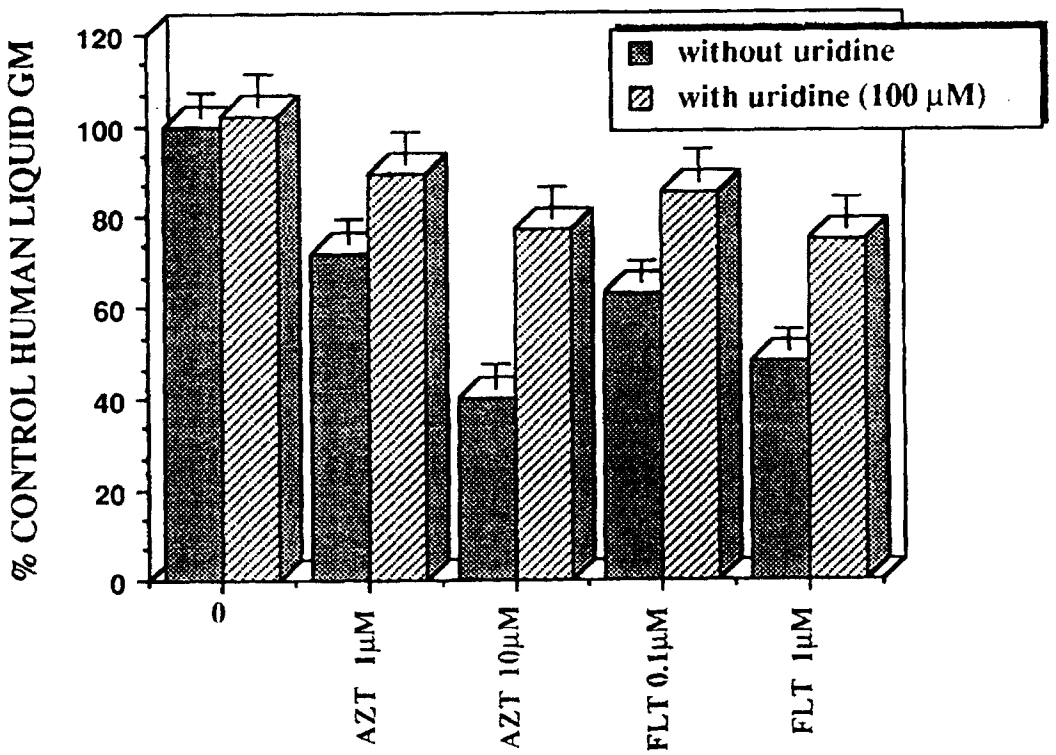

Fig. 2. Effect of AZT and FLT on human granulocyte-macrophage lineage cells in liquid cultures in the presence or the absence of uridine (100 μM) after 14 days of incubation. The experimental conditions are reported elsewhere (Faraj et al., 1994); bars represent standard deviations of at least three separate experiments.

a tryptan blue exclusion method. The anti-HIV cell based assay measured the level of reverse transcriptase associated with the clarified supernatant obtained from cells infected with HIV as previously described (Sommadossi et al., 1988). Fig. 2 shows the protection of uridine at a concentration of 100 μM against the toxic effects of AZT and FLT in human granulocyte-macrophage lineage cell cultures after 14 days of incubation. The achieved protection was almost complete with 100 μM β-D-uridine when cells were exposed to either 1 μM AZT or 0.1 μM FLT, drug concentrations which approximate their toxic 30% inhibitory concentration (IC_{30}) values. The protective effect of 100 μM of β-D-uridine was also extremely pronounced in the presence of higher concentrations of tested drugs with a 40% protection against an IC_{60} of 10 μM of AZT and a 30% protection against an IC_{60} of 1 μM of FLT. However, at these higher drug concentrations, β-D-uridine protection was substantially incomplete, consistent with the multifactorial mechanism(s) of ddNs-induced host toxicity (Sommadossi, 1993). As previously demonstrated with AZT (Sommadossi et al., 1988), 10 μM of thymidine also reduced the toxicity induced by FLT (data not shown), possibly by competing for FLT phosphorylation, as suggested by the decreased anti-HIV activity of FLT in the presence of thymidine (Table 1). In contrast, a concentration of 0.01 μM of FLT which represented an antiviral 90% effective concentration (EC_{90}) value, was not affected by a concentration as high as 100 μM of uridine (Table 1). AMT, a metabolite of AZT detected in vitro (Cretton et al., 1991a), in monkeys (Cretton et al., 1991b) and in AZT-treated patients (Staag et al., 1992), was shown by our group to be at least 5–7-fold more toxic to

Table 1
Effect of thymidine and uridine on FLT anti-retroviral activity in HIV-infected human peripheral blood mononuclear cells

Treatment	Concentration(μM)	Inhibition(%)	EC_{50} [a](μM)
FLT	0.001	39.0	
	0.01	87.5	
	0.1	98.0	0.002
Thymidine	10	6.7	
	100	18.5	>100
Uridine	10	0	
	100	4.8	>100
FLT/Thymidine	0.01/10	70.1	
	0.01/100	24.0	
FLT/Uridine	0.01/10	94.3	
	0.01/100	82.9	

[a] EC_{50}, effective concentration of the drug that inhibits 50% of replicating HIV in human PBM cells. The experimental conditions were as described in detail by Sommadossi et al. (1988). The variability for the data was less than 15%.

both human CFU-GM and BFU-E when compared with AZT (Cretton et al., 1991a). Fig. 3 demonstrates that β-D-uridine was also able to protect human bone marrow cells against the toxicity induced by AMT, in a clonogenic assay, under conditions similar to those reported for AZT protection by uridine (Sommadossi et al., 1988).

A combination of FLT and AZT has been reported to exhibit a synergistic inhibition of HIV replication in vitro (Cox, 1992; Harmenberg et al., 1990). Therefore, it was of interest to evaluate whether β-D-uridine could still protect the marrow toxicity induced by the combination of these two hematotoxic compounds. As shown in Fig. 3, β-D-uridine exerted a protection by at least 20% against the toxicity induced by a combination of FLT and AZT at ratios of 1:10 and 1:1. Of particular importance is the finding that the toxicity induced in human bone marrow cells by a combination of FLT and AZT at these concentrations was no greater than that induced by either AZT 1 μM (IC_{40}), FLT 0.1 μM (IC_{30}) or FLT 1 μM (IC_{60}) alone, as shown in Fig. 3. Therefore, these data demonstrate that a concentration of 100 μM β-D-uridine can protect pronounced hematotoxicity, as assessed by liquid granulocyte-macrophage lineage cells and semi-solid CFU-GM assays, in the presence of FLT alone or in combination with AZT.

In the search for the mechanism(s) underlying the β-D-uridine reversal effects, we also attempted to protect ddN-induced toxicity with β-L-uridine or α-D-uridine, two stereoisomers of the natural form. No effect on AZT and FLT toxicity in human granulocyte-macrophage lineage cells was observed with either β-L-uridine or α-D-uridine, suggesting that β-D-uridine has to be metabolized to nucleotides to prodice a pharmacological effect. Consistent with that hypothesis, the uracil base, a degradation product of β-D-uridine, did not protect against the toxic effects of AZT or FLT. Furthermore, UMP-S, a prodrug of β-D-uridine-5′-monophosphate, successfully protected human granulocyte-macrophage lineage cells against the toxic effects of AZT in a dose-dependent manner (Fig. 4). The degree of protection of UMP-S was higher than that observed with an identical concentration of 100 μM of β-D-uridine. The SATE derivative represents a new prodrug concept which allows the intracellular delivery of the monophosphate derivative of the selected nucleoside (Lefebvre et al., 1995; Perigaud et al., 1993, 1994).

In conclusion, these data demonstrate that β-D-uridine, without interfering with ddN antiviral activity, substantially protects human granulocyte-macrophage lineage cells from the toxic effects of AMT, AZT, FLT and a combination of the latter two anti-HIV agents, suggesting that these compounds may exert their cytotoxicity

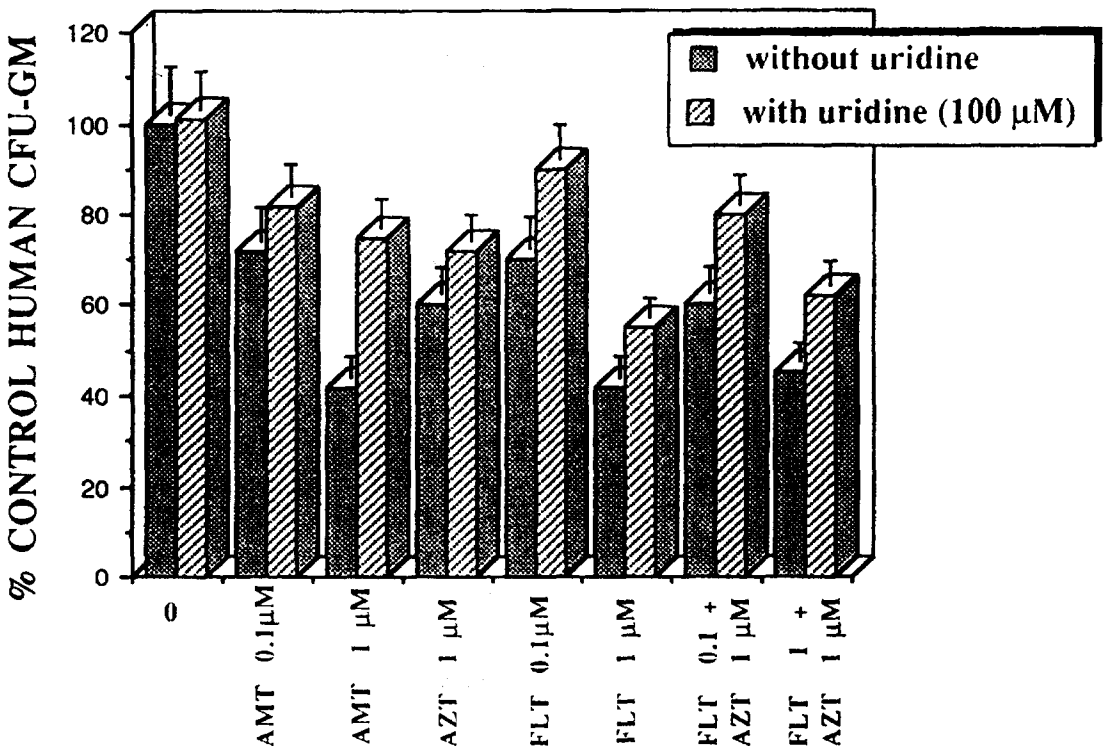

Fig. 3. Effect of AMT and a combination of AZT with FLT on human CFU-GM clonogenic assays (soft-agar) in the presence or the absence of uridine (100 μM) after 14 days of incubation. The experimental conditions are reported elsewhere (Sommadossi et al., 1988); bars represent standard deviations of at least three separate experiments.

events through a similar target site. The observed protection of UMP-S against AZT toxicity demonstrates in addition that β-D-uridine needs to be activated to its nucleotides to exert a pharmacological effect. Strategies are being investigated to further validate this selective chemotherapeutic protection, in order to decrease the hematotoxicity of these related compounds in AIDS patients (Sommadossi et al., 1995).

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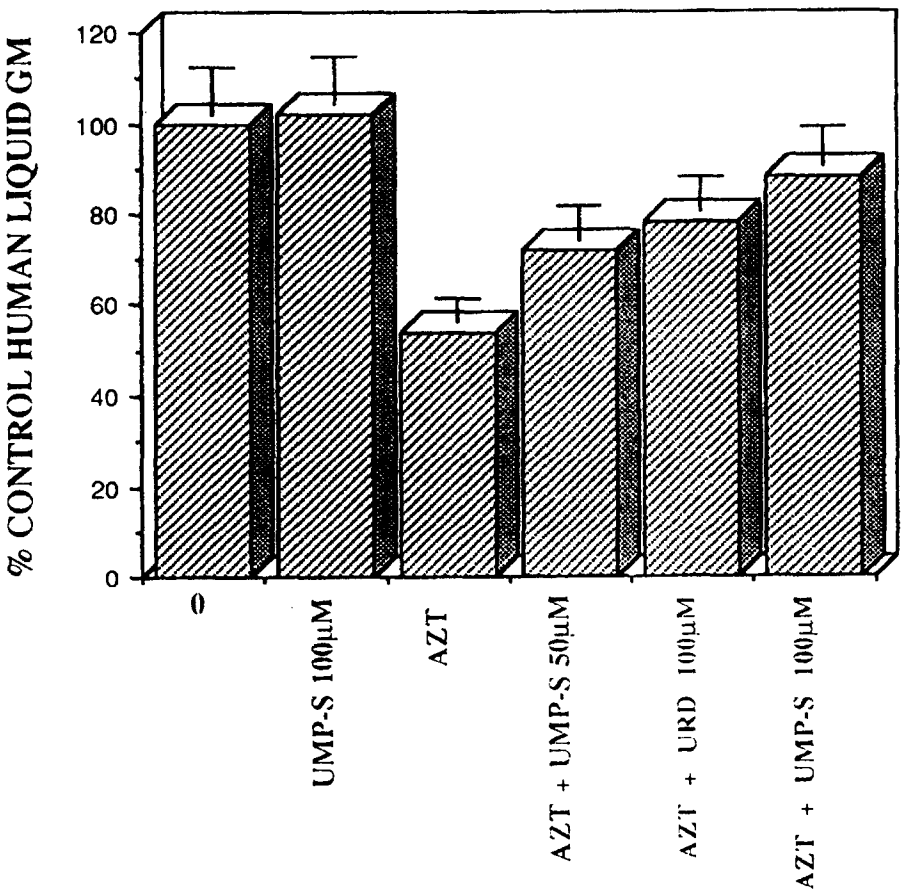

Fig. 4. Effect of 5 μM AZT on human granulocyte-macrophage lineage cells in liquid cultures in the presence or the absence of β-D-uridine-5′-bis(SATE)phosphotriester (UMP-S) or β-D-uridine after 14 days of incubation. The experimental conditions are reported elsewhere (Faraj et al., 1994); bars represent standard deviations of at least three separate experiments.

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