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# INHIBITION OF LYMPHOPROLIFERATION BY DIPYRIDAMOLE

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**Abstract**—Dipyridamole (DP, Persantin) was examined for its effects on the proliferation of mitogen-stimulated murine splenocytes and L1210 leukemia cells. In keeping with its reported activity as an inhibitor of nucleoside transport, DP inhibited incorporation by lymphoid cells of labeled thymidine and uridine into macromolecules. That this inhibition resulted from activities in addition to suppression of nucleoside transport was verified by measured decreases of cellular DNA and viable cell numbers. In addition, protein synthesis was also decreased as indicated by labeled valine incorporation and total protein content of the cells. The rapid accumulation of cAMP in phytohemagglutinin-stimulated splenocytes in the presence of DP may provide an explanation for the anti-proliferative effect of DP on lymphoid cells.

Dipyridamole [2,6-bis(diethanolamino)-4,8-dipiperidino-[5,4-*d*]pyrimidine] functions as an inhibitor of purine and pyrimidine nucleoside transport by mammalian cells [1], including lymphocytes [2]. Recently, Pazdur *et al.* [3] described the ability of dipyridamole (DP)† to inhibit the incorporation of [$^3$H]dTR into DNA by both PHA-stimulated and unstimulated human peripheral blood lymphocytes. The incorporation of [$^{14}$C]leucine into cellular protein, however, was unaffected by concentrations of DP which totally inhibited thymidine incorporation, suggesting that the apparent inhibition of mitogenesis induced by this agent was due to inhibition of thymidine transport. Additionally, these authors demonstrated the ability of DP to antagonize formation of rosettes between HPBL and SRBC.

The present study is concerned with the effect of dipyridamole on proliferation of normal and leukemic murine lymphoid cells. The results demonstrate that, in addition to its activity as an inhibitor of nucleoside transport, DP exerts suppressive effects on other metabolic systems and that they may be related to DP-induced elevation in intracellular cAMP.

## MATERIALS AND METHODS

### *Materials*

A/J mice were purchased from Jackson Laboratories. RPMI 1640 medium, fetal calf serum, penicillin–streptomycin solution and trypan blue were from GIBCO Laboratories (Grand Island, NY). Purified phytohemagglutinin (PHA) was from Wellcome Reagents (Greenville, NC), and Difco Products (Detroit, MI) supplied *Escherichia coli* 0127:B8 lipopolysaccharide (LPS). Microtiter plates and petri plates for tissue culture were from Falcon Laboratories (Scientific Products). Biuret reagent was purchased from Harleco, (Philadelphia, PA), and the protein standard (LabTrol) was a product of Dade Diagnostics (Miami, FL).

Labeled macromolecule precursors were obtained from the New England Nuclear Corp. (Boston, MA). [Me-$^3$H]Thymidine, 20 Ci/mmole, was diluted to 4 Ci/mmole with cold thymidine prior to use. [6-$^3$H]Uridine, 22.4 Ci/mmole, and [G-$^3$H]valine, 2 Ci/mmole, were used as supplied.

The cAMP assay kit was purchased from Amersham Radiochemicals (Arlington Heights, IL). Dipyridamole was the generous gift of Boehringer-Ingelheim, Ltd. (Ridgefield, CT).

### *Methods*

*Mitogen cultures.* Murine splenocytes were obtained by teasing apart spleens from male A/J mice in RPMI 1640. Debris was allowed to settle, and the supernatant fraction was transferred to a 15 ml conical centrifuge tube. Cells were subsequently treated with an ammonium chloride–Tris lysing buffer to remove RBC and were washed three times in RPMI 1640 prior to culture.

In experiments designed to evaluate incorporation of radiolabeled compounds, spleen cells ($3 \times 10^6$/ml) were cultured in 0.2 ml volumes in the wells of microtiter plates in RPMI 1640 supplemented with 5% fetal calf serum, 100 units penicillin/ml and 100 μg streptomycin/ml. Mitogen (1 μg PHA/ml or 33 μg LPS/ml) was added to culture in 0.02 ml volume. Unless otherwise indicated, cultures were incubated at 37° in a 5% $CO_2$ air atmosphere for 2 (LPS) or 3 (PHA) days.

Except where noted, [$^3$H]dTR (0.5 μCi/well) was added for the terminal 18 hr of the culture period.

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† Abbreviations: DP, dipyridamole; HPBL, human peripheral blood lymphocytes; SRBC, sheep erythrocytes; TCA, trichloroacetic acid; PHA, phytohemagglutinin; and LPS, lipopolysaccharide.

[$^3$H]UR or [$^3$H]valine (0.5 $\mu$Ci/well) was added 3 hr prior to culture harvest.

All cultures were harvested on a Mash II automatic cell harvester. To determine incorporation of [$^3$H]valine into protein, cold 5% TCA was substituted for the usual water wash during harvest. Incorporation of radioactivity was quantitated with a Beckman LS3150T liquid scintillation counter by placing the dry glass-fiber discs containing the harvested cells directly into a counting vial. Suppression of the response under study by dipyridamole was calculated as:

$$\% \text{ Suppression} = \left(1 - \frac{\text{cpm of cultures with dipyridamole}}{\text{cpm of cultures without dipyridamole}}\right) \times 100$$

Large numbers of mitogen-stimulated splenocytes were required for experiments involving measurement of DNA, protein and cAMP. Splenocytes were cultured in 3 ml volumes in 10 × 35 mm petri plates, with all other conditions remaining the same as for the microtiter cultures.

*L1210 cultures.* L1210 cells were maintained in ascitic form by serial passage in syngeneic DBA/2 carrier animals. Cells were harvested from the peritoneal cavities of carriers 7 days after transfer of $1 \times 10^4$ L1210 cells. Any contaminating RBC were removed with lysing buffer.

In studies designed to determine incorporation of radiolabeled compounds, L1210 cells ($1 \times 10^5$/well) were cultured in the wells of microtiter plates in 0.2 ml RPMI 1640 supplemented with 5% fetal calf serum, 100 units penicillin/ml, 100 $\mu$g streptomycin/ml, and $5 \times 10^{-5}$ M 2-mercaptoethanol. Unless otherwise indicated, cells were cultured at 37° for 24 hrs.

Labeling of cultures with macromolecule precursors was as described above for mitogen cultures, except that [$^3$H]dTR was present for the terminal 4 hr of leukemic cell culture.

Cells were harvested, and incorporation of label was determined as described above for splenocyte cultures. In experiments where large numbers of L1210 cells were required, e.g. protein determinations, cells were cultured in 5 ml volumes in 60 × 15 mm petri plates, in the medium used for micro cultures.

*Protein content of cultured cells.* Cultures were harvested after 24 hr of incubation and washed two times with 0.9% NaCl. Washed cells were resuspended in 0.5 to 1.0 ml saline and made 0.2 N in NaOH. Samples were immersed in boiling water for 15 min, and they were subsequently spun at 800 $g$ to pellet any insoluble debris. Protein was determined by biuret assay with LabTrol as the standard.

*DNA content of cultured cells.* PHA cultures, with and without DP, were harvested after 60 hr of culture and washed two times in 0.9% NaCl. Washed cells were resuspended in 0.5 ml of saline, and 2 ml of 10% TCA was added. After 45 min on ice, the precipitate was washed twice with cold 10% TCA and resuspended in a final volume of 1.25 ml of 5% TCA. This preparation was placed in a 90° water bath for 20 min. Following removal of debris by centrifugation, duplicate 0.5 ml aliquots were analyzed by mixing with 1 ml of diphenylamine reagent [4]. Incubation was at room temperature overnight in the dark; sample absorbance was determined at 600 nm, and the 2-deoxyribose content was determined by reference to a standard curve.

*Lymphocyte cAMP assay.* Large-scale PHA cultures, with and without dipyridamole, were harvested at the indicated times after culture initiation. An aliquot was withdrawn to determine cell recovery, and the remaining cell suspension was spun down and resuspended in 0.5 ml of cold 5% TCA. Samples were subsequently disrupted by sonication and centrifuged at 10,000 $g$ for 10 min to remove debris. Supernatant fractions were transferred to 12 × 75 mm glass tubes and extracted five times with 3 vol. of diethyl ether to remove TCA. Cell samples were then lyophilized and concentrated to 0.2 ml prior to assay.

The cAMP content of splenocyte cultures was determined with a protein binding assay kit purchased from Amersham Radiochemicals. The above extraction procedure results in greater than 95% recovery of cAMP from lymphocyte preparations spiked with a known amount of cold cAMP.

*Statistical methods.* As an indication of the variation observed among replicate data points in a given experiment, the maximum variation observed is noted in the appropriate figure legends. Here, variation is defined as the percent of the mean value which the standard error of the mean (S.E.M.) represents.

The statistical significance of differences observed between data points was evaluated by Student's $t$-test, and the resultant P values are presented in the accompanying figure legends.

## RESULTS

### *Effect of dipyridamole on incorporation of radiolabeled compounds by lymphoid cells*

In initial experiments, dipyridamole was examined for its effects on incorporation of radiolabeled precursors of macromolecules by various murine lymphoid cells.

*Effect of DP on [$^3$H]dTR incorporation.* (a) *Potency of inhibition.* Dose–response curves were constructed for the effect of DP on dTR incorporation by mitogen-stimulated splenocytes and L1210 leukemia cells. From the dose–response curves, the concentration of DP required to inhibit label incorporation by 50% ($I_{50}$) was determined. As shown in Table 1, mitogen-stimulated splenocytes, as well as L1210 cells, were about equally sensitive to inhibition of thymidine incorporation by DP.

(b) *Kinetics of inhibition.* Time courses were generated to determine the kinetics of DP inhibition of incorporation of labeled thymidine for both PHA and LPS cultures (Fig. 1). Significant inhibition of label incoporation was noted at the earliest time points examined, i.e. 6 hr for LPS cultures and 24 hr for PHA cultures.

(c) *Effects of delayed DP addition.* Delayed addition of 5 $\mu$M DP had a relatively minor effect on its capacity to inhibit either PHA- or LPS-stimulated cultures. For PHA cultures, inhibition

Table 1. Potency of dipyridamole inhibition of incorporation of radiolabeled nucleosides by lymphoid cells

| Culture | $I_{50}$* (μM) | |
|---|---|---|
| | [$^3$H]dTR incorporation | [$^3$H]UR incorporation |
| PHA-stimulated splenocytes | 2.0 ± 1.0 | 3.5 ± 1.4 |
| LPS-stimulated splenocytes | 3.0 ± 1.5 | 3.5 ± 1.7 |
| L1210 cells | 2.0 ± 0.1 | 1.8 ± 0.5 |

* Mean ± S.E.M. of two separate experiments.

decreased from 94% when DP was added at zero time to 79% when added at 50 hr, which is 69% of the culture period. Similarly for LPS cultures, inhibition decreased from 90 to 75% when DP addition was delayed 24 hr (50%) into the culture period.

(d) *Reversibility of DP inhibition.* Splenocytes ($3 \times 10^6$/ml) were incubated with and without 5 μM DP for 1 hr at 37°. Half of the cells were washed three times with culture medium, and the other half placed directly into culture. PHA was then added, and tritiated thymidine incorporation was assessed at 72 hr as usual. Continuous exposure of splenocytes to 5 μM DP resulted in 90% inhibition of the PHA response, while cells preincubated with this agent were suppressed by only 54%.

*Effect of dipyridamole on [$^3$H]UR incorporation.* (a) *Potency of inhibition.* Dose–response curves were constructed for the effect of DP on incorporation of the RNA precursor [$^3$H]UR by both mitogen-stimulated splenocytes and L1210 cells (Table 1). The dose–response curves generated for T and B cell mitogenesis were almost identical, with the $I_{50}$ of 3.5 μM being quite similar to the $I_{50}$ values for thymidine incorporation. L1210 cells appear slightly more sensitive than blasting lymphocytes to inhibition of uridine incorporation with an $I_{50}$ of 1.8 μM.

(b) *Kinetics of inhibition.* Time courses were generated to determine the kinetics of DP inhibition of uridine incorporation for both PHA and LPS cultures (Fig. 2). Using 3 hr pulses of labeled precursor, significant inhibition of uridine incorporation was noted after 24 hr of culture in the presence of either PHA or LPS.

*Effect of dipyridamole on [$^3$H]valine incorporation.* Protein synthesis was monitored in PHA and L1210 cultures at 24 hr by determining the incorporation of [$^3$H]valine into TCA-precipitable material. Dose–response curves for DP inhibition of this variable are presented in Fig. 3. The $I_{50}$ value for inhibition of label incorporation by PHA cultures was 8 μM, in contrast to the 1.8 μM value for inhibition of L1210 protein synthesis.

*Effect of dipyridamole on lymphoproliferation—assays independent of uptake of radiolabeled compounds*

Because of the reported ability of DP to inhibit nucleoside transport, it is not clear to what extent the foregoing results with labeled thymidine and uridine are due to this activity of DP. In the absence

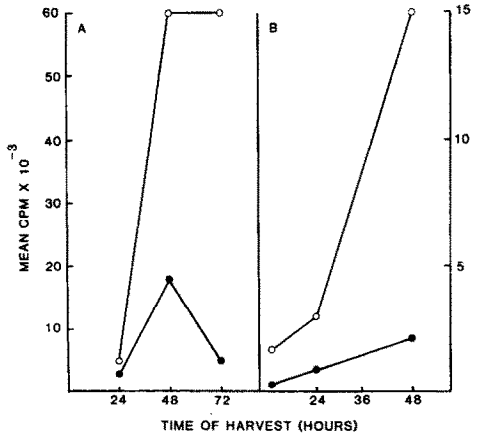


Fig. 1. Effect of DP on [$^3$H]dTR incorporation. Time courses were generated for the effect of 5 μM DP (●——●) on mitogen-induced [$^3$H]dTR incorporation (○——○). Cultures were pulsed with [$^3$H]dTR for the terminal 6 hr of the culture period. The variation among replicate mitogen cultures was less than 10%, and inhibition of thymidine incorporation observed in the presence of DP was statistically significant at all time points investigated ($P < 0.01$). The results presented in this figure are representative of two separate experiments. (A) PHA cultures. (B) LPS cultures.

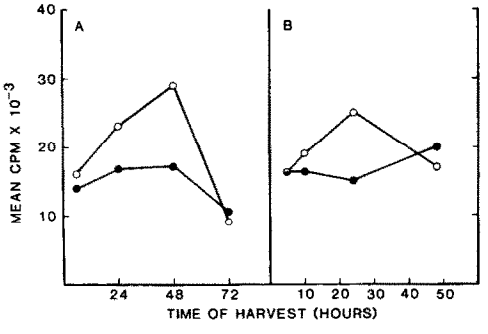


Fig. 2. Effect of DP on [$^3$H]UR incorporation. Time courses were generated for the effect of 5 μM DP (●——●) on mitogen-induced [$^3$H]UR incorporation (○——○). Cultures were pulsed with [$^3$H]UR for the terminal 3 hr of the culture period. The variation in the response of replicate mitogen cultures was less than 8%, and the results presented are representative of two separate experiments. (A) PHA cultures. Inhibition of uridine incorporation by DP is significant at 24 and 48 hr of culture ($P < 0.025$). (B) LPS cultures. Inhibition of uridine incorporation by DP was statistically significant only at 24 hr of culture ($P < 0.05$).

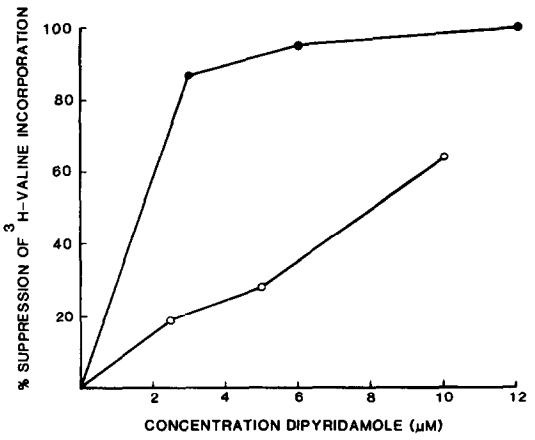


Fig. 3. Effect of DP on incorporation of [$^3$H]valine by PHA-stimulated splenocytes (○——○) and L1210 cells (●——●). Cultures were labeled with [$^3$H]valine for the terminal 3 hr of a 24-hr culture period, and label incorporation into TCA insoluble material was determined. The variation in response of replicate cultures was less than 10%.

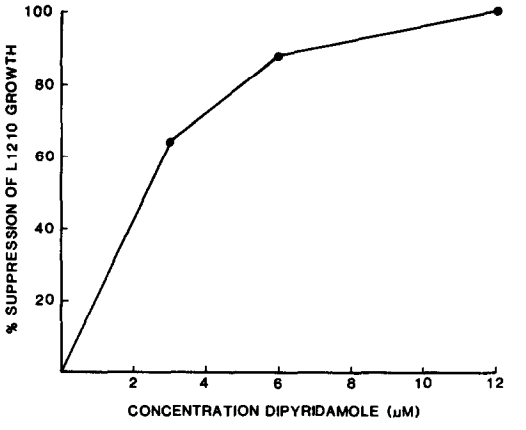


Fig. 5. Effect of DP on growth of L1210. Large-scale L1210 cultures containing the indicated concentrations of DP were harvested after 24 hr, and viable cell recovery was determined by trypan blue exclusion. Each value in this figure is the mean of duplicate determinations, with variation in cell counts of less than 10%.

of evidence that amino acid transport is similarly inhibited, it seems probable that inhibition of valine incorporation into cellular protein reflects true inhibition of protein synthesis by DP and suggests that this agent exerts effects on lymphoproliferation which are independent of inhibition of nucleoside uptake. To test this hypothesis, additional investigation of the effects of dipyridamole employed assays which do not depend on uptake of radiolabeled compounds.

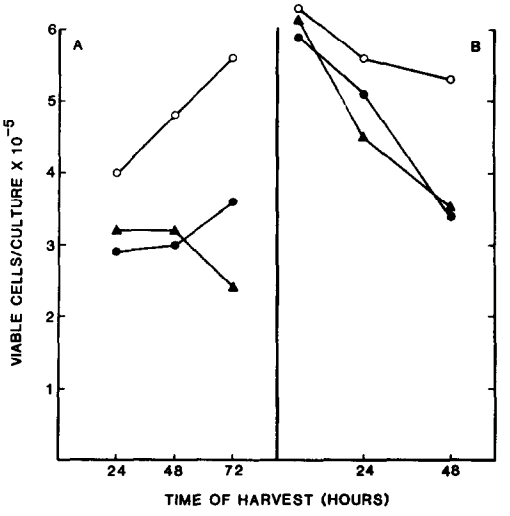


Fig. 4. Effect of DP on the survival of cultured cells. Replicate splenocyte cultures were harvested at the indicated times after culture initiation, and viable cell recovery was determined by trypan blue exclusion. Each value in this figure is the mean of duplicate determinations, with variation in cell counts of less than 10%, and is representative of two separate experiments: (○——○) mitogen-stimulated cultures; (●——●) mitogen-stimulated cultures +5 μM DP; and (▲——▲) unstimulated splenocytes. (A) PHA cultures. (B) LPS cultures.

*Effect of dipyridamole on the survival of cultured cells.* Two different approaches were used to evaluate DP effects on cell numbers in culture. First, the effect of 5 μM DP on cell recoveries from both PHA and LPS-stimulated splenocyte cultures was determined for several time points (Fig. 4). Inclusion of DP clearly gave decreased numbers of viable cells relative to cultures containing the mitogen alone. However, the cell content of DP-containing cultures did not decrease below that of unstimulated splenocyte cultures, suggesting that DP inhibited mitogenesis without producing cytotoxicity.

In a second type of experiment, a dose–response curve was constructed for the effect of DP on growth of L1210 (Fig. 5). Cells ($2.5 \times 10^6$) in 5 ml of medium, without and with different concentrations of DP, were seeded into petri plates and allowed to incubate at 37° for 24 hr. At the end of this period, cultures were harvested and the total viable cell recovery was determined by trypan blue exclusion. DP inhibited cell proliferation in L1210 cultures in a dose-dependent fashion (Fig. 5). Concentrations of DP greater than 3 μM actually decreased cell numbers below the number originally seeded into culture, indicating cytotoxicity was occurring. Fifty percent inhibition of L1210 growth was obtained at approximately 2 μM, a concentration comparable to the $I_{50}$ for thymidine incorporation (Table 1).

*DNA content of PHA-stimulated splenocytes.* Total DNA of PHA-stimulated cells was determined

Table 2. Effect of 5 μM DP on PHA-induced DNA synthesis

| Culture | Total μmoles of 2-deoxyribose* |
|---|---|
| PHA | 0.270 |
| PHA + DP | 0.158 |
| Unstimulated cells | 0.188 |

* Mean of duplicate determinations, with less than 1% variation in deoxyribose content.

Table 3. Effect of 5 μM DP on lymphocyte protein synthesis*

| Expt. No. | Culture | (μg Protein/$10^6$ cells) | Net increase over unstimulated (μg) |
|---|---|---|---|
| 1 | PHA | 43.8 | 22.2 |
| | PHA + DP | 22.6 | 1.0 |
| | Unstimulated | 21.6 | 0 |
| 2 | PHA | 59.8 | 15.9 |
| | PHA + DP | 47.9 | 4.0 |
| | Unstimulated | 43.9 | 0 |

* Protein content was determined at 24 hr by biuret assay. Each value is the mean of duplicate determinations, with less than 1% variation.

by the diphenylamine reaction. The increase in DNA content of splenocytes treated with PHA was abrogated by the presence of DP (Table 2). The DNA content of DP-treated cultures was only sightly less than that of unstimulated cells, again indicating minimal toxicity of DP for splenocytes and agreement with the data for viable cell recovery.

*Effect of dipyridamole on lymphoid cell protein content.* The effect of DP on the protein content of cultures of PHA-stimulated splenocytes was determined after 24 hr of incubation. The effect of 5 μM DP on PHA-induced protein synthesis is presented in Table 3. As no significant difference was noted in percent cell viability at 24 hr in the presence and absence of DP, results are expressed as μg protein per $10^6$ cells analyzed. The presence of DP clearly inhibited PHA-induced increases in total cellular protein, in agreement with the results of the [$^3$H]valine incorporation experiments.

The effect of various concentrations of DP on the protein content of L1210 cultures (Table 4) was determined. Because DP adversely affects L1210 cell viability, data are expressed as total protein/culture. Again, DP decreased the protein content of L1210 cultures, a reflection of its effect on cell survival.

*Effect of dipyridamole on cAMP accumulation in PHA cultures*

Because of a report that dipyridamole might inhibit cAMP phosphodiesterase, replicate PHA cultures, with and without 10 μM DP, were analyzed at indicated time points for cAMP content (Fig. 6). DP resulted in a time-dependent increase in the cAMP content of PHA-stimulated splenocytes, which was significant as early as 2 hr after culture initiation. The concentration of DP employed in this experiment inhibited thymidine incorporation by 100% in parallel cultures.

Table 4. Effect of dipyridamole on protein content of L1210 cultures*

| Concn of DP (μM) | Total biuret protein (μg/culture) |
|---|---|
| 0 | 862 |
| 3 | 584 |
| 6 | 313 |
| 12 | 281 |

* Protein was analyzed at 24 hr by biuret assay. Each value is the mean of duplicate determinations, with less than 1% variation.

## DISCUSSION

The effect of DP on proliferation of lymphoid cells was investigated by two separate approaches: (1) incorporation of radiolabeled precursors into cellular macromolecules, and (2) quantitation of proliferation by assay techniques that are independent of the transport of labeled compounds. The second approach was selected to eliminate conclusions which might arise as a result of inhibition of nucleoside transport and give the false appearance of inhibition of proliferation.

DP was found to be a potent inhibitor of [$^3$H]dTR incorporation by both mitogen-stimulated splenocytes and L1210 cells, in agreement with the findings of Pazdur *et al.* for PHA-stimulated HPBL [3]. However, murine lymphoid cells appear to be somewhat less sensitive, since the $I_{50}$ values obtained in the present study (2–3 μM) are somewhat higher than the 0.05 μM value reported for HPBL. Whether this difference reflects species differences or may be attributed to differences in culture conditions required for optimal cell proliferation has not been determined.

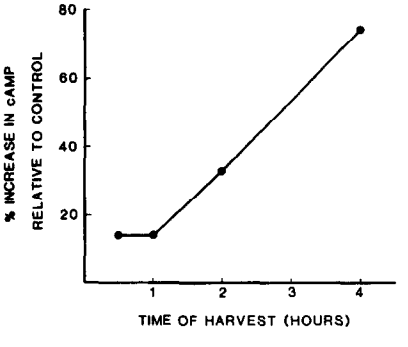


Fig. 6. Effect of DP on cAMP accumulation in PHA cultures. Replicate PHA cultures, with and without 10 μM DP, were harvested at the indicated times after culture initiation and were analyzed for cAMP content as described in Methods. The results are expressed as the percent increase in cAMP observed in DP-containing cultures, relative to cultures containing PHA alone. The latter exhibited progressively increasing values of 2.0, 2.5, 2.7 and 3.2 pmoles/$10^7$ cells for the indicated time points. Inclusion of DP in culture resulted in significant elevation of cellular cAMP at 2 and 4 hr ($P < 0.05$).

The validity of thymidine incorporation as an indicator of inhibition of cellular proliferation by DP was confirmed by measurements of total DNA and viable cell numbers for PHA-stimulated splenocytes and by viable cell recovery for L1210 cultures. While cell numbers in PHA-treated cultures were reduced significantly, they did not fall below the numbers in unstimulated cultures, which argues for an inhibitory effect on proliferation without concomitant cytotoxicity. In contrast, DP in excess of 3 $\mu$M was toxic for L1210 cells within 24 hr. These results clearly indicate that the effect of DP on lymphoid cells is not confined to inhibition of nucleoside transport.

Dipyridamole was also found to inhibit UR incorporation into RNA of mitogen-stimulated splenocytes and L1210 cells. The $I_{50}$ values of 2–3 $\mu$M are comparable to the values for dTR incorporation (Table 1). The kinetic data show the inhibitory effect to be most pronounced during the mid-portion of the culture period.

Protein synthesis in proliferating cells was examined by measuring the incorporation into protein of [$^3$H]valine, transport of which would not be expected to be inhibited by the nucleoside transport inhibitor, and by measuring the protein content of the cells. Dose–response studies revealed that both PHA-stimulated splenocytes and proliferating L1210 cells exhibited inhibition of protein synthesis, as monitored by valine incorporation, with L1210 cells being significantly more sensitive. Although Pazdur *et al.* [3] reported that DP does not inhibit [$^{14}$C]leucine incorporation by PHA-stimulated HPBL, the concentrations employed were lower than those required for inhibition of protein synthesis in the murine cells, and incorporation was measured only for the terminal 4 hr of 72-hr cultures. The inhibitory effect of DP on protein synthesis was confirmed in the present study by measurement of total protein of PHA-stimulated splenocytes and L1210 cells. These determinations were made at 24 hr, a time when protein synthesis is quite active.

Hait and Weiss [5] have reported that dipyridamole can inhibit the major form of cAMP phosphodiesterase in splenocyte homogenates. Considering the known anti-proliferative effect of cAMP on lymphoid cells [6], it was thought that DP might have been inhibiting lymphoproliferation as a consequence of inhibition of cyclic nucleotide catabolism. To address this question, the effect of DP on the cAMP content of PHA-stimulated splenocytes was determined. DP was found to induce significant increases in intracellular cAMP within 2 hr after culture initiation. Furthermore, although the data have not been presented, preliminary results suggest that the phosphodiesterase inhibitor theophylline can enhance the ability of DP to inhibit the PHA response, indicating that cAMP elevation plays a role in DP inhibition of lymphoproliferation.

It should be pointed out that cAMP modulation by DP provides an alternative explanation for the observation that DP can decrease the ability of HPBL to form rosettes with SRBC [3]. It is well known that agents which elevate cAMP, e.g. theophylline, inhibit lymphocyte rosette formation [7]. Hence, the ability of DP to antagonize rossette formation may be a consequence of cAMP elevation, rather than the result of direct alteration of the SRBC receptor by DP, as suggested by Pazdur *et al.* [3].

In summary, dipyridamole functions as an *in vitro* inhibitor of proliferation of both mitogen-stimulated splenocytes and L1210 leukemia cells. These findings have potential clinical relevance, in that this agent is employed in the long-term treatment of chronic angina pectoris, by virtue of its activity as a coronary vasodilator. It is not clear at present whether the administration of therapeutic doses of dipyridamole over prolonged periods of time could compromise the immune system. Micromolar concentrations of dipyridamole have been reported in the plasma of treated patients [8], and these levels are clearly immunosuppressive *in vitro*. Studies are currently underway to evaluate the effect of chronic DP administration on *in vivo* immune responses in aminal model systems and on the proliferative capacity of lymphocytes from patients undergoing dipyridamole therapy.

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