Biochemical Pharmacology, Vol. 40, No. 12, pp. 2695–2700, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
© 1990. Pergamon Press plc

EFFECTS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR ON 3′-AZIDO-3′-DEOXYTHYMIDINE UPTAKE, PHOSPHORYLATION AND NUCLEOTIDE RETENTION IN HUMAN U-937 CELLS

RAJEEV K. DHAWAN, SURENDER KHARBANDA, MARIKO NAKAMURA, TSEUNYA OHNO and DONALD KUFE*

Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, U.S.A.

(*Received* 8 *March* 1990; *accepted* 24 *July* 1990)

Abstract—Previous studies have demonstrated that granulocyte-macrophage colony-stimulating factor (GM-CSF) both increases and decreases levels of 3′-azido-3′-deoxythymidine (AZT) nucleotides in certain human myeloid cells. The present studies have examined the effects of GM-CSF on AZT metabolism in U-937 cells. The results demonstrate that GM-CSF stimulated AZT nucleotide formation in these cells. This stimulation was detectable during concurrent exposure to GM-CSF and AZT or as a result of pretreatment with GM-CSF. The GM-CSF-induced enhancement in AZT nucleotide formation was associated with a 4-fold increase in AZT uptake. The finding that uptake of AZT into U-937 cells was only partially sensitive to 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (NBMPR) suggested a process primarily involving nonfacilitated diffusion. The results also demonstrate that treatment of U-937 cells with GM-CSF was associated with nearly a 2-fold increase in thymidine kinase activity. Moreover, the findings indicate that retention of AZT-MP and AZP-TP was prolonged significantly ($P < 0.05$ and $P < 0.01$ respectively) in association with GM-CSF treatment. Taken together, these results suggest that GM-CSF enhances the formation of AZT nucleotides by increasing AZT uptake and phosphorylation, as well as increasing retention of phosphorylated derivatives.

3′-Azido-3′-deoxythymidine (AZT†) is a potent inhibitor of human immunodeficiency virus (HIV) replication in human T lymphocytes and monocytes [1–3]. The effects of AZT are mediated by conversion of this nucleoside to the active triphosphate metabolite AZT-TP. AZT-TP has been shown to act as both an inhibitor and a substrate of HIV reverse transcriptase [4–7]. The basis for the selectivity of AZT against HIV replication is related to the finding that AZT-TP binds more efficiently to reverse transcriptase than cellular DNA polymerase alpha [4].

Various strategies have been examined to enhance the intracellular formation of AZT-TP. Recent studies have demonstrated that treatment of monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF) is associated with increased intracellular levels of AZT and its nucleotides [8]. This effect of GM-CSF on AZT anabolism also results in an enhancement of anti-HIV activity [8]. In contrast, other studies in human bone marrow cells have indicated that GM-CSF significantly decreases intracellular AZT-TP levels [9]. These findings have suggested that the effects of GM-CSF on the intracellular pharmacology of AZT are dependent in part on cell type.

The present work has examined the effects of GM-CSF on the uptake and phosphorylation of AZT in the human U-937 monocyte-like cell line [10]. The results demonstrate that GM-CSF increases AZT nucleotide formation in these cells and that this effect is associated with increases in AZT uptake and thymidine kinase activity. The results also demonstrate that retention of AZT nucleotides is prolonged in association with GM-CSF treatment.

MATERIALS AND METHODS

Cell culture. U-937 cells (American Type Culture Collection, Bethesda, MD) were maintained at a density of 1–2 × 10^5 cells/mL in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine. U-937 cells chronically infected with HIV-1 IIIB (provided by Dr. J. Groopman, Boston, MA) were grown under similar conditions. The cells were exposed to various concentrations of AZT (Burroughs Wellcome Co., Research Triangle Park, NC). GM-CSF (Genetics Institute, Boston, MA) was used at a final concentration of 100 units/mL. Cell viability was monitored by trypan blue exclusion.

Measurement of intracellular AZT nucleotides. Cells were incubated with 3′-azido-3′-[5-^{3}H]deoxythymidine (15 Ci/mmol; Moravek Biochemicals, Brea, CA), washed twice with ice-cold phosphate-buffered saline, and then extracted with perchloric acid [11]. The acid-soluble fraction was treated with

* Author to whom correspondence should be addressed.

† Abbreviations: AZT, 3′-azido-3′-deoxythymidine; GM-CSF, granulocyte-macrophage colony-stimulating factor; NBMPR, 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine; HIV, human immunodeficiency virus; AZT-TP, AZT-triphosphate; AZT-MP, AZT-monophosphate; and AZT-DP, AZT-diphosphate.

0.5 M sodium periodate to remove ribonucleotides [12]. AZT nucleotides were analyzed on a Waters M510 high pressure liquid chromatography (Waters, Milford, MA) equipped with an SAX anion exchange column using a linear gradient of 0.015 M KH_2PO_4 (pH 3.5) to 1.0 M KH_2PO_4 (pH 3.5) at a flow rate of 1.0 mL/min. The eluant fractions were collected, and tritium was determined by liquid scintillation counting.

Measurement of AZT uptake. Cells were incubated with [^{3}H]AZT for intervals up to 60 sec. At the indicated times, the cells were centrifuged through a 4:1 (v/v) mixture of di-*n*-butyl phthalate and di-*n*-octyl phthalate (Aldrich Chemical Co., Milwaukee, WI) at a density of 1.03 g/mL [13]. The cell pellets were solubilized in 0.9 M NaOH and 0.6% sodium lauryl sulfate, neutralized, and then monitored for tritium. The intracellular water space was determined in similar experiments using cells incubated in 5 μCi/mL [^{3}H]H_2O (5 mCi/mL; Amersham) for 5 min at 37° as described [14] and centrifuged through the oil mixture. The trapped extracellular space was determined with 5 μCi/mL [^{14}C]sucrose (540 μCi/mL; Amersham) in corresponding tubes. Cell pellets were solubilized in 0.9 N NaOH, and the intracellular water space was calculated [13].

Measurement of NBMPR binding. Cellular NBMPR binding was determined by incubating U-937 cells with 0.1 to 8.0 nM [^{3}H]NBMPR for 25 min at 37° [15]. The cells were sedimented through the phthalate oil mixture, and specific binding in the presence of unlabeled NBMPR was used to calculate NBMPR binding sites and binding affinity by Scatchard analysis.

Measurement of thymidine kinase activity. Thymidine kinase activity was assayed as described [16] with certain modifications. The U-937 cells ($3–4 \times 10^7$) were suspended in hypotonic buffer containing 0.01 M Tris–HCl (pH 7.5), 0.01 M KCl, 1 mM $MgCl_2$ and 1 mM dithiothreitol. The cell suspension was incubated on ice for 20 min and then disrupted with a Dounce homogenizer. The homogenate was centrifuged at 25,000 *g* for 25 min and the supernatant adjusted to 10% glycerol, 0.15 M KCl and 0.2 mM phenylmethylsulfonyl fluoride. Phosphorylation reactions were performed in the presence of 0.1 mM [^{3}H]AZT or [*methyl*-^{3}H]thymidine (63 Ci/mmol; Moravek Biochemicals), 50 mM Tris–HCl (pH 7.5), 5 mM ATP-Mg^{2+} and the enzyme preparation at 37° for 30 min. The reactions were terminated by heating the tubes at 95° for 2 min. After centrifugation at 25,000 *g* for 3 min, the supernatants were collected and analyzed by high pressure liquid chromatography (HPLC) for conversion of nucleoside to the monophosphate derivative.

RESULTS

The effect of AZT on the growth of U-937 cells was monitored over 5 days. While 0.1 μM AZT partially slowed proliferation of these cells, exposure to 1 μM AZT was associated with nearly complete inhibition of growth (Fig. 1). In contrast, treatment with 10 μM AZT was cytotoxic (Fig. 1). Consequently, all subsequent experiments were performed with 1 μM AZT. There was no detectable stimulation of U-937 cell growth in the presence of GM-CSF.

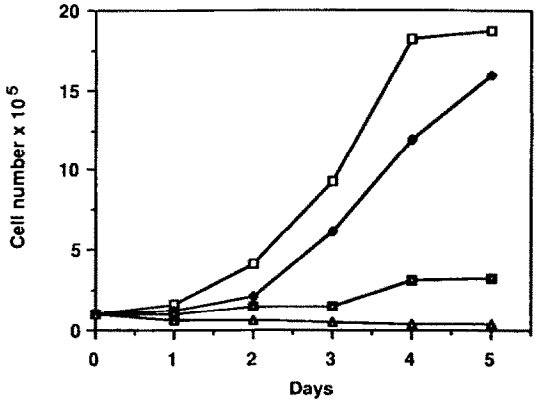

Fig. 1. Effects of AZT on U-937 cell growth. U-937 cells were seeded at 1×10^5/mL and grown in the absence (□) and presence of 0.1 μM (◆), 1 μM (□) or 10 μM (△) AZT. Cell counts were monitored at the indicated times. Viability as determined by trypan blue exclusion was over 95% for the cells treated with 1 μM AZT.

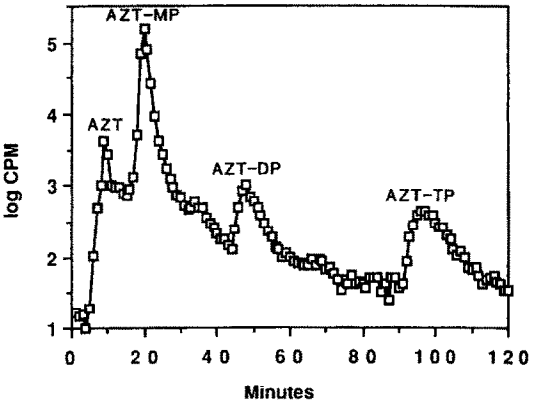

Fig. 2. Representative HPLC profile of AZT and AZT-nucleotides in U-937 cell extracts. U-937 (5×10^7) cells were exposed to 1 μM [^{3}H]AZT for 24 hr. The cells were extracted with perchloric acid, and the acid-soluble fraction was analyzed on an SAX anion exchange column using a linear KH_2PO_4 gradient.

The intracellular formation of AZT nucleotides in U-937 cells was similar to that reported for human T-cells and mononuclear phagocytes [8]. For example, formation of AZT-monophosphate (AZT-MP) was greater than that of the diphosphate (AZT-DP) and triphosphate (AZT-TP) derivatives (Fig. 2). Accumulation of the phosphorylated derivatives was maximal at 24 hr of AZT treatment (Fig. 3). Moreover, similar findings were obtained in uninfected and chronically HIV-1-infected U-937 cells (data not shown).

The effects of GM-CSF on the intracellular pharmacology of AZT were initially studied by simultaneously adding both agents. GM-CSF increased the formation of AZT-MP as compared to that in control (Fig. 3A). This effect was maximal

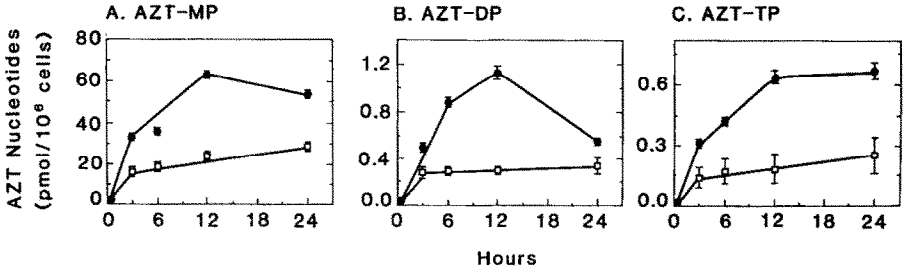

Fig. 3. Effects of GM-CSF on the formation of AZT nucleotides. U-937 cells were exposed to 1 μM [^{3}H]AZT in the absence (□) and presence (●) of GM-CSF. At the indicated times, the cells were extracted and monitored by HPLC for AZT nucleotide formation. These results, expressed as the mean ± SD of three determinations, are representative of five separate experiments.

Table 1. Effects of GM-CSF pretreatment on AZT nucleotide formation

GM-CSF pretreatment (hr)	Nucleotide (pmol/10^6 cells)		
	AZT-MP	AZT-DP	AZT-TP
0	54.6 ± 9.8	0.40 ± 0.01	0.22 ± 0.01
6	97.3 ± 27.1	0.70 ± 0.27	0.42 ± 0.13
12	131.4 ± 20.9	0.95 ± 0.22	0.53 ± 0.14
24	87.7 ± 19.4	0.59 ± 0.05	0.33 ± 0.03

U-937 cells were pretreated with GM-CSF for the indicated times. The cells were then washed and incubated with 1 μM AZT. After 24 hr, the cells were monitored for AZT nucleotide pools. Values are averages ± range for two separate experiments.

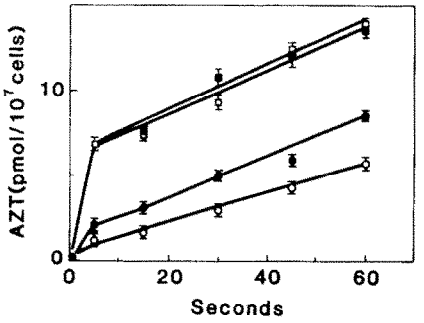

Fig. 4. Effects of GM-CSF pretreatment on uptake of AZT at 37°. U-937 cells were pretreated with GM-CSF for 0 (○), 6 (●), 12 (■) and 24 (□) hr. The cells were washed and then incubated with 1 μM [^{3}H]AZT. At the indicated times, the cells were sedimented through phthalate, solubilized, and then monitored for tritium uptake. These results are the means ± SD for three separate experiments each performed in triplicate.

at 12 hr of drug exposure. Similar increases were obtained for AZT-DP (Fig. 3B), while 12–24 hr of exposure was required for maximal AZT-TP formation (Fig. 3C). These findings suggested that, although GM-CSF increases the formation of AZT nucleotides, this effect is maximal by 12–24 hr of stimulation with this cytokine. Consequently, we also pretreated U-937 cells for 6, 12 and 24 hr with GM-CSF, washed the cells, and then monitored phosphorylation of AZT. While pretreatment with GM-CSF for 6 hr clearly had an effect as compared to control, pretreatment for 12 hr was associated with maximal increases in levels of AZT nucleotides (Table 1). Taken together, these findings suggested that 12- to 24-hr exposures to GM-CSF are associated with maximal formation and/or retention of AZT nucleotides.

To examine the effects of GM-CSF on the formation of AZT nucleotides, we first analyzed influx of this nucleoside into U-937 cells. Cells were incubated with 1 μM [^{3}H]AZT for 5–60 sec at 37°. Influx was linear during this period, and total uptake at 60 sec was 5.8 pmol/10^7 cells (Fig. 4). Cells were also pretreated with GM-CSF for 6, 12 and 24 hr before monitoring AZT uptake. The results indicated that GM-CSF treatment is associated with increased rates of AZT influx. For example, total uptake at 60 sec was 8.7, 13.5 and 13.8 pmol/10^7 cells after 6-, 12- and 24-hr GM-CSF exposures (Fig. 4).

Cell size was examined as a possible factor determining the amount of AZT uptake. However, intracellular water space was increased by up to approximately 15% (12.3 and 15.8% in two separate experiments) in cells treated with GM-CSF for 24 hr as compared to untreated cells. Consequently, we also studied the effects of GM-CSF on nucleoside transport capacity. GM-CSF had no detectable effect on either the number of [^{3}H]NBMPR binding sites or binding affinity of this nucleoside as compared to that for untreated cells (data not shown). In other experiments, 4 μM NBMPR was used to inhibit binding of AZT to nucleoside transport sites. NBMPR had a minimal but significant effect on uptake of AZT into untreated U-937 cells (Fig. 5A). Similar findings were obtained with GM-CSF-treated cells (Fig. 5B). Taken together, these findings suggested that AZT enters U-937 cells predominantly by a mechanism independent of nucleoside transport sites.

Previous studies have demonstrated that AZT is phosphorylated by thymidine kinase [4]. Consequently, we monitored the specific acitivty of this enzyme in untreated and GM-CSF-treated cells. AZT was phosphorylated at a rate of approximately 8 nmol/mg protein/hr using the cytosolic fraction of untreated U-937 cells. In contrast, this activity was

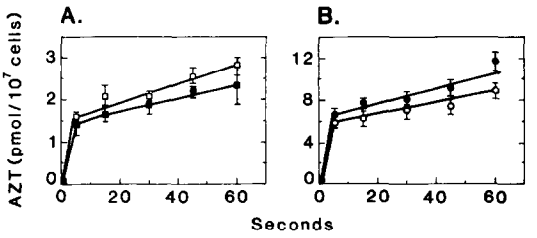

Fig. 5. Effects of NBMPR on uptake of AZT. (A) U-937 cells were exposed to 1 μM [^{3}H]AZT in the absence (□) and presence (■) of 4 μM NBMPR. (B) U-937 cells were pretreated with GM-CSF for 24 hr. The cells were washed and then exposed to 1 μM [^{3}H]AZT in the absence (●) and presence (○) of 4 μM NBMPR. At the indicated times, the cells were sedimented through phthalate, solubilized, and then monitored for tritium uptake. The results are the means ± SD of two separate experiments each performed in triplicate. Comparison (ANOVA, two-way analysis) of the data points for cells treated in the absence and presence of NBMPR indicated significant differences (A, $P < 0.05$; B, $P < 0.01$).

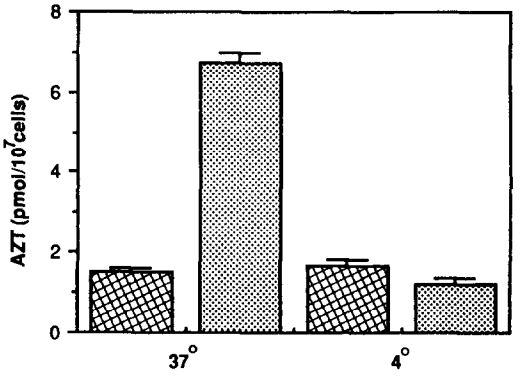

Fig. 6. Uptake of AZT in untreated and GM-CSF-treated cells at 4°. U-937 cells were grown in the absence (hatched) and presence (shaded) of GM-CSF for 24 hr. The cells were washed and monitored at 37° or 4° before adding 1 μM [^{3}H]AZT. AZT uptake was monitored at 5 sec. The results are the means ± SD of three separate experiments each performed in triplicate.

Table 2. Effect of GM-CSF on thymidine kinase activity in U-937 cells

	Specific activity (nmol nucleoside phosphorylated/mg protein/hr)	
Treatment	AZT	Thymidine
Control	8.0 ± 0.2	7.2 ± 0.9
GM-CSF	14.6 ± 0.7	13.3 ± 0.1

U-937 cells were treated with GM-CSF for 24 hr. The results are the averages ± range for two separate experiments.

increased nearly 2-fold in cells treated with GM-CSF for 24 hr (Table 2). Similar findings were obtained for phosphorylation of [^{3}H]thymidine (Table 2). These results suggested that the effects of GM-CSF on AZT nucleotide formation are, at least in part, related to increases in the specific activity of thymidine kinase.

An increase in phosphorylation of AZT to AZT-MP could contribute to the enhanced uptake of AZT into GM-CSF-treated cells. We therefore monitored AZT uptake at 4° to inhibit thymidine kinase activity. Uptake of AZT at 37° was increased in cells pretreated with GM-CSF (Fig. 6). Moreover, there was no detectable effect of temperature on AZT uptake into control cells. However, uptake of AZT into GM-CSF-treated cells was inhibited at 4° and declined to levels comparable to that in control cells (Fig. 6). Under these conditions, intracellular AZT was predominantly in the nucleoside form (data not shown). In addition to that, at 4°, the accumulated AZT consisted of both nucleoside and nucleotide forms. These findings suggested that while uptake of AZT into U-937 cells is insensitive to decreases in temperature, the enhanced uptake associated with GM-CSF-treatment is temperature sensitive and thus possibly related to inhibition of thymidine kinase activity.

We also examined the effects of GM-CSF on retention of AZT nucleotides. The half-lives of AZT-MP, AZT-DP and AZT-TP were 1.1, 3.0, and 3.1 hr respectively (Fig. 7). Similar findings were obtained in HIV-1-infected U-937 cells (data not shown). Moreover, treatment of these cells with GM-CSF for 24 hr was associated with significant increases in the half-lives of AZT-MP and AZT-TP, but not the diphosphate (Fig. 7). Taken together, these findings indicated that the increases in AZT nucleotide levels associated with GM-CSF treatment are related to both increased formation and retention.

DISCUSSION

Previous studies have demonstrated that treatment of human monocytes with GM-CSF for 5 days is associated with increased formation of AZT-TP [8]. In contrast, exposure of normal human myeloid progenitor cells to GM-CSF and AZT for 6 hr results in decreased formation of the triphosphate derivative [9]. In the present work with U-937 cells, simultaneous exposure to GM-CSF and AZT for 6 hr was associated with detectable increases in AZT nucleotide levels compared to cells treated with AZT alone. Moreover, longer exposures of 12 and 24 hr to these agents were associated with maximal

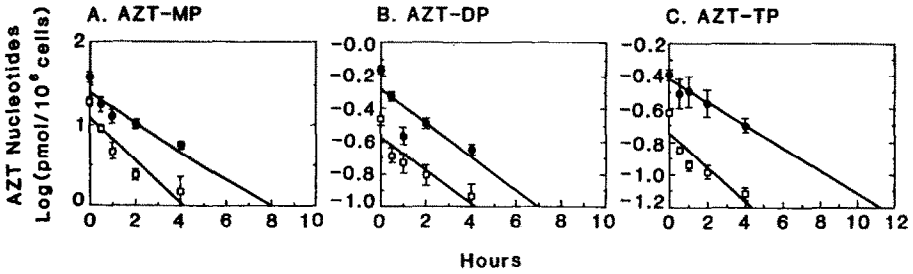

Fig. 7. Effects of GM-CSF on retention of AZT nucleotides. U-937 cells were exposed to 1 μM [^{3}H]AZT in the absence (□) and presence (●) of GM-CSF for 24 hr. After washing and incubating in the absence of drug for the indicated times, the cells were extracted and monitored by HPLC for AZT nucleotide pools. The results are the means ± SD for three experiments. The calculated half-lives (hr) were: (A) AZT-MP: control, 1.1 ± 0.1; GM-CSF-treated, 1.6 ± 0.2 ($P < 0.05$, Student's t-test); (B) AZT-DP: control, 3.0 ± 0.3; GM-CSF-treated, 2.9 ± 0.2 ($P < 0.5$); and (C) AZT-TP: control, 3.0 ± 0.6; GM-CSF-treated, 4.4 ± 0.4 ($P < 0.01$).

increases in pools of AZT-MP, AZT-DP and AZT-TP. These findings indicated that the effect of GM-CSF were time dependent. Consequently, other studies were performed to examine the effects of pretreatment with GM-CSF. In concert with the initial findings, while 6 hr of GM-CSF pretreatment clearly had an effect, longer GM-CSF exposures before adding AZT maximally increased AZT nucleotide pools. Taken together, these results suggested that a period of 12–24 hr of GM-CSF treatment is required to increase AZT nucleotide pools in these cells.

AZT permeates the membranes of human erythrocytes and lymphocytes primarily by nonfacilitated diffusion and not, as in the case of thymidine, via the nucleoside transporter [17]. This distinction between AZT and thymidine has been attributed to an increase in lipophilicity as a result of replacing the 3′-hydroxyl group with an azido moiety. The present results also support uptake of AZT primarily by nonfacilitated diffusion. However, less than 10% of the AZT uptake in U-937 cells was inhibited by NBMPR. These findings suggested that facilitated uptake may play a minor but nonetheless significant role in the uptake of AZT. Of further interest was the finding that GM-CSF pretreatment was associated with apparent increases in AZT uptake. While pretreatment with GM-CSF for 6 hr had a partial effect, exposures of 12 and 24 hr to this cytokine maximally increased total AZT uptake as compared to control. The GM-CSF-induced increases in AZT were also partially sensitive to NBMPR. Moreover, the increases in AZT uptake could not be attributed to increases in intracellular volume associated with GM-CSF treatment. These findings suggested that AZT chiefly permeates both control and GM-CSF-treated U-937 cells by nonfacilitated diffusion.

Several different types of carrier-mediated mechanisms have been described for nucleoside uptake into mammalian cells. In addition to the widely studied nucleoside transport system which is energy-independent and sensitive to NBMPR [18, 19], another nonconcentrative transporter has been described which is insensitive to the effects of this inhibitor [20, 21]. Moreover, an energy-dependent nucleoside transport system has been identified which is insensitive to NBMPR [22]. The uptake of AZT into U-937 cells at 5 sec was similar at 4° and 37°. In contrast, the stimulation of AZT uptake into GM-CSF-treated cells observed at 37° was inhibited at 4°. Thus, AZT uptake at 4° was similar in both control and GM-CSF-treated cells. The basis for this temperature effect is unclear, and further studies are required to characterize more precisely this uptake process. However, taken together with the other uptake experiments, the results suggest that GM-CSF treatment is associated with increases in AZT uptake through the activity of a temperature-dependent and NBMPR-insensitive process.

AZT is phosphorylated to AZT-MP by thymidine kinase and thus increases in the activity of this enzyme could account for enhanced formation of AZT nucleotides. Indeed, the present results demonstrate that GM-CSF treatment was associated with approximately 2-fold increases in thymidine kinase activity. Of interest, in other studies with 1-β-D-arabinofuranosylcytosine (ara-C) in U-937 cells, there was no detectable effect of GM-CSF on the formation of ara-C nucleotides, thus suggesting that deoxycytidine kinase activity is not stimulated by this cytokine. The basis for this selective increase in enzyme activity was examined by monitoring thymidine kinase mRNA levels in untreated and GM-CSF-treated U-937 cells. There was no detectable effect of GM-CSF on levels of RNA transcripts that hybridize to a human thymidine kinase probe [23] (data not shown). Thus, GM-CSF-induced increases in the activity of this enzyme are probably related to changes at the protein level. These increases in thymidine kinase activity thus provide one explanation for the effects of GM-CSF on AZT nucleotide formation. Moreover, the increases in thymidine kinase activity could contribute to the increase in uptake of AZT into GM-CSF-treated cells. In this regard, while AZT permeates cells primarily by nonfacilitated diffusion [18], rapid phosphorylation of AZT, particularly in the presence of increased thymidine kinase activity, would increase AZT nucleotide formation and apparent AZT uptake by a temperature-dependent process.

Finally, the present results demonstrate that GM-CSF treatment was associated with a significant prolongation in the half-life of AZT-MP. While the basis for this effect is unclear, increased retention in GM-CSF-treated cells would contribute to the expansion of AZT-MP pools. Furthermore, although retention of AZT-DP was similar in both untreated and GM-CSF-treated cells, expansion of the AZT-TP pools may also be related, at least in part, to increased retention of the triphosphate. Taken together, the present findings suggest that the GM-CSF increases AZT nucleotides by multiple mechanisms involving increased uptake, increased phosphorylation to AZT-MP, and increased retention of nucleotide derivatives.

Acknowledgements—This investigation was supported by the AIDS Clinical Trial Group, NIAID, DHHS, and by a Burroughs Wellcome Award in Clinical Pharmacology (D. K.).

REFERENCES

1. Mitsuya H, Weinhold KJ, Furman PA, St. Clair MH, Nusinoff-Lehrman S, Gallo RC, Bolognesi DP, Barry DW and Broder S, 3'-Azido-3'-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphoadenopathy-associated virus *in vitro*. *Proc Natl Acad Sci USA* **82**: 7096–7100, 1985.
2. Mitsuya H and Broder S, Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphoadenopathy-associated virus (HTLV-III/LAV), by 2',3'-dideoxy nucleosides. *Proc Natl Acad Sci USA* **83**: 1911–1915, 1986.
3. Perno CF, Yarchoan R, Cooney DA, Hartman NR, Gartner S, Popovic M, Hao Z, Gerrard TL, Wilson YA, Johns DG and Broder S, Inhibition of human immunodeficiency virus (HIV/1/HTLV-III$_{Ba-L}$) replication in fresh and cultured human peripheral blood monocyte/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. *J Exp Med* **168**: 1111–1125, 1988.
4. Furman PA, Fyfe JA, St. Clair MH, Weinhold K, Rideout JL, Freeman GA, Nusinoff-Lehrman S, Bolognesi DP, Broder S, Mitsuya H and Barry DW, Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc Natl Acad Sci USA* **83**: 8333–8337, 1986.
5. Ono K, Ogasawara M, Iwata Y, Nakane H, Fujii T, Sawai K and Saneyoshi M, Inhibition of reverse transcriptase activity by 2',3'-dideoxythymidine-5'-triphosphate and its derivatives modified on the 3'-position. *Biochem Biophys Res Commun* **140**: 498–507, 1986.
6. St. Clair MH, Richards CA, Spector T, Weinhold KJ, Miller WH, Langlois AJ and Furman PA, 3'-Azido-3'-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. *Antimicrob Agents Chemother* **31**: 1972–1977, 1987.
7. Cheng Y-C, Dutchman GE, Bastow KF, Sarngadharan MG and Ting RYC, Human immunodeficiency virus reverse transcriptase. General properties and its interactions with nucleoside triphosphate analogs. *J Biol Chem* **262**: 2187–2189, 1987.
8. Perno CF, Yarchoan R, Cooney DA, Hartman NR, Webb D, Hao Z, Mitsuya H, Johns DG and Broder S, Replication of human immunodeficiency virus in monocytes. Granulocyte/macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3'-azido-2',3'-dideoxythymdiine (AZT) and other dideoxynucleoside congeners of thymidine. *J Exp Med* **169**: 933–951, 1989.
9. Bhalla K, Birkhofer M, Grant S and Graham G, The effect of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) on 3'-azido-3'-deoxythymidine (AZT)-mediated biochemical and cytotoxic effects of normal human myeloid progenitor cells. *Exp Hematol* **17**: 17–20, 1989.
10. Sundstrom C and Nilsson K, Establishment and characterization of a human histiocytic lymphoma cell linc (U-937). *Int J Cancer* **17**: 565–570, 1976.
11. Bhalla K, Nayak R and Grant S, Isolation and characterization of a deoxycytidine kinase deficient human promyelocytic leukemic cell line highly resistant to 1-β-D-arabinofuranosylcytosine. *Cancer Res* **44**: 5029–5036, 1984.
12. Garrett C and Santi DV, A rapid and sensitive high pressure liquid chromatographic assay for deoxyribonucleoside triphosphates in cell extracts. *Anal Biochem* **99**: 268–273, 1979.
13. Wiley JS, Jones SP, Sawyer WH and Paterson ARP, Cytosine arabinoside influx and nucleoside transport sites in acute leukemia. *J Clin Invest* **69**: 479–489, 1982.
14. Andreasson PA and Schaumburg BP, A rapid technique for the separation of thymocytes from suspensions by centrifugation through silicone oil. *Anal Biochem* **59**: 610–616, 1974.
15. White JC, Rathmell JP and Capizzi RL, Membrane transport influences the rate of accumulation of cytosine arabinoside in human leukemic cells. *J Clin Invest* **79**: 380–387, 1987.
16. Fyfe JA, Keller PM, Furman PA, Miller RL and Elion GB, Thymidine kinase from Herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl) guanine. *J Biol Chem* **253**: 8721–8727, 1978.
17. Zimmerman TP, Mahoney WB and Prus KL, 3'-Azido-3'-deoxythymidine: An unusual nucleoside analog that permeates the membrane of human erythrocytes and lymphocytes by nonfacilitated diffusion. *J Biol Chem* **262**: 5748–5754, 1987.
18. Plagemann PGW and Wohlhueter RM, Permeation of nucleosides, nucleic acid bases, and nucleotides in animal cells. *Curr Top Membr Transp* **14**: 225–330, 1980.
19. Paterson ARP, Kolassa N and Cass CE, Transport of nucleoside drugs in animal cells. *Pharmacol Ther* **12**: 515–536, 1981.
20. Belt JA, Heterogeneity of nucleoside transport in mammalian cells: Two types of transport activity in L1210 and other cultured neoplastic cells. *Mol Pharmacol* **24**: 479–484, 1983.
21. Plagemann PGW and Wohlhueter RM, Nucleoside transport in cultured mammalian cells. Multiple forms with different sensitivity to inhibition by nitrobenzylthioinosine or hypoxanthine. *Biochim Biophys Acta* **773**: 39–52, 1984.
22. Darnowski JW and Handschumacher RE, Tissue uridine pools: Evidence *in vivo* of a concentrative mechanism for uridine uptake. *Cancer Res* **46**: 3490–3494, 1986.
23. Lin P, Zhao S and Ruddle FH, Genomic cloning and preliminary characterization of the human thymidine kinase gene. *Proc Natl Acad Sci USA* **80**: 6528–6532, 1983.