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THE EFFECT OF THE NUCLEOSIDE TRANSPORT INHIBITOR DIPYRIDAMOLE ON THE INCORPORATION OF [^{3}H]THYMIDINE IN THE RAT

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Abstract—Dipyridamole is a non-specific inhibitor of nucleoside transport into mammalian cells. It is currently undergoing clinical evaluation in combination with various antimetabolites in an attempt to enhance the activity of these anticancer drugs by blocking the salvage of extracellular nucleosides, an important determinant of their cytotoxicity. In the present study, the effect of i.v. infusions of dipyridamole on [^{3}H]thymidine incorporation into DNA has been examined in the anaesthetized rat. The tissues studied were bone marrow, gastrointestinal tract epithelium and the ascitic form of the Walker carcinosarcoma. Dipyridamole at 10 mg/kg, given over 3 hr, led to plasma levels of < 5 μM and did not reduce [^{3}H]thymidine incorporation into any of the tissues studied. At 40 mg/kg dipyridamole (plasma levels 10–15 μM) [^{3}H]thymidine incorporation into the DNA of bone marrow and gastrointestinal tract epithelium was reduced to 20–30% of control values. Increasing the dose to 100 mg/kg did not lead to a further suppression of incorporation. Measurement of [^{3}H]thymidine plasma pharmacokinetics and the intracellular distribution of tritium suggested that the inhibition of [^{3}H]thymidine incorporation was due to reduced cellular uptake. In contrast to the effects on normal tissues, even at a lethal dose (200 mg/kg) dipyridamole did not significantly inhibit [^{3}H]thymidine incorporation into Walker tumour cells. The levels of dipyridamole found in the ascitic fluid, at 100 mg/kg approximately half those in plasma, argue against a pharmacokinetic basis for this difference. Dipyridamole was found to bind extensively (97%) to rat plasma proteins, which may explain the discrepancy between the concentrations of dipyridamole required to inhibit nucleoside incorporation *in vitro*, in serum-free media, and those needed *in vivo*. From a comparison of the plasma levels of dipyridamole which cause an inhibition of [^{3}H]thymidine incorporation in the rat with those which can be achieved safely in patients, it is concluded that dipyridamole is unlikely to markedly reduce nucleoside salvage in man.

Dipyridamole (Persantin, Fig. 1) was originally introduced into clinical practice for the treatment of angina pectoris [1]. It produces a range of pharmacological effects, the most important being vasodilation and the prevention of platelet aggregation, and has biochemical properties which include the blockade of nucleoside transport and the inhibition of cyclic AMP phosphodiesterase [2]. The property of dipyridamole which has attracted cancer chemotherapists is its ability to inhibit nucleoside transport across the cell membrane.

Nucleoside transport is important in cancer chemotherapy firstly because it mediates the cellular uptake of cytotoxic nucleosides [3] and secondly because it represents the first step in the salvage pathway for the utilization of extracellular nucleosides [4]. The salvage pathway is in turn an important determinant of the cytotoxicity of antimetabolites which act by the inhibition of *de novo* nucleotide biosynthesis [5].

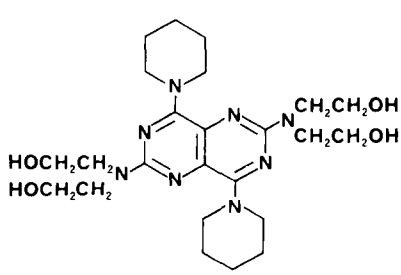

Fig. 1. The structure of dipyridamole.

When used in combination with certain cytotoxic nucleosides, inhibitors of nucleoside transport can reduce whole animal toxicity [6] which in some instances leads to an increased therapeutic index in experimental tumour systems [7, 8]. Studies with combinations of nucleoside transport inhibitors and antimetabolite inhibitors of *de novo* nucleotide biosynthesis have been performed in most cases *in vitro*. Thus against cultured tumour cells the cytotoxicity of a range of antimetabolites can be maintained, despite the presence of extracellular nucleosides, by the addition of a nucleoside transport inhibitor. A number of studies have utilized dipyridamole as the nucleoside transport inhibitor in combination with, for example, methotrexate [9–11], PALA [12], acivicin [13] and N^{10}-propargyl-5,8-dideazafolic acid [14]. In *in vivo* experiments dipyridamole has been shown to inhibit thymidine uptake into mouse blood cells [9] and to deplete ribonucleotide and deoxyribonucleotide levels in a rat hepatoma [15]. When used in combination with methotrexate, dipyridamole produces enhanced

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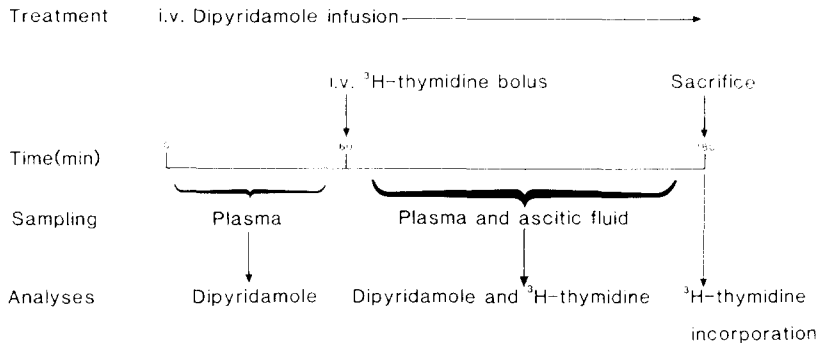

Fig. 2. Outline of the experimental protocol used.

whole animal toxicity and a slight increase in antitumour activity against the Ridgway osteogenic sarcoma but not against the L1210 leukaemia [9]. Similarly, dipyridamole enhances the toxicity of PALA in nude mice [12].

On the basis of the above results clinical trials have been initiated of dipyridamole given in combination with inhibitors of *de novo* nucleotide biosynthesis [16–18]. However, from the literature, the relationship of dipyridamole dose and pharmacokinetics to the inhibition of nucleoside utilization *in vivo* is not clear. The experiments reported in the present study were conducted in order to clarify this point. Investigations were performed in rats so as to facilitate surgery and with [^{3}H]thymidine as the nucleoside because of its relatively simple intracellular fate and its importance in DNA synthesis. The tissues studied were bone marrow, gastrointestinal tract epithelium and a transplantable rodent tumour, the Walker 256 carcinosarcoma. From the results obtained it has been possible to correlate dipyridamole dose and pharmacokinetics with the inhibition of [^{3}H]thymidine incorporation into the DNA of normal tissues. In addition, it has been found that the Walker carcinosarcoma is insensitive to dipyridamole-induced inhibition of [^{3}H]thymidine incorporation *in vivo*.

MATERIALS AND METHODS

Materials. Experiments were performed using Walker tumour-bearing female Wistar rats, body weight 200–250 g, supplied by the Institute of Cancer Research breeding colony. The ascitic form of the Walker 256 carcinosarcoma (5×10^5 cells) was implanted by i.p. injection five days prior to the experiment with the result that on the day of the experiment the cell concentration within the peritoneal cavity was within the range $2–6 \times 10^7$/ml ascitic fluid. Dipyridamole and sodium pentobarbital were purchased from the Sigma Chemical Company (Poole, Dorset, U.K.). [Methyl-^{3}H]thymidine (25 Ci/mmol) and [^{3}H]hexadecane were supplied by Amersham International (Amersham, Bucks, U.K.) and Cocktail T scintillation fluid by BDH Chemicals (Poole, Dorset, U.K.). All other chemicals were of analytical grade where available and obtained from standard suppliers.

Constant rate i.v. infusions were given using Sage Model 352 pumps (Orion Res. Inc. Cambridge, MA, U.S.A.) and all HPLC separations performed using a Waters Associates chromatograph (Harrow, London, U.K.).

Animal experiments. The protocol for the experiments performed is outlined in Fig. 2. Rats were anaesthetized with 50 mg/kg sodium pentobarbital i.p. (50 mg/ml in 0.15 M NaCl) and the trachea, carotid artery and left femoral vein cannulated using polyethylene tubing. When not in use the patency of the carotid and femoral cannulae was maintained with 50 iu/ml heparin in 0.15 M NaCl. Throughout the experiment the body temperature was monitored with a rectal thermometer and maintained by means of a heating lamp at 37 ± 1°. Additional doses of sodium pentobarbital were given as necessary to maintain satisfactory anaesthesia.

Once the body temperature had stabilized following surgery a constant rate (3 ml/hr) i.v. infusion into the femoral vein was started. The infusion fluid was either 0.15 M NaCl pH 2.0 (control rats) or dipyridamole solution in the same solvent so as to give total doses over 3 hr of 10, 40, 100 or 200 mg/kg dipyridamole. Sixty minutes after the start of the i.v. infusion the rats received an i.v. bolus dose of 1 mCi/kg [^{3}H]thymidine (1.0 mCi/ml in H_2O) into the femoral vein. 180 min after the start of the i.v. dipyridamole infusion, i.e. 120 min after the i.v. [^{3}H]thymidine dose, the animals were sacrificed by an i.v. bolus dose of KCl.

For blood sampling during the course of the experiment samples (0.3 ml) were taken from the carotid artery and heparinized plasma (10 iu/ml) prepared by centrifugation at room temperature for 2 min. Ascitic fluid (0.5 ml) was taken directly from the peritoneal cavity with a syringe and treated in a similar manner. Plasma and ascitic fluid were stored at −20° prior to dipyridamole and [^{3}H]thymidine analyses.

Following sacrifice, the right femur, a 10 cm length of small intestine taken 5–15 cm below the pyloric sphincter and a 0.5 ml aliquot of the total ascitic tumour cell suspension were removed. The tumour cells were layered over Ficoll-Paque (Pharmacia, Milton Keynes, Bucks, U.K.) and centrifuged at 1500 g for 10 min at 4°. The tumour cell concentrate was removed and placed in 5 ml 0.15 M NaCl. The

segment of small intestine was rinsed clean with 0.15 M NaCl, slit open up the entire length and the inner epithelial surface scraped off with a scalpel blade into 5 ml 0.15 M NaCl. Finally, both ends of the femur were removed and the bone marrow washed out into 5 ml 0.15 M NaCl. The above procedures were completed within 30 min of sacrifice, with all samples being placed and kept on ice as soon as possible. Once the samples from one rat had been removed, the cells were sedimented by centrifugation at 1000 *g* for 15 min at 4°. The resultant pellets were used to analyse the [^{3}H]content of the acid soluble, RNA, DNA and protein fractions.

Estimation of [^{3}H]*thymidine incorporation into DNA.* This was accomplished by an adaption of the method of Schmidt and Thannhauser [19]. To the pellets derived, as described above, from bone marrow, gastrointestinal tract epithelium and Walker tumour cells, 2 ml of ice-cold 0.2 M perchloric acid was added. Samples were mixed, centrifuged and the supernatants, representing the acid soluble fraction, removed (i.e. tritium not associated with macromolecules such as free nucleosides and nucleotides). To the pellet 1.0 ml of 0.3 M KOH was added and, after mixing, samples incubated at 37° for 1 hr following which they were cooled and 0.65 ml 2 M perchloric acid added with mixing. Samples were left at room temperature for 30 min and then centrifuged. The resultant supernatant was decanted and combined with two further washings of the pellet, each with 2 ml 0.2 M perchloric acid, to yield the RNA fraction. To the pellet remaining following the extraction of RNA, 2.5 ml of 0.5 M perchloric acid was added and, following mixing, the samples incubated at 70° for 30 min. Following centrifugation the supernatant was removed, the procedure repeated twice, and the three 70° 0.5 M perchloric acid extracts pooled to give the DNA fraction. Finally, the pellet remaining following the extraction of DNA was digested in 2 ml 0.1 M NaOH at room temperature to produce the protein fraction. To determine the tritium present in each fraction a 0.1 ml aliquot was removed and placed in 10 ml Cocktail T scintillant. Quench correction was achieved using [^{3}H]hexadecane. The DNA concentration in the DNA fraction was assayed using the method of Burton [20] and [^{3}H]thymidine incorporation expressed as nCi/μg DNA. All centrifugation was performed at 1000 *g* for 10 min at 4°.

Estimation of plasma [^{3}H]*-thymidine levels.* This was performed by an adaption of the method of Taylor *et al.* [21]. Plasma samples were thawed and a 0.1 ml aliquot removed to which 0.02 ml 1.2 M perchloric acid was added with vigorous mixing. The precipitate was removed by centrifugation at 1500 *g* for 15 min at 4° and 0.02 ml of the supernatant analysed by HPLC within 1 hr. Samples were applied to a 25 × 0.46 cm APEX ODS column (Jones Chromatography Ltd., Llanbradach, Glamorgan, U.K.) fitted with a 5 × 0.21 cm CO:PELL ODS precolumn (Whatman, Maidstone, Kent, U.K.) and eluted with 0.1 M NH_3CH_3COOH/methanol (85/15 v/v) at a flow rate of 1.5 ml/min. 0.75 ml fractions of the column effluent were collected into scintillation vials and 10 ml Cocktail T scintillant added. From the resultant radiochromatogram, the radioactivity associated with the fractions co-chromatographing with thymidine was calculated and, following quench correction using [^{3}H]hexadecane, the plasma [^{3}H]thymidine concentration determined. Using this method the radiochemical purity of the [^{3}H]thymidine used in these studies was found to be 96%.

Estimation of dipyridamole in plasma and ascitic fluid. The assay of dipyridamole was accomplished by HPLC with fluorescence detection. 0.05 ml aliquots of plasma were vigorously mixed with 0.15 ml methanol and the precipitate removed by centrifugation at 1500 *g* for 10 min at 4°. 0.05 ml of the resultant supernatant was applied to a 25 × 0.46 cm μBondapak C18 column (Waters Associates, Harrow, London, U.K.) fitted with a 5 × 0.21 cm CO:PELL ODS precolumn (Whatman, Maidstone, Kent, U.K.) and eluted with methanol/H_2O/acetic acid 60/39/1 (v/v) at 2 ml/min. Dipyridamole was detected in the column effluent by fluorescence at 500 nm following excitation at 395 nm. The assay was validated by the addition of dipyridamole solutions (0.01 ml) to heparinized (10 iu/ml) rat plasma (1 ml) to give concentrations of 5–200 μM dipyridamole. Over this range the recovery of dipyridamole was complete (101 ± 4%) and the assay linear ($r = 0.99$ $P < 0.001$). Ascitic fluid was treated in an identical manner.

Protein binding studies. The binding of dipyridamole to rat plasma proteins was examined *in vitro* using the Amicon Centrefree system (Amicon Ltd., Stonehouse, Gloucestershire, U.K.). Aliquots of dipyridamole solutions (0.01 ml) were added to 1 ml samples of heparinized rat plasma (10 iu/ml) to give concentrations of 20–200 μM dipyridamole. The 1 ml of rat plasma was then placed in the upper part of the centrifuge tube and ultrafiltrates prepared by centrifugation at 1500 *g* for 20 min at 15°. 0.02 ml of the ultrafiltrate was analysed by HPLC as described above with the exception that detection was achieved using the more sensitive Perkin–Elmer LS4 fluorimeter (Perkin-Elmer, Beaconsfield, Berks, U.K.) which allowed a limit of detection of 0.1 μM dipyridamole.

Pharmacokinetic and statistical analyses. A two-compartment open pharmacokinetic model was fitted to the plasma [^{3}H]thymidine levels using a non-linear least-squares method [22] with an error weighting of $1/(y + \hat{y})^2$ [23, 24]. From the fitted equation, i.e.

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C is the plasma concentration at time t and A, B and α, β are the concentration and rate constants, respectively; the half-lives and volumes of distribution were calculated [25]. The [^{3}H]thymidine total plasma clearance was calculated as dose/area under the plasma concentration vs time curve (AUC). The AUC was calculated from the computer fit by the equation $AUC = A/\alpha + B/\beta$ [25].

Throughout the text and in tables values are given as the mean ± the standard error. The number of rats in each group were control, 7; dipyridamole 10 mg/kg, 5; 40 mg/kg, 4; 100 mg/kg, 5; and 200 mg/kg, 5. Differences between groups were demonstrated using Student's *t*-test and linearity by linear regression analysis.

Table 1. The pharmacokinetics of 1 mCi/kg [^{3}H]thymidine in control rats and in rats receiving dipyridamole infusions at 40 or 200 mg/kg

Group	Control	Dipyridamole 40 mg/kg	Dipyridamole 200 mg/kg
t_1^{α} (min)	3.6 ± 0.5*	3.4 ± 0.2	3.5 ± 0.3
t_1^{β} (min)	34 ± 1	34 ± 3	43 ± 3
V_1 (l/kg)	0.65 ± 0.10	0.55 ± 0.044	0.54 ± 0.06
V_{ss} (l/kg)	1.68 ± 0.06	1.44 ± 0.13	1.36 ± 0.08
Clearance (ml/kg)	48 ± 2	41 ± 3	27 ± 1

* All values are the mean ± SE.

RESULTS

To validate the experimental protocol used (Fig. 2) the plasma levels of [^{3}H]thymidine were studied following an i.v. bolus of 1 mCi/kg [^{3}H]thymidine in rats receiving an i.v. infusion of the dipyridamole vehicle (0.15 M NaCl pH 2.0). Plasma levels of [^{3}H]thymidine decayed biphasically with first-order kinetics. The plasma half-lives, volumes of distribution and clearance values are given in Table 1. At the end of the experiment, i.e. 120 min after [^{3}H]thymidine administration only 2.7 ± 0.4% of the total plasma [^{3}H] was in the form of [^{3}H]thymidine.

The incorporation of [^{3}H]thymidine into the acid soluble, RNA, DNA and protein fractions of Walker tumour cells, bone marrow and gastrointestinal tract epithelium was also studied in animals receiving the dipyridamole infusion vehicle. As shown in Table 2, the specific activities of the DNA, i.e. [^{3}H]thymidine incorporation, in the three tissues were similar. Furthermore, the majority of the [^{3}H] in each tissue was associated with the DNA fraction. Thus on the basis of the above data, it was concluded that 120 min after administration was a suitable time point at which to determine [^{3}H]thymidine incorporation, i.e. the majority of the [^{3}H]thymidine had been cleared from the plasma with intracellular [^{3}H] associated primarily with the DNA fraction.

These experiments were repeated in rats receiving constant rate i.v. infusions of dipyridamole at doses of 10, 40, 100 and 200 mg/kg. The effect of dipyridamole on [^{3}H]thymidine pharmacokinetics was investigated at two dose levels, 40 and 200 mg/kg dipyridamole. As shown in Table 1, 40 mg/kg dipyridamole did not alter [^{3}H]thymidine pharmacokinetics, whereas 200 mg/kg dipyridamole did influence [^{3}H]thymidine levels during the disposition phase. Thus the t_1^{β} was somewhat increased and the steady-state volume of distribution decreased, with the overall plasma clearance of [^{3}H]thymidine significantly reduced when compared to the control rats ($P \leq 0.001$). However, it should be noted that 200 mg/kg dipyridamole was found to be lethal within 3 hr in 5/5 rats. Although the cause of death in these animals was not determined, observations indicated a fall in body temperature and a reduced rate of respiration prior to death. In the 5 rats treated with 200 mg/kg dipyridamole, death occurred 80, 80, 90, 95 and 115 min after [^{3}H]thymidine administration, i.e. the animals did receive the major part of the dipyridamole dose.

The effect of dipyridamole on [^{3}H]thymidine incorporation into the DNA of Walker tumour cells, bone marrow and gastrointestinal tract epithelium is shown in Fig. 3. Again data for the 200 mg/kg dipyridamole dose shoule be interpreted cautiously since these rats did not receive a full exposure to either dipyridamole or [^{3}H]thymidine. At the lowest dose (10 mg/kg) dipyridamole did not significantly alter [^{3}H]thymidine incorporation in any of the tissues studied ($P \geq 0.05$). At all of the higher doses, i.e. 40, 100 and 200 mg/kg dipyridamole, [^{3}H]thymidine incorporation into the DNA of bone marrow and gastrointestinal tract epithelium was significantly decreased ($P \leq 0.05$). The degree of inhibition of [^{3}H]thymidine into these tissues was not increased by using doses of greater than 40 mg/kg dipyridamole, nor could it be suppressed to below 20–30% of the control value. In contrast to the results in bone marrow and gastrointestinal tract epithelium, [^{3}H]thymidine incorporation into the DNA of Walker tumour cells was not significantly altered by dipyridamole at any of the doses studied.

Table 2. The incorporation of [^{3}H]thymidine into the DNA of Walker tumour cells, bone marrow and gastrointestinal tract epithelium in control rats

Tissue	[^{3}H]Thymidine incorporation (mCi/μg DNA)	% of total tissue [^{3}H] in Acid-soluble pool	RNA	DNA	Protein
Walker tumour	0.13* ± 0.02	17 ± 1%	< 1%	78 ± 2%	5 ± 1%
Bone marrow	0.14 ± 0.01	< 1%	< 1%	91 ± 2%	9 ± 2%
Gastro intestinal tract epithelium	0.14 ± 0.02	9 ± 2%	4 ± 1%	80 ± 2%	7 ± 1%

* All values are the mean ± SE.

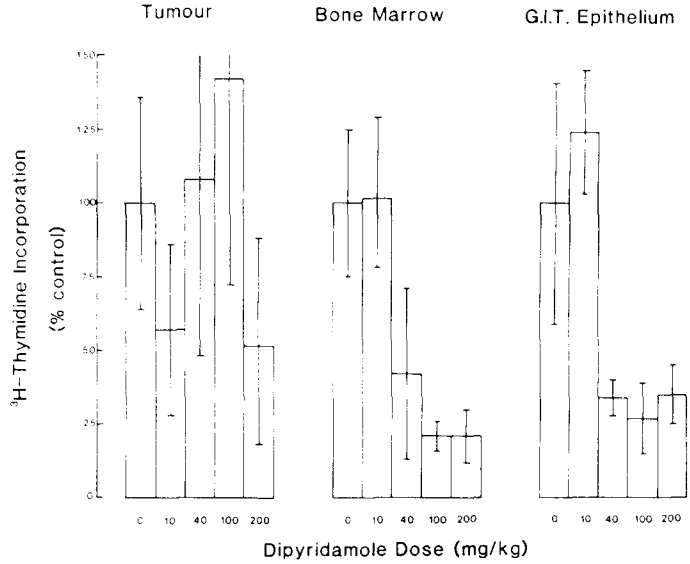

Fig. 3. The effect of dipyridamole given as a constant rate intravenous infusion on the incorporation of [^{3}H]thymidine into the DNA of bone marrow, gastrointestinal tract epithelium and Walker tumour cells. Bars are the 95% confidence limits of the means.

The distribution of [^{3}H] into the acid soluble, RNA, DNA and protein fractions was studied for each tissue in every experiment. Following dipyridamole, in Walker tumour cells and bone marrow, the percentage of the total tissue [^{3}H] content present in the DNA fraction was not significantly altered from control values (Table 2) with the exception of Walker cells following 40 mg/kg (53 ± 4% [^{3}H] in the DNA fraction) and bone-marrow after 10 mg/kg dipyridamole (76 ± 3% [^{3}H] in the DNA fraction). However, dipyridamole did consistently reduce the percentage of the total tissue [^{3}H] present in the DNA fraction of the gastrointestinal tract epithelium, i.e. to 59 ± 4%, 56 ± 8%, 45 ± 4% and 52 ± 5% at 10, 40, 100 and 200 mg/kg respectively. Thus this effect was observed at the lowest dose and was no more pronounced at higher doses.

To determine the drug concentrations associated with the effects on [^{3}H]thymidine incorporation, dipyridamole plasma levels were measured at all four dose levels and ascitic fluid levels during the 100 mg/kg infusion. At all time points during the 10 mg/kg dipyridamole infusion plasma levels were below the limit of detection (< 5 μM) whilst at higher doses dipyridamole was readily detectable. These data are shown in Fig. 4. At all three dose levels, plasma dipyridamole concentrations increased throughout the infusion although the rate of increase was more marked prior to the administration of [^{3}H]thymidine at 60 min. Dipyridamole plasma levels tended to

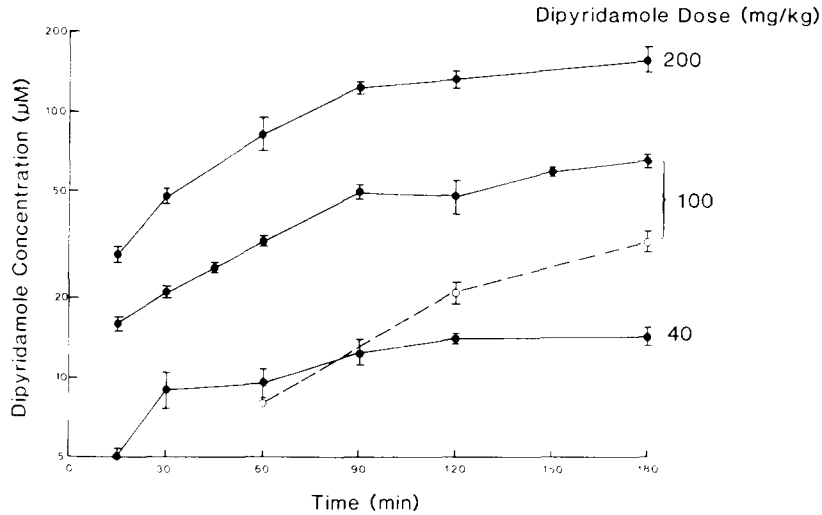

Fig. 4. Dipyridamole plasma (———) and ascitic fluid (– – –) levels during constant rate i.v. infusions of 40, 100 or 200 mg/kg dipyridamole. Bars are the standard errors of the means.

plateau during the period of exposure to [^{3}H]thymidine, most notably at the 40 mg/kg dose. The overall mean plasma dipyridamole levels for the period from the administration of the [^{3}H]thymidine to the end of the experiment were 13 ± 1, 52 ± 6 and 125 ± 16 μM at 40, 100 and 200 mg/kg dipyridamole, respectively.

At the time points studied, levels of dipyridamole in the ascitic fluid of rats treated with 100 mg/kg dipyridamole were approximately half those found in plasma (Fig. 4). Despite this, the levels observed in the ascitic fluid were in excess of those in plasma during 40 mg/kg dipyridamole, i.e. concentrations which gave rise to significant inhibition of [^{3}H]thymidine incorporation into bone marrow and gastrointestinal tract epithelium DNA.

The binding of dipyridamole to plasma proteins over the concentration range 20–200 μM dipyridamole was studied using heparinized rat plasma. Binding was found to be linear ($r \geq 0.99$, $P \leq 0.001$) at 97.1 ± 0.3%. Thus dipyridamole binds extensively to rat plasma proteins.

DISCUSSION

The aim of the study reported in this paper was to determine the effect of dipyridamole on the incorporation of [^{3}H]thymidine into the DNA of rat tissues and to define the plasma levels of dipyridamole associated with this effect. As shown in Fig. 3, dipyridamole induced a dose-dependent inhibition of [^{3}H]thymidine incorporation into the DNA of bone marrow and gastrointestinal tract epithelium. In contrast, incorporation into Walker tumour cell DNA was not significantly altered even at a lethal dose of dipyridamole.

It is unlikely that the failure to suppress [^{3}H]thymidine incorporation into Walker cells was due to inadequate dipyridamole exposure since levels in the ascitic fluid following 100 mg/kg dipyridamole exceeded those observed in plasma following 40 mg/kg (Fig. 4), a dose which induced suppression in normal tissues. Thus the basis for the differential sensitivities of normal tissues and tumour cells to dipyridamole probably lies at the cellular level. It is known that cultured Walker cells have a nucleoside uptake mechanism which, unlike most cell types, is insensitive to the nucleoside transport inhibitor *p*-nitrobenzyl mercaptopurine riboside [6, 26]. However, comparison with data from other cell lines would suggest that nucleoside uptake, and hence thymidine incorporation, should still be sensitive to dipyridamole [27].

Regardless of the exact type of nucleoside transport mechanism involved, the differential sensitivities of bone marrow, gastrointestinal tract epithelium and Walker tumour cells to nucleoside transport inhibition offers an explanation for the observations of Jakobs and Paterson [28]. These authors found that the therapeutic index of the cytotoxic nucleoside tubercidin was increased when it was given in combination with the transport inhibitor *p*-nitrobenzyl mercaptopurine riboside-5′-phosphate. From the data reported in the present study it would be predicted that tubercidin uptake into normal tissues might be reduced by a nucleoside transport inhibitor whilst uptake into Walker cells would not. If human tumour cells were found with nucleoside uptake mechanisms of the type found in Walker cells such combinations might warrant clinical evaluation.

The foregoing discussion makes the assumption that where a reduction in [^{3}H]thymidine incorporation into DNA was observed this was due to an inhibition of the cellular uptake of [^{3}H]thymidine. However, dipyridamole could produce the same result by other mechanisms. For example, it could inhibit the intracellular phosphorylation of thymidine to thymidine triphosphate or the subsequent action of DNA polymerase. Alternatively dipyridamole could enhance [^{3}H]thymidine plasma clearance thereby reducing tissue exposure and [^{3}H]thymidine incorporation. It is unlikely that the former occurred since there should have been a concomitant rise in the radioactivity associated with the acid soluble fraction. This effect was only seen in the gastrointestinal tract epithelium and then it did not correlate with the inhibition of [^{3}H]thymidine incorporation into DNA. With regard to the second possibility, if anything, dipyridamole decreased the plasma clearance of [^{3}H]thymidine (Table 1). Taken together the above evidence strongly suggests that the decrease in [^{3}H]thymidine incorporation into the DNA of bone-marrow and gastrointestinal tract epithelium resulted from an inhibition of uptake rather than a stimulation of plasma clearance or a reduction in intracellular phosphorylation or DNA polymerization.

The relationship between dipyridamole dose, pharmacokinetics and the inhibition of [^{3}H]thymidine incorporation is of interest, particularly with regard to the clinical trials of dipyridamole given in combination with antimetabolites, i.e. methotrexate [17], PALA [18] and acivicin [16]. In the present study, inhibition of [^{3}H]thymidine incorporation was not observed during the infusion of 10 mg/kg dipyridamole, a dose which gave rise to plasma levels of < 5 μM. When levels of 10–15 μM dipyridamole were achieved during the 40 mg/kg infusion significant inhibition of [^{3}H]thymidine incorporation into the DNA of bone marrow and gastrointestinal tract epithelium was observed. From the data available on the kinetics of dipyridamole in man (e.g. [29–32]), it is unlikely that plasma levels of greater than 4 μM dipyridamole are being achieved in the clinical trials using oral administration [17, 18]. Furthermore, in one study, where an i.v. infusion of dipyridamole was employed, steady state dipyridamole levels of < 1 μM were reported [16]. Comparison of these clinical data with those reported herein would suggest that higher doses of dipyridamole will be required in order to inhibit markedly the salvage of extracellular nucleosides in patients. However, elevation of the dipyridamole dose may not be possible because of the hypotensive properties of the drug [2].

In terms of the concentrations of dipyridamole required to inhibit nucleoside utilization *in vitro* and those needed to suppress [^{3}H]thymidine incorporation *in vivo*, there is clearly a large discrepancy. In serum-free media, nucleoside uptake or incorporation *in vitro* is reduced by dipyridamole at 1 μM

or less (e.g. [12, 13, 26, 27, 33]) whereas *in vivo* plasma levels of 10–15 μM dipyridamole were required. The most likely explanation for this apparent discrepancy lies in the extensive plasma protein binding of dipyridamole. In the present study dipyridamole was found to be 97% bound to rat plasma proteins. Similar or higher values have been reported for the binding of dipyridamole to human plasma proteins [31, 34, 35]. Thus dipyridamole would appear to be an example of a drug whose high affinity for plasma proteins is a significant determinant of *in vivo* potency.

In conclusion, dipyridamole was able to reduce [^{3}H]thymidine incorporation into the DNA of rat bone marrow and gastrointestinal tract epithelium, probably by inhibiting the cellular uptake of [^{3}H]thymidine. However, a dose of 40 mg/kg dipyridamole, with associated plasma levels of 10–15 μM, was required to produce this effect. Dipyridamole at 10 mg/kg, plasma levels < 5 μM, did not reduce [^{3}H]thymidine incorporation. In contrast to the results in normal tissues, [^{3}H]thymidine incorporation into Walker carcinosarcoma cells was not suppressed, even at a lethal dose of dipyridamole (200 mg/kg, plasma levels 80–160 μM). The reason for this difference is unclear although it is unlikely to have a pharmacokinetic basis. From a comparison of the data reported herein with those on the clinical pharmacokinetics of dipyridamole it seems unlikely that levels of dipyridamole can be achieved readily in man which will markedly inhibit nucleoside salvage.

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