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POTENTIATING EFFECT OF {2-[2-[(2-AMINO-1,6-DIHYDRO-6-OXO-9H-PURIN-9-YL)METHYL]-PHENYL]ETHENYL}-PHOSPHONIC ACID (MDL 74,428), A POTENT INHIBITOR OF PURINE NUCLEOSIDE PHOSPHORYLASE, ON THE ANTIRETROVIRAL ACTIVITIES OF 2′,3′-DIDEOXYINOSINE COMBINED TO RIBAVIRIN IN MICE

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Abstract—2′,3′dideoxyinosine (ddI) has potent activity against human immunodeficiency virus (HIV) but is rapidly metabolized by erythrocytic purine nucleoside phosphorylase (PNP), and therefore has a very short plasma half-life in rodents, monkeys and in patients with acquired immunodeficiency syndrome. It is now reported that 100 μM {2-[2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methyl]-phenyl]ethenyl}-phosphonic acid (MDL 74,428), a very potent inhibitor of PNP blocks the intracellular phosphorolysis of ddI in cultured human red blood cells, in T leukemic CEM lymphoblasts and prolongs ddI plasma effective concentration in mice at a dose of 250 mg/kg body weight given i.p. In MDL 74,428-treated CEM cells, despite marked reduction of ddI catabolism, neither further accumulation of ddATP, the active antiviral metabolite of ddI, nor potentiation of the activity of ddI against HIV cytopathogenicity is observed. MDL 74,428 does not also affect the inhibitory effect of ddI combined with ribavirin on the transformation *in vitro* of C3H/3T3 cells by Moloney murine sarcoma virus (MSV). In mice, on the contrary, MDL 74,428 (200 mg/kg body weight, i.p.) is effective at potentiating the effect of ribavirin used either alone, or combined with ddI on MSV-induced tumour formation and associated mortality. However, in the absence of ribavirin, co-administration of MDL 74,428 with ddI affords, no chemotherapeutic advantage.

Key words: 2′,3′-dideoxyinosine; ribavirin; human immunodeficiency virus; murine sarcoma virus; purine nucleoside phosphorylase inhibitor

A number of 2′,3′-dideoxynucleosides inhibit the *in vitro* infectivity and cytopathic effects of HIV,‡ the causative agent of AIDS. Of the dideoxynucleosides, ddI exhibits favourable therapeutic ratios *in vitro* [1, 2], has demonstrated positive responses in AIDS-suffering patients [3] and has been recently approved to market by U.S. Food and Drug Administration for treatment of adult and paediatric AIDS patients who are intolerant to AZT.

The antiviral activity of ddI is assumed to be mediated by its intracellular conversion to ddATP [2, 4], a potent inhibitor of HIV reverse transcriptase [5]. Conversion of ddI to ddATP is, however, rate-limiting but is improved by combination of ddI with ribavirin [6, 7], an IMP dehydrogenase inhibitor possessing a broad antiviral spectrum. Intracellular accumulation of IMP, a phosphate donor in the enzymatic transformation of ddI to its monophosphate derivative, has been proposed as the mechanism of action of the ribavirin potentiating effect [6].

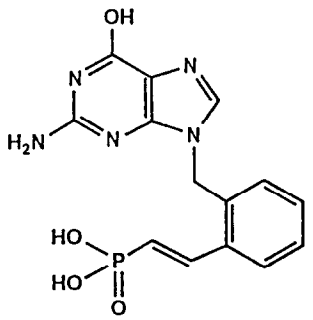

Fig. 1. Structure of MDL 74,428.

However, ddI is a substrate of purine nucleoside phosphorylase (PNP) both *in vitro* [8] and in cultured cells [4]. These observations are likely to be the biochemical basis for the short plasma half-life of ddI in rodents [9, 10], humans [11] and monkeys [12], due to its rapid and extensive catabolism by

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‡Abbreviations: PNP, purine nucleoside phosphorylase (EC. 2.4.2.1.); ddI, 2′,3′-dideoxyinosine; ddATP, 2′,3′-dideoxyadenosine-5′-triphosphate; MDL 74,428, {2-[2-[(2-amino - 1,6 - dihydro - 6 - oxo - 9H - purin - 9 - yl)methyl] - phenyl]ethenyl}-phosphonic acid; HIV, human immunodeficiency virus; MSV, Moloney murine sarcoma virus; AIDS, acquired immunodeficiency syndrome; AZT, 3′-azido-2′,3′-dideoxythymidine

erythrocytic PNP [13]. Therefore, a potent inhibitor of PNP, such as MDL 74,428 [14] (Fig. 1), should potentiate the antiviral activity of ddI by inhibition of both lymphocytic and erythrocytic PNP.

It is reported here that MDL 74,428 blocks ddI catabolism both in human leukemic and red blood cells cultured *in vitro*, prolongs ddI plasma concentration in mice but fails to potentiate the antiviral activity of ddI in mice infected with MSV. However, in this *in vivo* model of retrovirus infection, MDL 74,428 enhances the antiviral activity of ribavirin and, more markedly, of ribavirin co-administered with ddI.

MATERIALS AND METHODS

Chemicals. Bovine insulin, human transferrin, bovine serum albumin and [8-^{14}C]ddI (59.7 Ci/mol) were from Sigma Chemical Co. (St Louis, MO, U.S.A.).[2′,3′-^{3}H]ddI (34.2 Ci/mmol) was from Amersham International (Amersham, U.K.). RPMI 1640 medium and foetal calf serum were from Gibco (Gaitherbourg, MD, U.S.A.). Unlabelled ddI, MDL 74,428 and 9-(5,5-difluoro-5-phosphonopentyl) guanine were synthesized at Marion Merrell Dow Research Institute (Strasbourg, France) by Dr S. Halazy. Stock solutions (10 mM) of MDL 74,428 were prepared by dissolving the compound in 0.05 N NaOH for cell culture studies. For *in vivo* experiments 1.7% (w/v) MDL 74,428 solution in 3% $NaHCO_3$ was used.

Cells. Human T leukemic CCRF-CEM lymphoblasts were routinely grown in suspension in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum in a humidified incubator under CO_2/air atmosphere (5%, v/v) at 37°. For ddI metabolic studies, cells were cultured in serum-free medium but supplemented with 20 μg/mL bovine insulin, 50 μg/mL human transferrin and 2 mg/mL bovine serum albumin to prevent extracellular catabolism of ddI by contaminating PNP present in serum. Under this condition cells grew with a population doubling time of 24 hr.

As described [15, 16], fresh blood from healthy volunteers was collected on heparin, centrifuged at 200 *g*, washed twice at room temperature in Puck's saline and then once with Ca^{2+}-free Tyrode solution supplemented with 17.9 mM $NaHCO_3$, 1 g/L glucose and 2 g/L bovine serum albumin pre-equilibrated at 37° in a CO_2/air atmosphere (5%, v/v) in a humidified incubator. Cultures were then established by resuspension of the cell pellet in Tyrode solution at a cell density of 5×10^9 cells/mL.

Animals. Male CD_1-mice (30–40 g; Charles River, St-Aubin-les-Elbeuf, France) were used for acute toxicity and pharmacokinetic studies of ddI and MDL 74,428. The animals were housed in metal cages with free access to food and water, under constant temperature (21–23°), humidity (45–55%) and a 12 hr light/12 hr dark cycle. Newborn NMRI mice were used for MSV infection studies.

Metabolism of ddI in cultured cells. CCRF-CEM cell cultures (2×10^5 cells/mL) were pre-incubated for 24 hr in the presence or absence of 100 μM MDL 74,428. Thereafter, cultures were labelled for 24 hr with [2′,3′-^{3}H]ddI for the determination of intracellular dideoxynucleoside and its phosphorylated derivatives, i.e. ddATP or [8-^{14}C]2′,3′-ddI for the measurement of purine nucleotides derived from ddI. At the end of labelling, cells were chilled on ice, centrifuged and the cell pellet washed twice with cold PBS. Cellular nucleotides were extracted overnight at −20° by 60% methanol, as described elsewhere [17]. Following evaporation of the methanolic extracts and their solubilization in water, nucleotides and ddI were analysed by HPLC according to Stocchi *et al.* [18] and Osborne *et al.* [19], respectively. Nucleotide—and ddI—associated radioactivities were measured by the flowthrough method using a Berthold LB 506-C, HPLC radioactivity monitor (Berthold France, S.A. Elancourt, France) equipped with an Epson PcAX computer.

Human red blood cell cultures (5×10^9 cells/mL) were pre-incubated for 3 hr in a Wheaton roller (Wheaton Instruments Millville, NJ, U.S.A.) at 37° under a CO_2/air atmosphere (5%, v/v) in the presence or absence of varying concentrations of MDL 74,428. Then, cultures were labelled for 3 hr with an initial concentration of 10 μM [8-^{14}C]2′,3′-ddI (59.7 Ci/mol). At given intervals, one aliquot part of the cell culture was mixed with two parts of cold methanol and centrifuged at 4°. Supernatants were then evaporated under nitrogen and analysed by HPLC [19]. ddI radioactivity was determined by the flowthrough method as described above.

HIV-induced cytopathogenicity and MSV infectivity in vitro. HIV-1-induced cytopathogenicity was studied in CCRF-CEM cells as previously described [6, 7]. For MSV infectivity, murine C3H3/3T3 cells were seeded in 1 cm^2 wells at a cell density of 5×10^5 cells/mL and cultured for 1 day. At that time cultures were infected with 80 focus-forming units of MSV for 1 hr at 37°, as described elsewhere [7, 21]. Thereafter, the medium was changed and the cells were incubated in the presence or absence of the drugs for 6 days. Transformation was determined visually by light microscopy.

Pharmacokinetics of MDL 74,428 or ddI in mice. MDL 74,428 (250 mg/kg body weight, i.p.) or ddI (25 mg/kg body weight, s.c.) was administered to CD_1 mice (30–40 g). After mouse decapitation, 1 mL blood was collected in tubes at 0° containing 20 μL 0.25 M EDTA, pH 7.4, and 10 μL 1 mM 9-(5,5-difluoro-5-phosphonopentyl) guanine, a PNP inhibitor [14]. Tubes were then centrifuged at 1000 *g* for 20 min at 4°, and plasma kept frozen at −20°. For determination of MDL 74,428 or ddI, plasma was first submitted to pre-column purification under vacuum on to an Analytichem Bond Elut C18 extraction column (Varian, Harbor City, CA, U.S.A.) [20] and then analysed by reverse-phase HPLC, as described for plasma nucleoside and base analyses [19]. Under this condition ddI and MDL 74,428 eluted at 22 and 31 min, respectively. MDL 74,428 and ddI concentrations were calculated with reference to calibration curves obtained by post-column UV recording at 254 nm of respective standard concentration solutions. Recoveries of MDL 74,428 and ddI were 90 and 95%, respectively.

Antiviral activity in MSV-infected NMRI mice. Two- or three-day-old NMRI mice (2 g) were

Table 1. Effects of MDL 74,428 on ddI metabolism in CCRF-CEM leukemic cells cultured *in vitro*

Addition	Intracellular radiolabelled metabolites (pmol/10^6 cells)						
	DDI	ddATP	ADP	ATP	GTP	Unknown	Total
Experiment 1							
5 μM ddI	3.7 ± 0.03 (3.8)	0.11 ± 0.01 (0.1)	18.5 ± 1.4 (19.1)	51.7 ± 2.6 (53.3)	7.2 ± 0.4 (7.4)	15.8 (16.3)	97.0
5 μM ddI+ 100 μM MDL 74,428	6.2 ± 0.5 (87.6)	0.13 ± 0.01 (1.8)	ND*	0.75 ± 0.1 (10.6)	ND	ND	7.1
Experiment 2							
0.5 μM ddI	0.2 (8.0)	0.007 (0.2)					
5.0 μM ddI	2.3 (7.3)	0.09 (0.2)					
0.5 μM ddI+ 100 μM MDL 74,428	0.5 (82.0)	0.005 (0.6)					
5.0 μM+ 100 μM MDL 74,428	5.9 (80.0)	0.08 (0.7)					

* ND, not detected.

Cell cultures (2×10^5 cells/mL) were pre-incubated for 24 hr in the presence or absence of 100 μM MDL 74,428. Thereafter, cultures were further incubated for 24 hr in the presence of [8-^{14}C]2',3'-ddI (59.7 Ci/mol) for the determination of ddI and purine nucleotides or [2',3'-^{3}H]ddI (34.2 Ci/mmol) for determination of [2',3'-^{3}H]ddATP (experiment 1).

In experiment 2, cells were identically manipulated except that tritiated ddI only was used. Results of experiment 1 are the means of triplicate cultures run in parallel ± SE. For experiment 2 results are representative of a typical experiment and are the means of duplicate cultures; identical samples differed by no more than 10%. Numbers in parentheses represent the percentage relative to total radiolabelled metabolites

inoculated s.c. in the left hind leg with 50 μL MSV (100 focus-forming units, as measured by *in vitro* determination of the virus-induced transformation of murine C3H/3T3 embryo fibroblast cells). At 4–5 days after infection, tumours developed and rapidly increased in volume upon further ageing of the mice. The initial tumour formation was scored by visual inspection of the left hind legs of the MSV-infected mice. Within 10 to 12 days after infection, mice died from viral infection. Drug treatment started 2–3 hr before virus inoculation and was continued, once daily, for 4 days.

RESULTS

Inhibition of ddI catabolism in CCRF-CEM cells

As seen in Table 1 (experiment 1), when cells were incubated for 24 hr in the presence of 5 μM radiolabelled ddI, most of the intracellular radioactivity (80%) was associated with ribonucleotides, ATP being formed predominantly (52 pmol/10^6 cells). The remainder of the ddI-derived metabolites were found as ADP, GTP and, as little as 0.10 pmol/10^6 cells, as ddATP. ddI, the parent compound, accounted only for 3.8% of the total radioactivity. Pre-incubation of the cells for 24 hr in the presence of 100 μM MDL 74,428, followed by addition of ddI, reduced by 98.5% the formation of radiolabelled ATP. Under this condition most (80–88%) of the intracellular radioactivity remained as ddI, and no radiolabelled ADP or GTP were detected. MDL 74,428 reduced intracellular radioactivity by 93% and elevated only 1.7–2.6-fold the absolute ddI content of the cells. This increase did not, however, cause further accumulation of ddATP, relative to MDL 74,428- untreated cells. This was also true when ddI was used at 10-times lower extracellular concentration (0.5 μM) (Table 1, experiment 2) or when the medium was supplemented with 10% (v/v) foetal calf serum (not shown). In the second experiment, using ddI tritiated at the dideoxyribose moiety, some radioactivity was found associated with ribonucleotides but was not determined. Similar observations have been made previously [4]. It is likely that this radioactivity resulted from some contaminating ddI labelled at the purine moiety since a marked reduction was observed in the presence of MDL 74,428. Under all experimental conditions, labelled ddAMP and ddADP could not be determined with sufficient accuracy due to chromatographic interferences.

Effect of MDL 74,428 on the activity of ddI against HIV-induced cytopathogenicity in CCRF-CEM lymphoblasts and against transformation by MSV of C3H/3T3 cells

ddI protected lymphoblasts against the cytopathic effect of HIV at a 50% effective concentration (EC_{50}) of 4 μM. MDL 74,428, up to 500 μM, added 30 min or 17 hr before ddI administration, did not modify this EC_{50} value.

ddI exerted modest inhibitory effects on the transformation of murine C3H/3T3 cells by MSV with an EC_{50} value of about 85 μM. Ribavirin (8 μM)

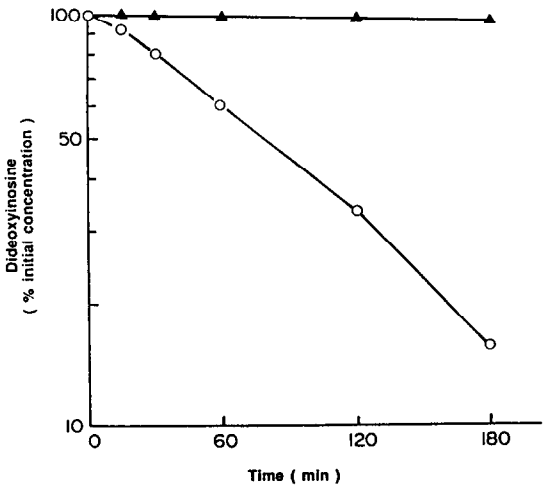

Fig. 2. Inhibition by MDL 74,428 of ddI catabolism by human red blood cells cultured *in vitro*. Human red blood cell cultures (5×10^9 cells/mL) were first pre-incubated for 3 hr in the absence (○) or presence (▲) of 100 μM MDL 74,428, and further incubated with an initial concentration of 10 μM [8-^{14}C]2′,3′-ddI (59.7 Ci/mol) (100%). At the indicated time, aliquot parts of the cell cultures were withdrawn and processed, as described in Materials and Methods, for the determination of ddI.

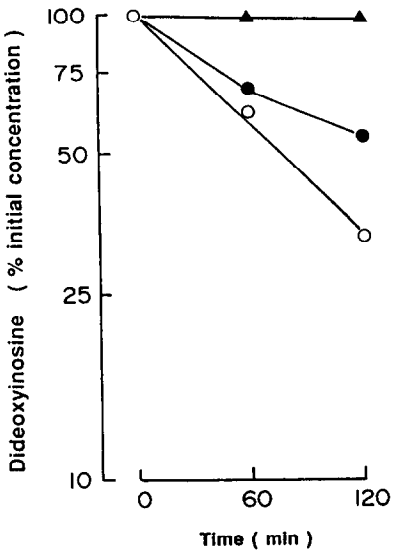

Fig. 4. Effects of the pre-incubation time on inhibition of ddI catabolism by MDL 74,428 in human red blood cells cultured *in vitro*. Human red blood cell cultures were pre-incubated in the absence (○) or presence (▲) of 100 μM MDL 74,428 for 3 hr. At that time 100 μM MDL 74,428 was added to portions of MDL 74,428-untreated cultures (●) and ddI catabolism determined as described in the legend to Fig. 2.

potentiated this activity, reducing 4-fold the EC_{50} value for ddI. MDL 74,428, at a concentration up to 250 μM, did not affect the antiviral activity of ddI used either alone or combined with ribavirin.

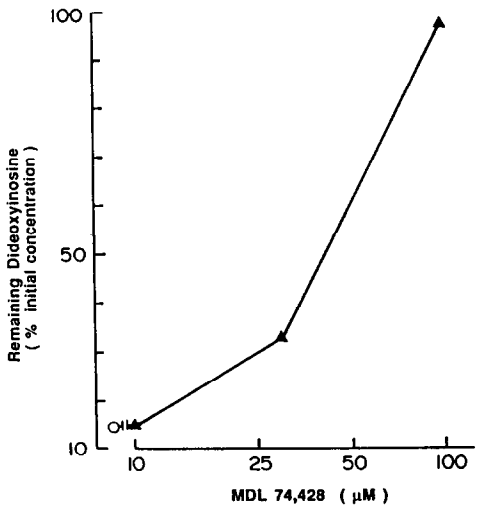

Fig. 3. Dose-related effects of MDL 74,428 on catabolism of ddI in human red blood cells cultured *in vitro*. Human red blood cell cultures (5×10^9 cells/mL) were first pre-incubated for 3 hr in the presence of increasing concentrations of MDL 74,428 and further incubated for 3 hr with 10 μM radiolabelled ddI as described in the legend to Fig. 2.

Inhibition of ddI catabolism in human red blood cells cultured in vitro

ddI (10 μM) was catabolized by human red blood cells cultured *in vitro* with a half-life of 75 min (Fig. 2). MDL 74,428 (100 μM), added to the cell culture 3 hr before ddI, entirely blocked ddI catabolism during the following 3 hr incubation period. This protective effect was dose-related (Fig. 3) and dependent on the duration of cell pre-incubation in the presence of MDL 74,428 (Fig. 4).

Effect of MDL 74,428 *on plasma concentration of ddI in* CD_1 mice

In a preliminary study, plasma concentration of MDL 74,428 was determined following i.p. injection to CD_1 mice of MDL 74,428 (250 mg/kg body weight) (Fig. 5A). Maximum concentration of MDL 74,428 (350 μM) was reached at 30 min, and was maintained for up to 80 min, after administration. Thereafter, MDL 74,428 declined with a half-life of 25 min.

As shown in Fig. 5B, the plasma concentration of ddI was 50 μM 10 min after s.c. administration of 25 mg/kg body weight to CD_1 mice. Thereafter, the ddI concentration decreased rapidly with an elimination half-life of 9 min to reach a value of 3.6 μM 40 min after administration. MDL 74,428 (250 mg/kg body weight, i.p.) given 60 min before ddI maintained the ddI plasma concentration above

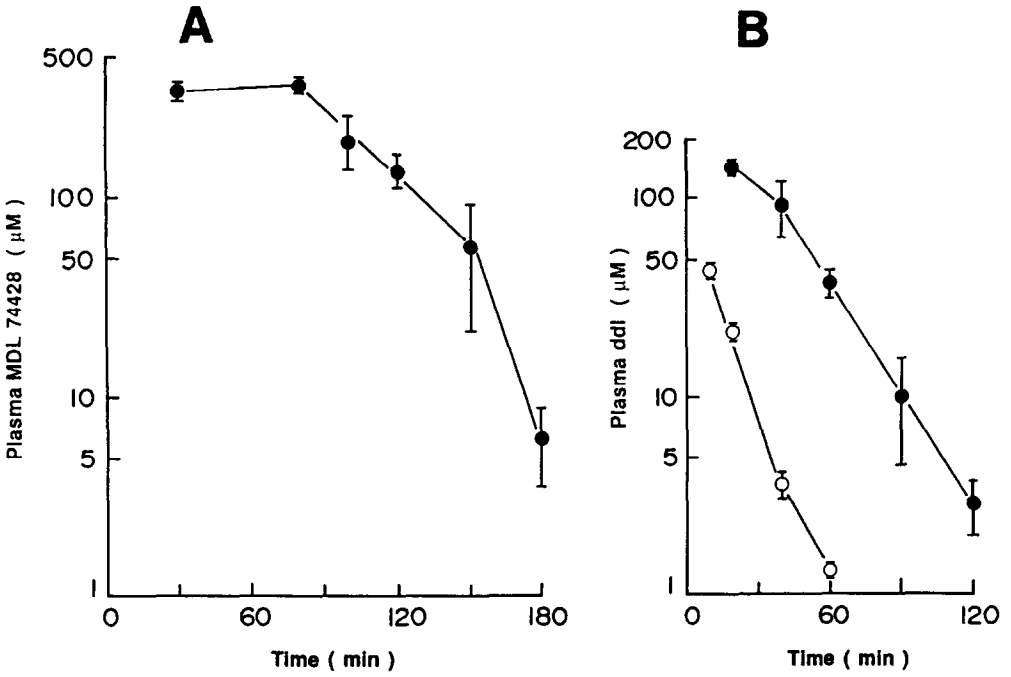

Fig. 5. Plasma concentration of MDL 74,428 (A) and of ddI in CD_1 mice following i.p. administration of MDL 74,428 (B). A. MDL 74,428 (250 mg/kg body weight) was injected i.p. to groups of CD_1 mice (N = 6). At the indicated time, MDL 74,428 plasma concentration was determined as described in Materials and Methods. Results are the means ± SE corrected from the recovery of MDL 74,428. B. CD_1 mice were given s.c. injections of ddI (○) (25 mg/kg body weight). When combined with ddI (●) MDL 74,428 (250 mg/kg body weight) was injected i.p. 60 min prior to ddI. Plasma ddI was determined at the indicated time, as described in Materials and Methods. Results are the means ± SE (N = 6).

100 μM for at least 40 min. Plasma ddI then decreased with an elimination half-life of 16 min.

Potentiation by MDL 74,428 of ddI or ribavirin antiviral activity in MSV-infected newborn NMRI mice

As shown in Table 2, ddI (100 mg/kg body weight, i.p., once daily for 4 days), MDL 74,428 (200 mg/kg body weight, i.p., once daily for 4 days) or a combined treatment by ddI and MDL 74,428 caused no delay in tumour appearance and did not affect the survival time of MSV-infected mice. Combination of ddI with ribavirin (40 mg/kg body weight, i.p. once daily for 4 days) delayed slightly, but significantly ($P < 0.05$), tumour initiation (T/C = 1.28) and increased the life span of the mice (T/C = 1.11). This combined treatment was not, however, significantly more effective than ribavirin used alone. On the contrary, MDL 74,428 co-administered with ribavirin was significantly ($P < 0.05$) more effective than ribavirin used alone and potentiated markedly the antiviral activity of ribavirin plus ddI, this triple combination being the most effective treatment tested. None of the treatment regimens showed signs of overt toxicity.

DISCUSSION

The present study demonstrates that MDL 74,428, a potent PNP inhibitor [14] protects ddI—a 2',3'-dideoxynucleoside presently undergoing clinical trials for the treatment of AIDS—against extensive catabolism both *in vitro* and *in vivo*, and potentiates *in vivo* the antiretroviral activity of the purine nucleoside when combined with ribavirin.

The results reported herein show that ddI is drastically metabolized by human red blood cells cultured *in vitro* and is rapidly eliminated from the plasma when administered to mice, in agreement with recently reported findings [9, 13]. This is likely due to its phosphorolysis by the very abundant circulating erythrocytic PNP [22], even if ddI is a weak substrate of PNP relative to inosine [8]. In fact, MDL 74,428 entirely blocks ddI breakdown by human erythrocytes cultured *in vitro* and causes maintenance of plasma ddI concentration in mice well above that required for maximum antiviral effect.

Duration of action of MDL 74,428 correlates well with its effective plasma concentration (100 μM), which is maintained at least for 2 hr after administration. That blockade of ddI catabolism results from inhibition of intraerythrocytic PNP, and not from complete inhibition of ddI cellular uptake, and is demonstrated by the fact that MDL 74,428 does not afford similar protection when added concomitantly with ddI to the erythrocyte culture. Moreover, this result suggests relatively slow intra-erythrocytic accumulation of MDL 74,428.

Table 2. Inhibitory effects of ddI, ribavirin, MDL 74,428, administered alone or in combination, on mean tumour initiation and survival times in MSV-infected newborn NMRI mice

Compound		Mean tumour initiation time (days)	T/C	Mean survival time (days)	T/C
None	(N = 59)	4.7 ± 0.1		10.5 ± 0.1	
ddI	(N = 30)	5.1 ± 0.2	1.09	10.7 ± 0.2	1.02
Ribavirin	(N = 26)	5.7 ± 0.2*	1.21	11.3 ± 0.2*	1.08
ddI + ribavirin	(N = 23)	6.0 ± 0.2*	1.28	11.7 ± 0.2*	1.11
MDL 74,428	(N = 39)	4.5 ± 0.1	0.96	10.2 ± 0.2	0.97
MDL 74,428 + ribavirin	(N = 35)	6.4 ± 0.2*†	1.36	12.1 ± 0.2*†	1.15
MDL 74,428 + ddI	(N = 20)	5.0 ± 0.2	1.06	10.9 ± 0.2	1.04
MDL 74,428 + ddI + ribavirin	(N = 48)	7.6 ± 0.2*†‡§	1.62	13.7 ± 0.2*†‡§	1.30

$P < 0.05$

* Relative to control.

† Relative to ribavirin.

‡ Relative to ribavirin + MDL 74,428.

§ Relative to ddI + ribavirin.

Newborn NMRI mice were inoculated by MSV, as described in Materials and Methods. Animals were given i.p. injections of MDL 74,428 (200 mg/kg body weight), ribavirin (40 mg/kg body weight), ddI (100 mg/kg body weight) once daily for 4 days, beginning 3 hr before virus inoculation. When combined with ribavirin, MDL 74,428 was administered 1–2 hr prior to ddI. Results are the means ± SE of three separate experiments. Statistical analysis was performed by Tukey's Studentized Range (HSD) test on the variation of mean tumour initiation time and mean survival time.

ddI exerts its potent activity against HIV due to its intracellular conversion to ddATP [2, 4], a powerful inhibitor of reverse transcriptase [5]. However, as shown in the present study, and in agreement with previous findings [4], only a tiny amount of the ddI added to the culture medium of cultured T-lymphoblasts is transformed to ddATP. This data demonstrates that, under this condition, ddI is indeed mostly metabolized to ribonucleotides after phosphorolysis by cellular PNP. Inhibition of PNP activity by MDL 74,428 markedly reduces ddI cellular catabolism, but only moderately increases ddI intracellular concentration. The 14-fold reduction of intracellular ddI-derived radioactivity observed in MDL 74,428-treated cultures may not be related to a direct inhibitory effect of the PNP inhibitor on ddI cellular penetration. In the absence of the PNP inhibitor most of the ddI entering the cells by passive diffusion [4] is converted mainly to phosphorylated metabolites (i.e. ATP, GTP) which remain trapped in the intracellular compartment. On the contrary, in the presence of MDL 74,428, which blocks this metabolic pathway, most of the unphosphorylated ddI-derived radioactivity may diffuse in and out of the cells until equilibrium between the intracellular and the extracellular compartments is reached. 8-Aminoguanosine, another inhibitor of PNP, has been reported to exert similar effects on the uptake of 2′,3′-dideoxyguanosine in cultured H9 cells [23]. Moreover, the fact that ddATP does not further accumulate in the presence of MDL 74,428 suggests that intracellular IMP, the phosphate donor for ddI phosphorylation catalysed by 5′-nucleotidase [2], rather than ddI, is rate-limiting for the formation of ddIMP. However, it should also be considered that a PNP inhibitor may not only block the phosphorolysis of ddI to hypoxanthine but also that of inosine. Since ddI shows maximum velocity of phosphorylation of 2% the rate observed with inosine [24], possible increases of inosine content should compete favourably with ddI for phosphorylation by 5′-nucleotidase. Altogether these considerations may give an explanation for the failure of MDL 74,428 to potentiate the *in vitro* activity of ddI against HIV.

In vivo, but not *in vitro*, MDL 74,428 potentiates the activity of ddI against MSV, but solely in combination with ribavirin. Ribavirin markedly enhances the *in vitro* and *in vivo* antiviral effects of several 2′,3′-dideoxynucleosides [25], including ddI [7]. This potentiating effect is supposed to be due to ribavirin-induced intracellular elevation of the IMP content [7], and thus facilitating conversion of ddI to ddATP. However, the mechanism of action of ribavirin remains controversial and a certain number of hypotheses, which are not mutually exclusive, have been proposed for its broad-spectrum antiviral activity (reviewed recently in [26]). Since the anti-MSV activity of ddI combined with ribavirin is enhanced by MDL 74,428 *in vivo* but not *in vitro*, it may tentatively be suggested that the potentiating effect of MDL 74,428 observed in mice results partly from its effectiveness at inhibiting *in vivo* erythrocytic PNP, and thus at maintaining circulating ddI at effective concentrations. However, MDL 74,428 also potentiates the *in vivo* antiviral effect of ribavirin in absence of ddI, although to a lesser extent than in its presence. At the present state of this groups investigations these findings do not allow firm conclusions regarding the mechanisms of action involved in the beneficial antiviral effect of the PNP inhibitor *in vivo* to be drawn.

It still remains to be determined by other

appropriate experimental models whether or not PNP inhibition might be relevant to the treatment of viral diseases, of various autoimmune and purine metabolism-associated diseases and useful in adjuvant cancer therapy [27, 28].

Note added in proof:

During submission of this manuscript two reports have appeared which show that 9-benzyl-9-deazaguanine, another potent inhibitor of PNP, doubles the half-life of ddI when administered i.v. to rats but has, like MDL 74,428, a very rapid plasma elimination half-life [29, 30]. No data regarding a potentiating effect on the antiviral activity of ddI have been reported.

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