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MODULATION OF DIPYRIDAMOLE ACTION BY α_1ACID GLYCOPROTEIN

REDUCED POTENTIATION OF QUINAZOLINE ANTIFOLATE (CB3717) CYTOTOXICITY BY DIPYRIDAMOLE

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Abstract—Dipyridamole potentiates the cytotoxicity of N^{10}-propargyl-5,8-dideazafolic acid (CB3717), an antifolate inhibitor of thymidylate synthase, by inhibiting both thymidine (TdR) salvage and deoxyuridine (UdR) efflux. Dipyridamole binds to the serum component $\alpha_1$acid glycoprotein (α_1AGP) and hence the effects of α_1AGP on dipyridamole-induced changes in nucleoside transport and CB3717 cytotoxicity have been investigated. Using A549 lung cancer cells *in vitro*, α_1AGP reduced the inhibition of nucleoside transport by dipyridamole in a concentration-dependent manner. Between 10 and 200 times the concentration of dipyridamole was needed to inhibit TdR uptake to the same degree in medium containing 1 mg/ml α_1AGP (a physiological concentration) when compared to the uptake in α_1AGP-free medium. Although dipyridamole inhibited UdR efflux more than TdR efflux, inhibition of UdR efflux was reduced less than the inhibition of TdR efflux in the presence of 1 mg/ml α_1AGP. Thus, clinically achievable levels of dipyridamole (2.5–7.5 μM), even in the presence of physiological α_1AGP concentrations, caused significant inhibition of nucleoside uptake and efflux. The cytotoxicity of CB3717 was increased 2–3-fold by 3 and 10 μM dipyridamole in α_1AGP-free medium, whereas dipyridamole did not significantly ($P \geq 0.05$) potentiate CB3717 cytotoxicity in the presence of 1 mg/ml α_1AGP. Measured free dipyridamole levels indicated that the impaired inhibition of nucleoside transport and the lack of potentiation of CB3717 cytotoxicity in the presence of α_1AGP was due solely to the binding of dipyridamole to α_1AGP. It is concluded that α_1AGP levels will be a major determinant of the ability of dipyridamole to modulate the activity of antimetabolites *in vivo*.

Dipyridamole is a commonly used vasodilatory and antiplatelet drug [1]. Peak plasma levels are in the range 2.5–7.5 μM after normal dosing schedules [1, 2] with oral bioavailability varying between 30–75% in patients [1, 3] and a final elimination half-life of about 12 hr [2]. Animal studies have shown it to be concentrated in the liver, kidneys and lung [4] and, due to excretion through the bile, it undergoes enterohepatic circulation [1].

Dipyridamole interacts with the high affinity (nitrobenzyl thioinosine-sensitive) nucleoside transporter of cell membranes [5, 6] with K_i for deoxynucleoside transport 1–2 μM [7]. This property of dipyridamole may be of use in antimetabolite cancer chemotherapy. Dipyridamole enhances the toxicity of antimetabolites that block *de novo* nucleotide synthesis by preventing salvage of exogenous nucleosides both *in vitro* [8–13] and *in vivo* [13–15]. Dipyridamole can potentiate other antimetabolite action by preventing the efflux of accumulated toxic nucleosides [16–19].

Using the antifolate inhibitor of thymidylate synthase, N^{10}-propargyl-5,8-dideazafolic acid (CB3717)‡ [20], we have shown that dipyridamole potentiates CB3717 cytotoxicity via inhibition of nucleoside transport [21]. Inhibition of thymidylate synthase, the rate-limiting step in *de novo* dTTP synthesis, causes a reduction in thymidine deoxynucleotide levels, accumulation of uracil deoxynucleotides [22, 23], misincorporation of dUMP into DNA [24, 25] and DNA strand breakage [26]. The cytotoxicity of CB3717 may be prevented by the salvage of exogenous TdR [22]. In the presence of 1 μM dipyridamole, CB3717 cytotoxicity *in vitro* is enhanced by inhibition of salvage of exogenous TdR and prevention of efflux of UdR [21].

Dipyridamole is significantly bound to the serum component α_1AGP (99.13 ± 0.24% [27]) at two binding sites, one high affinity and one low affinity [28]. α_1AGP is the major serum binding component for several basic drugs [29–31] and progesterone [32]. The molecular weight of human α_1AGP is 44,000, it contains approximately 45% carbohydrate including 12% sialic acid and it is the most negatively charged plasma protein [33] which accounts for much of its binding properties. It is an acute phase protein [34], the levels of which increase during inflammatory disease and cancer [35–37]. There is a wide variation in α_1AGP levels in humans, with normal levels in the range 0.12–1.68 mg/ml and levels in cancer patients in the range 0.36–2.7 mg/ml [36, 37]. Levels in normal subjects are usually < 0.8 mg/ml and in cancer patients > 0.8 mg/ml [36].

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‡ α_1AGP, $\alpha_1$acid glycoprotein; TdR, deoxythymidine; UdR, deoxyuridine; HPLC, high performance liquid chromatography; FBS, foetal bovine serum; CB3717, N^{10}-propargyl-5,8-dideazafolic acid.

It seems likely, therefore, that dipyridamole will be significantly less effective in patients than in *in vitro* systems. We report here our investigations of the effect of physiological levels of α_1AGP on the inhibition of nucleoside transport and the potentiation of CB3717 cytotoxicity by dipyridamole.

MATERIALS AND METHODS

Materials

CB3717 disodium salt was a gift from ICI Pharmaceuticals (Macclesfield, U.K.); it was dissolved in 0.05 M NaOH, filter sterilized and stored at 0–4° in the dark. TdR, UdR, dipyridamole and α_1AGP were purchased from Sigma Chemical Co. (Poole, U.K.). Dipyridamole was dissolved in 0.1 M HCl, filter sterilized and stored at 0–4° in the dark. [Methyl-^{3}H]TdR (44 Ci/mMol; 1 Ci/ml) was purchased from Amersham International (Amersham, U.K.) and [5-^{3}H]UdR (23 Ci/mMol; 1 Ci/ml) was obtained from ICN Radiochemicals (Eschwege, F.R.G.). All routine chemicals were of AnalaR grade and purchased from BDH (Poole, U.K.). Cell culture media, trypsin and foetal bovine serum were obtained from either Gibco (Paisley, U.K.) or Northumbria Biologicals (Cramlington, U.K.). Dulbecco's modified phosphate buffered saline without magnesium and calcium (DulA), Titertek cell harvester and filters came from Flow Laboratories (Irvine, U.K.) and plastic culture dishes were supplied by Nunc (Gibco: 6-well plates) or Falcon (Becton-Dickinson, Oxford: flat bottomed 24-well and 96-well plates).

Cell culture

A549 lung carcinoma cells [38, 39] were grown in RPMI + 10% foetal bovine serum (FBS: heat-inactivated 56°, 30 min) with 500 I.U./ml penicillin and 500 μg/ml streptomycin at 37° in an atmosphere of 5% CO_2 in air.

[Methyl-^{3}H]TdR uptake

Cells were seeded at 2–2.5 × 10^4 cells/well in 0.125 ml RPMI + 10% FBS in 96-well trays. The following day the medium was removed, the cells washed with DulA and then given 80 μl serum-free medium supplemented with CB3717 and/or dipyridamole and/or α_1AGP where indicated for a 2 hr preincubation period. Then 20 μl 500 nM [^{3}H]TdR (final concentration = 100 nM) was added for 2 hr, after which the medium was rapidly aspirated, the cells washed twice with ice-cold DulA and solubilised with 0.5 M NaOH and transferred to glass fibre filters using the Titertek apparatus. (Blanks were: no cells, no [^{3}H]TdR or 2 mM non-radioactive TdR.) The filters were dried and counted in 5 ml scintillant (Optiphase-safe: LKB, South Croydon, U.K.) in a LKB 1217 liquid scintillation counter. The measured radioactivity represents incorporation after uptake. Replicate wells were trypsinized and counted using a Coulter counter (Coulter Electronics, Luton, U.K.). Percentage inhibition of uptake was calculated as follows:

$$\%\ \text{inhibition} = 100 - \left[100 \times \left(\frac{\text{uptake} + \text{dipyridamole} \pm \alpha_1\text{AGP} \pm 30\ \mu\text{M CB3717}}{\text{uptake} \pm \alpha_1\text{AGP} \pm 30\ \mu\text{M CB3717}}\right)\right].$$

Nucleoside efflux

[Methyl-^{3}H]TdR. Subconfluent monolayers of A549 cells in 6-well plates were incubated for 1 hr at 37° with 1 ml serum-free medium containing 100 nM [^{3}H]TdR. The wells were then washed three times with ice-cold DulA and 1 ml serum-free medium with or without 1 mg/ml α_1AGP and 0, 1 or 10 μM dipyridamole (warmed to 37°) was added. Aliquots (50 μl) were withdrawn immediately and at 5, 10, 20 and 30 min intervals thereafter.

[5-^{3}H]UdR. As [5-^{3}H]dUMP derived from [5-^{3}H]UdR may be converted to dTMP and [^{3}H]H_2O by the action of thymidylate synthase, all incubations were carried out in the presence of 30 μM CB3717 to inhibit this enzyme. The experimental procedure was the same as described above for [^{3}H]thymidine, except that 100 nM [5-^{3}H]UdR was used and all aliquots for scintillation counting were evaporated to dryness to eliminate [^{3}H]H_2O arising from residual thymidylate synthase activity. Percentage inhibition of efflux of TdR and UdR by dipyridamole was calculated as follows:

$$\%\ \text{inhibition} = 100 - \left[100 \times \left(\frac{\text{rate of efflux + dipyridamole}}{\text{rate of efflux no dipyridamole}}\right)\right].$$

Cell growth inhibition studies

Cells were seeded in 24-well dishes at approximately 1.25 × 10^4 cells/well in 0.5 ml RPMI + 10% FBS. The following day, the medium was replaced with fresh control medium or that containing α_1AGP at a final concentration of 1 mg/ml. Dipyridamole and/or CB3717 were added to give the final concentrations as indicated in the figures. The volume of control or experimental medium in the wells was 0.3 ml. Replicate wells were trypsinized and counted immediately. After 72 hr incubation at 37°, the cells were trypsinized and counted on a Coulter counter.

The effect of α_1AGP *per se* on cell growth was assayed as follows: 24 well dishes were seeded with 1 × 10^4 cells/well in 0.5 ml RPMI + 10% FBS. The next day, five wells were trypsinized and counted, medium containing varying concentrations of α_1AGP was added to replicate wells which were trypsinized and counted 72 hr later.

Analysis of dipyridamole in medium and medium ultrafiltrates

Dipyridamole, prepared as above, was added to RPMI medium or RPMI medium + 10% FBS containing 0, 1 or 2 mg/ml α_1AGP to give final dipyridamole levels of 1, 3 or 10 μM. Samples were stored frozen (−20°) until analysed and then thawed to room temperature. A 0.5 ml aliquot was removed and placed in the upper chamber of an Amicon Centrefree Micropartition Unit. Ultrafiltrates were prepared by centrifugation for 20 min at 15° at 1,000 *g* on an MSE Chilspin centrifuge equipped with an angle rotor. Aliquots (20 μl) of the ultrafiltrate were analysed for free dipyridamole levels using HPLC

with fluorescence detection. To measure total dipyridamole levels, a 0.1 ml aliquot of medium was mixed with 0.2 ml methanol and the precipitated protein removed by centrifugation at 1,000 *g* for 15 min at 4°. The supernatant was removed and aliquots (20 μl) analysed by HPLC as described below. A standard curve was prepared by dissolving dipyridamole at 1 mg/ml in 0.01 M HCl and then dilution in 0.15 M NaCl.

HPLC was performed on a Waters Associates chromatograph equipped with a Perkin Elmer LS4 fluorescence detector. Dipyridamole was separated on a 15 × 0.46 cm μBondapak C18 column (Waters Associates), eluted isocratically with methanol/water/glacial acetic acid (50/50/1) at a flow rate of 2 ml/min. Dipyridamole eluted with a retention volume of 8 ml and was detected by fluorescence at 490 nm (slit width 20 nm) following excitation at 280 nm (slit width 15 nm). UV absorbance at 280 nm was also recorded and dipyridamole in medium samples identified by the ratio of the fluorescence to UV absorbance and by co-chromatography with authentic dipyridamole. Quantitation was achieved using the output of the fluorescence detector by means of a computing integrator.

Over the free dipyridamole concentration range 0.05–2 μM the recovery of drug from the membranes of the Centrefree Micropartition units was 99.2 ± 4.0% (mean ± SD, N = 10) and the response of the fluorescence detector was linear ($r = 0.999$). Estimation of total dipyridamole was linear over the concentration range 0.1–10 μM ($r = 1.000$). For both free and total dipyridamole analyses all unknown samples were analysed in a single run and the intra-assay coefficients of variation were 5% and each analysis was performed in quadruplicate.

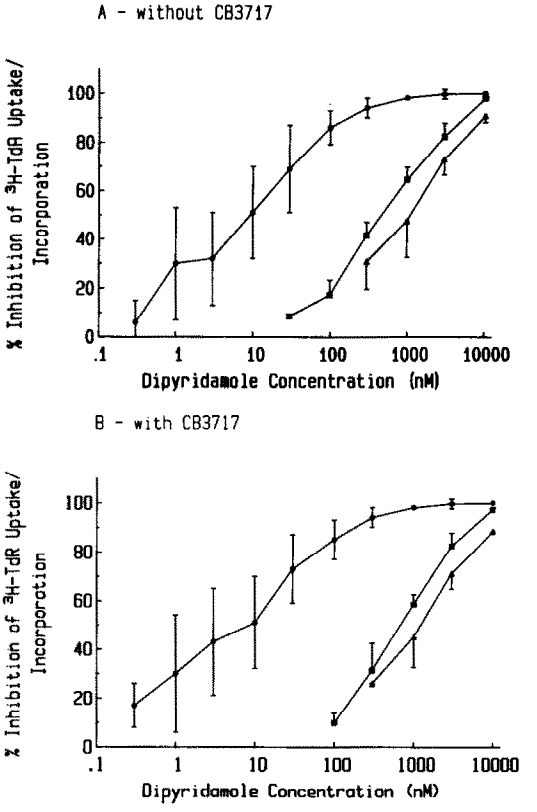

Fig. 1. Inhibition of TdR uptake/incorporation by dipyridamole with or without 1 or 2 mg/ml α_1AGP. Control α_1AGP-free medium, ●; 1 mg/ml α_1AGP, ■; 2 mg/ml α_1AGP, ▲. (A) Without CB3717. (B) In the presence of 30 μM CB3717. Figures are means ± SD of 4–11 experiments.

RESULTS

[^{3}H]*TdR uptake/incorporation*

The inhibition of TdR uptake by increasing concentrations of dipyridamole in the presence or absence of α_1AGP is shown in Fig. 1. The degree of inhibition by dipyridamole was essentially the same in the presence or absence of 30 μM CB3717. Significant inhibition of TdR uptake was observed at all concentrations above 1 nM dipyridamole in the absence of α_1AGP ($P < 0.05$). However, inhibition of TdR uptake was not significant ($P > 0.05$) below 100 nM dipyridamole in the presence of 1 mg/ml α_1AGP or 300 nM dipyridamole in the presence of 2 mg/ml α_1AGP. Thus, dipyridamole was approximately 100–200-fold less effective in the presence of 1 or 2 mg/ml α_1AGP than in control medium at concentrations of 100–300 nM. Bovine serum albumin (2 mg/ml) did not have any effect on the inhibition of TdR uptake by dipyridamole (data not shown).

The effect of increasing concentrations of α_1AGP on the inhibition of TdR uptake by 1 μM dipyridamole was investigated. As shown in Fig. 2, the degree of inhibition was again very similar in the presence or absence of 30 μM CB3717. For each α_1AGP concentration, a control was included which contained the same amount of α_1AGP (± CB3717) but no dipyridamole. As shown in Fig. 2, 1 μM DP induced a significant ($P < 0.05$) inhibition of TdR uptake/incorporation. This effect was reduced in a concentration-dependent manner by α_1AGP, such that at 1.5 mg/ml α_1AGP inhibition of TdR uptake was only 50%. However, increasing the α_1AGP concentration above 1.5 mg/ml failed to suppress further the effect of dipyridamole. This result was explained by the unexpected observation that α_1AGP alone was able to suppress TdR uptake. Thus, at α_1AGP concentrations of 1.5 mg/ml and above, the inhibition of TdR uptake is due solely to the presence of α_1AGP, i.e. there was no significant difference between the samples with or without dipyridamole ($P > 0.05$). We were unable to detect binding of [^{3}H]TdR to α_1AGP using Amicon ultrafiltration cones (data not shown).

Nucleoside efflux

The rate of efflux of both [5-^{3}H]UdR and [methyl-^{3}H]TdR and the inhibition by dipyridamole is shown in Table 1. Dipyridamole inhibited the efflux of UdR more than TdR. The inhibition of UdR efflux by dipyridamole was slightly reduced in the presence of 1 mg/ml α_1AGP. However, there was a greater reduction in the inhibition of TdR efflux by dipyridamole in the presence of 1 mg/ml α_1AGP.

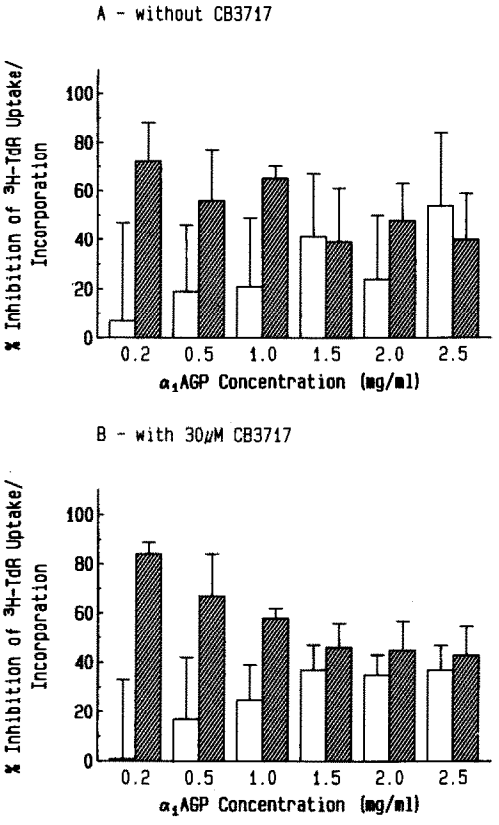

Fig. 2. Inhibition of TdR uptake/incorporation by α_1AGP in the absence (open bars) and presence (closed bars) of 1 μM dipyridamole. (A) Without and (B) with 30 μM CB3717. Figures are means ± SD of 3–7 experiments.

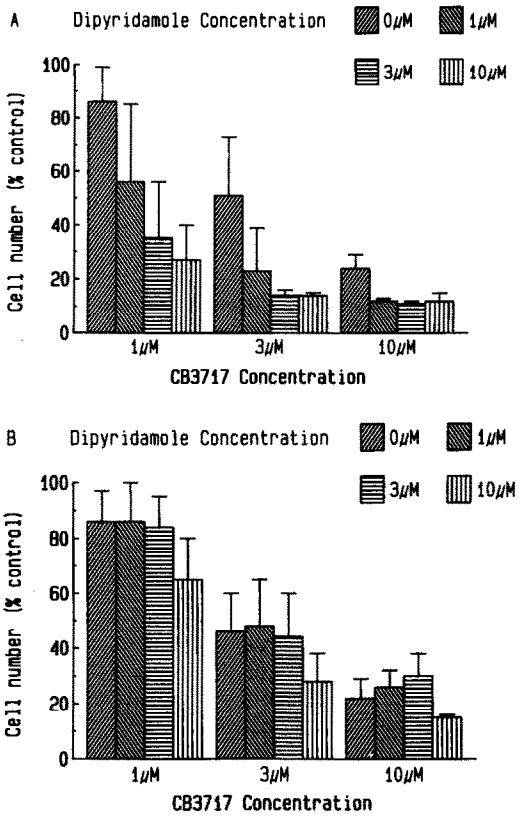

Fig. 3. Growth inhibition assay of A549 cells exposed to combinations of CB3717 and dipyridamole for 72 hr in the absence (A) and presence (B) of 1 mg/ml α_1AGP. Figures are means ± SD of cell number as a percentage of controls (in the absence of CB3717) of four experiments.

Growth inhibition studies

The inhibition of growth of cells exposed for 72 hr to varying concentrations of CB3717 and dipyridamole in the presence or absence of 1 mg/ml α_1AGP is shown in Fig. 3. In the absence of α_1AGP (Fig. 3A), 3 and 10 μM dipyridamole significantly potentiated ($P < 0.05$) the growth inhibition of 1, 3 and 10 μM CB3717 by 2–3-fold. There was a less marked potentiation by 1 μM dipyridamole ($0.05 > P < 0.1$). In the presence of 1 mg/ml α_1AGP (Fig. 3B), 1 and 3 μM dipyridamole failed to enhance the growth inhibitory effect of CB3717 and there was only a slight potentiation by 10 μM dipyridamole ($0.05 < P < 0.1$). In two experiments we coincubated cells for 72 hr with varying concentrations of CB3717 and dipyridamole and 1 mg/ml bovine serum albumin. No effect of albumin on CB3717 growth inhibition or its potentiation by dipyridamole was detected (data not shown). Again unexpectedly, we observed a concentration-dependent growth inhibitory effect of α_1AGP alone (Fig. 4).

HPLC analysis of free dipyridamole levels in α_1AGP-containing medium

Table 2 shows the free dipyridamole levels in RPMI or RPMI + 10% FBS media containing 0, 1 or 2 mg/ml α_1AGP. Over the dipyridamole concentration range (1–10 μM), binding to the 10% FBS supplement was within the range 34–57%. Following the addition of 1 mg/ml α_1AGP, free dipyridamole could only be detected (> 0.05 μM) at the highest concentration (10 μM) and hence, in both serum-free and medium supplemented with 10% FBS, dipyridamole was >95% bound. Following the addition of 2 mg/ml α_1AGP, free dipyridamole could only be detected in serum-free medium at 10 μM where it was approximately 99% bound.

DISCUSSION

The presence of physiological concentrations of α_1AGP markedly reduced the inhibitory effect of dipyridamole on TdR uptake/incorporation. This was particularly noticeable at concentrations of dipyridamole less than 1 μM. The concentration of dipyridamole required to produce an equivalent inhibitory effect in the presence of α_1AGP was 100–200 times greater than that needed in its absence. Similarly, others have found that in whole blood, 1 μM dipyridamole was ineffective in inhibiting ADP-induced platelet aggregation (mediated by adenosine transport) and that 10 μM was needed [40]. The concentration of dipyridamole needed to be increased approximately 100-fold to achieve the

Table 1. Nucleoside efflux

	[5-^{3}H]UdR		[Methyl-^{3}H]TdR	
	Rate of efflux (pmol/10^6 cells/hr)	% Inhibition of efflux	Rate of efflux (pmol/10^6 cells/hr)	% Inhibition of efflux
Control	21.38 ± 14.64		3.11 ± 1.03	
+ 1 μM dipyridamole	1.68 ± 0.54*(a)	89.3 ± 8.1	0.79 ± 0.28*(a)	76.5 ± 1.7*(c)
+ 10 μM dipyridamole	0.46 ± 0.29*(a)	97.6 ± 0.8	0.36 ± 0.37*(a)	89.7 ± 8.5
1 mg/ml α_1AGP	24.85 ± 12.93		2.22 ± 0.61	
+ 1 μM dipyridamole	5.48 ± 3.38*(a)	78.9 ± 7.9†(b)	1.43 ± 0.6*(a)	44.0 ± 6.6*(b) *(c)
+ 10 μM dipyridamole	1.66 ± 1.16*(a)	93.9 ± 1.7†(b)	0.95 ± 0.71*(a)	66.3 ± 9.1*(b) *(c)

Figures are mean ± SD of four experiments. Percentage inhibition was calculated for each experiment, prior to pooling data, as described in Materials and Methods.

Significant differences (Student's t-test) are given by: * $P < 0.05$; † $P < 0.1$ for (a) with and without dipyridamole, (b) with and without α_1AGP and (c) TdR compared to UdR.

Table 2. The effect of α_1AGP on free dipyridamole levels in RPMI and RPMI + 10% FBS

Total dipyridamole concentration (μM)	Free dipyridamole concentration (μM) in medium containing: 0 mg/ml α_1AGP	1 mg/ml α_1AGP		2 mg/ml α_1AGP	
	RPMI + 10% FBS	RPMI	RPMI + 10% FBS	RPMI	RPMI + 10% FBS
10	6.6 ± 0.8	0.31 ± 0.14	0.25 ± 0.2	0.12 ± 0.04	ND
3	1.6 ± 0.05	ND	ND	ND	ND
1	0.43 ± 0.03	ND	ND	ND	ND

ND = not detected (< 0.05 μM). Results are the mean ± SD of four determinations. In all samples, total dipyridamole was within 5% of the added concentration.

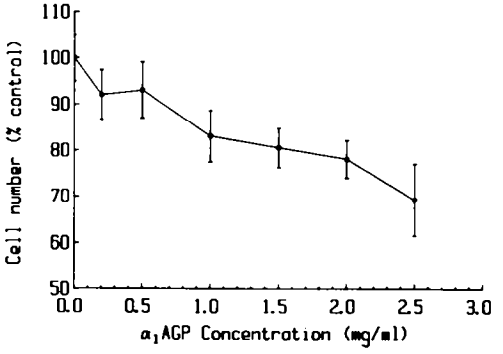

Fig. 4. Growth of A549 cells exposed to varying concentrations of α_1AGP for 72 hr. Figures are mean ± SD of five observations in a single experiment.

same level of inhibition of aggregation in plasma compared to washed platelets [41] and 50–100 μg/ml α_1AGP neutralised the effect of 0.1–1 μM dipyridamole. Also, inhibition of adenosine uptake by platelets was reduced 1,000-fold by 1 mg/ml α_1AGP [42]. This would be expected if the binding of dipyridamole to α_1AGP is greater than 99%.

At higher concentrations of dipyridamole, the reduction in potency by α_1AGP was less marked. For example, the presence of 1 mg/ml α_1AGP or 2 mg/ml α_1AGP rendered 10 μM dipyridamole only 10–30-fold or 50–100-fold less effective, respectively. These results can be explained by the free dipyridamole levels detected in serum-free RPMI media (Table 2). Thus, protein binding alone is sufficient to explain the α_1AGP-induced modulation of dipyridamole effects on thymidine uptake/incorporation.

As expected, there was an inverse relationship between α_1AGP concentration and the inhibition of TdR uptake by a single concentration of dipyridamole (1 μM). However, even at the highest concentration of α_1AGP used (2.5 mg/ml), TdR incorporation was still inhibited by 40–50%. Parallel studies demonstrated that at α_1AGP concentrations of ≥ 1.5 mg/ml inhibition of TdR uptake could be accounted for by α_1AGP alone, with dipyridamole not contributing to the effect. That some inhibition of nucleoside transport is still apparent in the presence of physiological levels of α_1AGP is presumably a reflection of the reversible equilibrium between the binding of dipyridamole to α_1AGP and cell membranes, dependent on their relative affinities for dipyridamole, as has been suggested for platelets [41, 42].

At peak plasma levels of dipyridamole routinely observed in patients on normal dosing regimes (2.5–7.5 μM) TdR incorporation was inhibited by between 70 and 90% in the presence of 1 mg/ml α_1AGP and between 65 and 85% in the presence of 2 mg/ml α_1AGP (Fig. 1). Similarly, it has been reported that therapeutic concentrations of dipyridamole following oral dosage of patients resulted in an 80% inhibition of adenosine uptake by platelets [40] and 10 μM dipyridamole inhibited [^{14}C]adenosine uptake

by red blood cells by 80% [43]. Furthermore, plasma concentrations of 2.5 μM dipyridamole following an i.v. dose of 0.4 mg/kg in human volunteers doubled the plasma adenosine concentration [44].

Nucleoside efflux was also inhibited by dipyridamole and, as we have previously shown [21], the efflux of UdR is inhibited more than the efflux of TdR. The effect of dipyridamole on UdR efflux was only slightly reduced by α_1AGP, but the inhibition of TdR efflux by dipyridamole was reduced to a greater extent by 1 mg/ml α_1AGP. In comparison to nucleoside uptake, the relatively minor effect of α_1AGP on dipyridamole-induced alterations in nucleoside efflux may be due to the relative concentrations of the nucleosides, dipyridamole and α_1AGP, intracellularly when compared to those present extracellularly. Thus, even in the presence of physiological concentrations of α_1AGP, dipyridamole may exacerbate a nucleotide pool imbalance caused by CB3717 by virtue of its effect on nucleoside transport.

Despite the significant effect (65–85% inhibition) of 1 and 3 μM dipyridamole on TdR uptake in the presence of α_1AGP (Fig. 1), α_1AGP completely abolished the potentiation of CB3717 cytotoxicity by these concentrations of dipyridamole (Fig. 3), and there was only a slight potentiation by 10 μM dipyridamole (approx. 95% inhibition of TdR uptake). From this, we conclude that TdR uptake must be inhibited by more than 95%, its efflux by more than 65% and UdR efflux by more than 85% to potentiate the toxicity of CB3717. Although this may be difficult to achieve *in vivo*, it may still be possible to improve CB3717 therapy with dipyridamole in patients for the following reason. *In vivo* CB3717 causes a dose-dependent decrease in plasma TdR [45], presumably because of increased utilization of the nucleoside and possibly because of reduced *de novo* synthesis and export from cells [46]. This latter source of circulating TdR might be decreased still further by dipyridamole, even in the presence of circulating α_1AGP, thereby a reduction in TdR available to rescue cells from CB3717 toxicity may be achieved. Thus reduced extracellular TdR, together with a decreased ability to take up the TdR, may enable dipyridamole to potentiate CB3717 toxicity *in vivo*. Chan and coworkers [47] have shown a synergistic lowering of plasma uridine by PALA and dipyridamole in patients and that dipyridamole reduced the LD_{50} of PALA by 50% in mice [14]. As patients have variable levels of serum TdR and α_1AGP, and tumours vary with respect to their thymidine kinase activity, some patients may derive more benefit from combined CB3717 and dipyridamole therapy than others.

We have also observed that α_1AGP alone affects both TdR incorporation following uptake and cell growth in a concentration-dependent manner. The inhibitory effect of α_1AGP *per se* on TdR incorporation (Fig. 2) was an unexpected finding and to our knowledge a previously unreported phenomenon, although it has been found that α_1AGP alone inhibits ADP-stimulated platelet aggregation [33, 48]. We have not determined whether this is due to interaction with the transport process or interference with the salvage pathway and DNA synthesis. Cell growth was also inhibited by α_1AGP (Fig. 4); however, a relationship between cell growth inhibition and reduced TdR uptake was not proven. Observations with regenerating rat liver have led to the suggestion that α_1AGP may be a primary mitotic inhibitor, whose intracellular concentration over a critical level inhibits cell division [49]. *In vitro* studies show that whereas low concentrations of α_1AGP (30 μg/ml) stimulated the growth of HeLa, human lymphoblastoid and human lung fibroblasts, higher concentrations (> 100 μg/ml) inhibited cell growth and α_1AGP had a dose-related suppressive effect on the growth of Yoshida sarcoma cells [50]. The varied effects of α_1AGP on cell growth may be related to the sequence similarity between α_1AGP and the C-terminal half of the epidermal growth factor receptor binding domain [51].

In conclusion, although the effect of dipyridamole on nucleoside transport and potentiation of CB3717 toxicity is reduced by α_1AGP, nucleoside uptake and efflux are still perturbed, particularly at higher dipyridamole concentrations. In selected patients with low α_1AGP levels, dipyridamole may enhance the effect of certain antimetabolites. Since some of the clinical effects of dipyridamole are due to blockade of adenosine uptake, then combination with an adenosine receptor antagonist may enable increased dosage. Alternatively, the use of well-tolerated drugs that bind to α_1AGP, e.g. propanolol [52], may be useful in increasing free dipyridamole levels.

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