Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1662–1666, March 1989
Medical Sciences

Anticancer activities of adenine nucleotides in mice are mediated through expansion of erythrocyte ATP pools

(experimental cancer therapy/blood plasma ATP levels)

ELIEZER RAPAPORT* AND JAMES FONTAINE

Department of Microbiology, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118

Communicated by Leon A. Heppel, December 5, 1988

ABSTRACT ATP and AMP exhibit significant anticancer activities against established footpad CT26 colon adenocarcinoma in $CB6F_1$ mice. Adenosine, inorganic phosphate, and inorganic pyrophosphate were without such effects under identical conditions. Daily intraperitoneal injections of adenine nucleotides in large volumes of saline, starting after the tumors became palpable, resulted in inhibition of tumor growth and a few "cures." The treatment was not toxic to the host as determined by changes in body weights. Weight loss observed in animals upon progression of the fast-growing CT26 tumors was slowed markedly in adenine nucleotide-treated mice. The inhibition of weight loss in tumor-bearing mice was shown to be neither the cause nor the effect of the inhibition of tumor growth. Intraperitoneal injections of AMP or ATP but not of adenosine yielded expansions of erythrocyte ATP pools in host animals. The expanded erythrocyte ATP pools are stable over a period of hours, while slowly releasing micromolar amounts of ATP into the blood plasma compartment, leading to several-fold increases in plasma (extracellular) ATP levels. Based on previous studies in which 1–5 μM extracellular ATP effectively inhibited the growth of a variety of tumor cells in several *in vitro* systems, it is suggested that similar levels of ATP in blood plasma account for the anticancer activities observed in a murine host.

Extensive *in vitro* studies by Heppel and his colleagues (1, 2) and our group (3, 4) have shown the cytostatic and cytotoxic properties of extracellular ATP against a wide variety of animal and human tumor cells under several culture conditions. Both the selectivity of the tumor growth-inhibitory properties of exogenous ATP against transformed but not normal cells (1–3) and the ability of low (1–5 μM) concentrations of ATP to effectively inhibit the growth of human tumor cells in soft agar cultures (4) suggested the possible therapeutic efficacy of ATP against experimental tumors grown in a host. One of us has shown (5) that daily i.p. injections of adenine nucleotides significantly inhibited the growth of murine CT26 colon adenocarcinoma in (BALB/c ♀ × C57BL/6 ♂) F_1 ($CB6F_1$) mice and human pancreatic adenocarcinoma, CAPAN-1, xenografts in athymic nude mice. Administration (i.p.) of AMP or ATP in large volumes of saline resulted in increases in levels of ATP in whole blood (cellular) and the plasma compartment (extracellular). Treatment of mice with AMP or ATP yielded increases in plasma levels of ATP from a basal level of about 0.8 μM to 2–5 μM. These expanded ATP pools were achieved for several hours after a single i.p. injection of AMP or ATP and were obtained without long-lasting toxicity toward the host (5). We suggested that the extracellular ATP generated in the blood plasma of tumor-bearing hosts affects the growth of tumor cells by mechanisms that were identified by the *in vitro* studies (1–4), although host-mediated mechanisms cannot be ruled out, since ATP is known to influence several physiological systems that may affect tumor growth (6).

We now have identified a biological mechanism that accounts for the generation of increased blood plasma ATP concentrations after i.p. administration of AMP or ATP (but not adenosine) into mice. The potential clinical utility of AMP or ATP treatments is demonstrated by the significant inhibition of CT26 colon adenocarcinoma growth in $CB6F_1$ mice when the treatment is initiated after the tumors became palpable. In addition, an unexpected effect of adenine nucleotides is their ability to slow the rate of weight loss in mice bearing relatively large CT26 tumors. This phenomenon is not related to the tumor growth-inhibitory effects of these compounds and may be related to an effective introduction of purines into the host and their effective distribution into host tissues, thus slowing down the nutritional depletion of host compartments by the fast-growing tumor (7).

MATERIALS AND METHODS

Solutions of nucleotides in saline were prepared as described (5). Culture conditions of CT26 cells and tumor inoculations in $CB6F_1$ mice (The Jackson Laboratory) followed published procedures (5).

Experimental Protocol. Tumors were inoculated by injections of 2.5×10^5 CT26 cells (>90% viability) in 50 μl of phosphate-buffered saline into the right hind footpad of $CB6F_1$ mice. Treatments were initiated at a variety of times after inoculation of the tumors. The injection schedules started either 1 or 5 days after tumor inoculation (when none of the tumors were palpable) or when the tumors were clearly palpable (average calculated weight of 100 mg), which occurred in 100% of the inoculated mice after 8–11 days. Mice were randomized, divided into groups, and injected daily with 2.2 ml of saline, adenosine, AMP, or ATP (compounds were in sterile saline solutions at concentrations of 25 mM, with AMP and ATP solutions adjusted to pH 6.2). Injections were administered i.p. with 30-gauge needles, and mice were lightly anesthetized with ether. Mice were weighed, and tumor sizes were measured before the start of the treatment schedule and every 3 days during and after the treatment schedule. These determinations were performed before injections during the treatment period.

Analyses of ATP Levels in Whole Blood, Plasma, and in Isolated Washed Erythrocytes (RBCs). Blood (0.25 ml) was collected into 1-ml syringes (26-gauge needles) containing either citrate/dextrose (0.05 ml of 93 mM sodium citrate/7 mM citric acid/140 mM dextrose, pH 6.5) or sodium heparin (0.05 ml of 3 units of sodium heparin in saline) from the inferior vena cava. Mice were anesthetized with ether during the procedure. Plasma or conditioned Hanks' balanced salt

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RBC, erythrocyte; HBSS, Hanks' balanced salt solution; $CB6F_1$, BALB/c ♀ × C57BL/6 ♂.

*To whom reprint requests should be addressed.

solution (HBSS) from the incubation of isolated RBCs was prepared by centrifugation of whole blood or RBCs, respectively, in a Beckman Microfuge (30 sec at 8000 × g), and samples of 100 μl were added to 1 ml of ice-cold 7% (wt/vol) trichloroacetic acid. RBCs were prepared by centrifugation of whole blood (1500 × g for 5 min at 4°C), and removal of plasma and the buffy coat was followed by a wash of the pelleted RBCs (from 250 μl of whole blood) in 5 ml of ice-cold HBSS. After centrifugations, the RBC pellet was resuspended in a volume of HBSS to yield the original hematocrit (percent of RBC volume in the whole blood). Aliquots of 20 μl of RBC suspensions or whole blood were added to 1 ml of ice-cold 7% trichloroacetic acid. Extraction of acid-soluble nucleotides and determinations of ATP levels by luminometry followed published procedures (5). Levels of [^{3}H]ATP in whole blood, RBCs, plasma, or RBC incubation medium (HBSS) were determined after incorporation of 250 μCi (1 Ci = 37 GBq) of [^{3}H]adenosine (specific radioactivity, 30 Ci/mmol) into whole blood or RBC suspensions (250 μl total), followed by immediate trichloroacetic acid extractions in the manner described earlier. Two-dimensional thin-layer chromatography on poly(ethylene)imine-cellulose with an unlabeled ATP carrier was performed as described by Bochner and Ames (8). For the determination of whole blood or isolated RBC [^{3}H]ATP pools, 10 μl of the 1-ml initial trichloroacetic acid extract was chromatographed. Determinations of [^{3}H]ATP levels in blood plasma or RBC incubation medium were performed on 300 μl of the initial 1-ml trichloroacetic acid extract, which was lyophilized and redissolved in 15 μl of water. After chromatography, the spots corresponding to the ATP carrier were cut and eluted with 0.5 ml of 4 M ammonium hydroxide, and radioactivity was determined in 10 ml of scintillation fluid.

RESULTS

Tumor Growth-Inhibitory Activities of Adenine Nucleotides. The therapeutic efficacy of adenine nucleotides was shown against footpad murine CT26 colon adenocarcinoma grown in CB6F$_1$ mice (Fig. 1). Daily i.p. administration of AMP and especially of ATP in 2.2 ml of saline (at pH 6.2) for 12 days starting after the tumors became palpable significantly inhibited the growth of this fast-growing tumor. Much weaker anticancer activities were exhibited by equimolar amounts of adenosine (Fig. 1), whereas equimolar amounts of either inorganic phosphate or inorganic pyrophosphate in saline at pH 6.2 were without any effects on the growth of CT26 tumors in CB6F$_1$ mice under identical conditions (unpublished data). The magnitude of the antitumor activities of AMP and ATP depended to a small extent on the pH of the solution administered i.p. (unpublished data). This property is probably related to the number of negative charges on the phosphate groups (the pK_a of the secondary phosphate is around 6.6). AMP and especially ATP have some buffering capacity, and at a pH < 6.6, they carry a smaller net charge than at a physiological pH. The lower charge of these molecules enhances the initial (first hour) transport of AMP and ATP across the peritoneal membrane and into the systemic circulation (9). No inhibition of tumor growth was exhibited when either AMP or ATP was administered by the same 12-consecutive-day schedule ending 1 day before tumor inoculation (unpublished data). The treatment schedule of palpable CT26 tumors, which is illustrated in Fig. 1, consistently yielded 10–20% of "cures." These mice remained tumor-free for at least 2 months (unpublished data). When applied to palpable tumors, inhibition of tumor growth by ATP was more pronounced than that obtained with AMP (Fig. 1). However, AMP is as effective as ATP in inhibiting tumor growth in the same model system when the treatment schedule is initiated 1 day after tumor inoculation (5).

Adenine Nucleotide-Mediated Inhibition of Weight Loss in Animals Bearing Relatively Large CT26 Tumors. Adenine nucleotides exhibited significant inhibition of the rate of weight loss in animals bearing relatively large tumors (Figs. 1 and 2). When treatment schedules ended long before the tumors became relatively large, the inhibition of the rate of weight loss could be correlated with the inhibition of tumor growth showing that adenosine was having only a small effect on either of the two phenomena (Fig. 2 *A* and *B*). When adenine nucleotides were administered only after the tumors became palpable, the CT26 tumors turned progressively larger during the treatment schedule (Figs. 1 and 2*C*). In this case, the inhibition of tumor growth could be separated from the inhibition of weight loss. When comparing tumors of similar sizes, the data in Fig. 1 indicate that adenosine and, to a somewhat larger extent, AMP and ATP inhibited weight

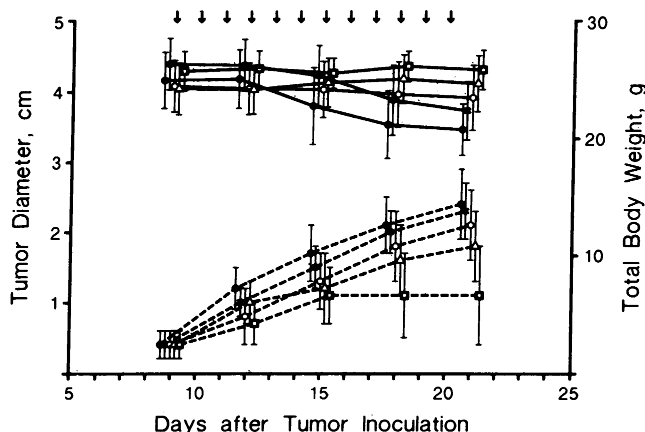

FIG. 1. Inhibition of tumor growth (---) and host weight loss (—) in tumor-bearing mice by treatment with adenine nucleotides. Mice (CB6F$_1$ males, 8 weeks old) bearing palpable CT26 footpad tumors (on day 9 after tumor inoculation) were treated with saline (●), adenosine (○), AMP (△), or ATP (□) as described. An untreated group was also included (×). Arrows indicate days of injection. This representative experiment included 10 animals per treatment group, and data points are expressed as the means ± SD. No deaths occurred before day 22 after tumor inoculation.

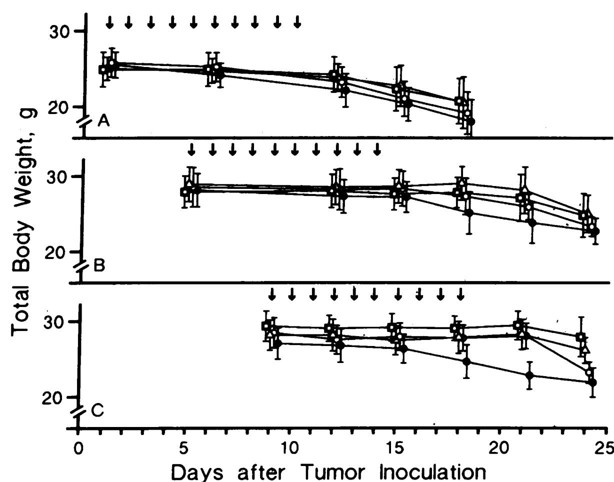

FIG. 2. Inhibition of host weight loss in tumor-bearing animals by treatment with adenine nucleotides. Mice (CB6F$_1$ males, 7 weeks old in *A* and 10 weeks old in *B* and *C*) were inoculated with CT26 tumors, and daily treatments (for 10 consecutive days) with saline (●), adenosine (○), AMP (△), or ATP (□) were initiated 1 day (*A*), 5 days (*B*), or 9 days (*C*) after tumor inoculation (when all of the tumors were palpable). Arrows indicate days of injection. Each treatment group included 10 animals, and data points are expressed as means ± SD. No deaths occurred before day 19 (*A*) or day 25 (*B* and *C*).

loss in a manner that could be dissociated from their inhibitory effects on tumor sizes. Another way of demonstrating the same phenomenon was by using a variety of treatment schedules among which one is completed before weight losses occur (Fig. 2*A*), another is completed at the time when weight losses are beginning to be observed (Fig. 2*B*), and the third treatment schedule is applied during the period of weight losses in tumor-bearing mice (Fig. 2*C*). We established that the largest inhibition of animal weight loss occurred when a 10-day daily injection schedule was initiated on day 9 after inoculation of the tumors, when they were palpable (Fig. 2*C*). Starting the same injection schedule earlier, on day 1 or day 5 after tumor inoculation, yielded lower levels of inhibition of weight loss (day 5) or of inhibition of weight loss resulting only from smaller tumors in the treated animals (day 1) (Fig. 2 *A* and *B*; ref. 5). One aspect of this phenomenon is probably related to the supply of purines because adenosine, which has only a small effect on the growth of the tumors, markedly inhibited the rate of weight loss in tumor-bearing animals (Fig. 2*C*). However, other activities specific to AMP and ATP must be involved because the inhibition of weight loss mediated by these compounds was distinctly larger than the inhibitory activities of adenosine (focusing on similar-size tumors).

Expansion of RBC ATP Pools After i.p. Injections of Adenine Nucleotides and the Slow Release of ATP from RBCs. Single i.p. injections of 2 ml of 35 mM AMP or ATP in saline at pH 6.2 into $CB6F_1$ mice resulted in expansion of RBC ATP pools in these mice (Table 1). The expansions of RBC ATP pools after i.p. injections of AMP or ATP could be detected 30 min after injections and lasted for ≈6–8 hr after the nucleotides were completely adsorbed across the peritoneal membrane and into the systemic circulation (unpublished data). The rates of adsorption of i.p. injected ATP solutions varied in different strains of mice (unpublished data). The increases in RBC ATP pools were comparable to the increases observed in total blood ATP levels after similar i.p. injections of AMP or ATP (5). Adenosine, at levels similar to those of AMP or ATP, was only slightly effective in expanding mouse RBC ATP pools (Table 1). Plasma (extracellular) ATP levels were markedly increased after i.p. administration of AMP or ATP. These plasma ATP levels originate in RBCs because they can be metabolically labeled by $[^3H]$adenosine. Plasma ATP pools, which originate in the dense granules of blood platelets, would not be radioactively labeled under the same conditions (10).

The experiments described in Tables 1 and 2 include uptake of $[^3H]$adenosine by whole blood (Table 1) or isolated washed RBCs (Table 2). In both cases, all of the extracellular ATP, determined seconds after the uptake of $[^3H]$adenosine, was metabolically labeled. The radioactively labeled $[^3H]$ATP in blood plasma (Table 1) or in the medium in which the washed RBCs were suspended (Table 2) showed good correlations with the actual ATP levels, which were determined by bioluminometry. Thus, we conclude that the origin of plasma ATP levels is in the RBC ATP pools. Metabolic radioactive labeling of RBC ATP pools was achieved by the incorporation of $[^3H]$adenosine of high specific radioactivity, representing actual levels of <25 μM adenosine. Since 1 mM adenosine was shown not to affect RBC ATP pools *in vitro* (Table 3), it was concluded that the radioactive labeling procedure does not affect the size of these pools either. The finding that the ratios of plasma or conditioned medium ATP levels to the cellular (RBC) ATP pools increased when RBC ATP pools were expanded suggests that the release of ATP from RBCs into the extracellular compartment is a specific process that is probably not related to hemolysis of RBCs. An equal degree of hemolysis of RBCs during the 40 sec (10 sec of labeling and 30 sec of centrifugation) that elapse from the time of uptake of $[^3H]$adenosine to the time of isolation and fixation of the extracellular medium would have led to similar

Table 2. Stability of expanded RBC ATP pools after i.p. injections of AMP and ATP into mice

Administered substance	RBC ATP pools,* mM		ATP levels in HBSS,* μM	$[^3H]$ATP levels in HBSS immediately after $[^3H]$adenosine incorporation into isolated washed RBCs,† μM
	0 min	120 min		
Saline	0.68	0.56	1.10	1.09
Adenosine	0.69	0.54	1.71	1.20
AMP	1.01	0.86	2.42	1.81
ATP	2.52	1.76	4.08	4.93

Blood was withdrawn into a syringe containing citrate/dextrose. Plasma and buffy coat were removed after centrifugation, and RBCs were washed with 5 ml of HBSS. RBCs were resuspended in a volume of HBSS to yield the original hematocrit, and $[^3H]$adenosine was taken up by the cells for 10 sec. An aliquot of the total cell suspension was fixed in 1 ml of trichloroacetic acid, and the remaining RBC suspension was used for the determination of the 120-min time point or was centrifuged for the isolation of the medium. ATP levels in the medium were determined at time 0. Determinations of $[^3H]$ATP levels in HBSS incubation medium were identical to determinations performed in blood plasma (described in the footnote to Table 1). Data represent the average of two experiments.
*Determined by bioluminometry.
†At time zero.

Table 1. Expansion of RBC ATP pools and blood plasma ATP levels 5 hr after i.p. injections of AMP and ATP into mice

Administered substance	RBC ATP pools,* mM	Plasma ATP levels,* μM	Ratio × 10^3 of plasma to RBC ATP levels	Plasma $[^3H]$ATP levels immediately after $[^3H]$adenosine incorporation into whole blood, μM
Saline	0.81 ± 0.14	0.74 ± 0.14	0.91	0.56 ± 0.22
Adenosine	1.10 ± 0.24	1.12 ± 0.28	1.02	0.77 ± 0.30
AMP	1.78 ± 0.32	3.10 ± 0.96	1.74	2.86 ± 1.06
ATP	2.49 ± 0.42	4.94 ± 1.17	1.98	5.75 ± 2.05

Blood was withdrawn into a syringe containing citrate/dextrose as an anticoagulant. $[^3H]$Adenosine was taken up by whole blood for 10 sec, and half of the blood sample was immediately centrifuged for plasma isolation. The other half was used for the isolation of washed RBCs, which were resuspended in a volume of HBSS to yield the original hematocrit before the removal of an aliquot for ATP determination. Plasma $[^3H]$ATP levels were calculated from the radioactivity of plasma $[^3H]$ATP after correlation with the specific radioactivity of RBC $[^3H]$ATP pools, which in turn were determined by bioluminometry and two-dimensional thin-layer chromatography. Data represent the means ± SD of three experiments.
*Determined by bioluminometry.

ratios of extracellular to RBC ATP levels for all forms of treatment. Mice injected with AMP or ATP showed not only expanded RBC ATP pools, as compared with saline- or adenosine-treated mice, but also higher plasma ATP levels relative to their (expanded) RBC ATP pools (Table 1).

The stability of the expanded RBC ATP pools *in vitro* also was demonstrated. Two-hour incubations of washed isolated RBCs containing normal or expanded ATP pools in HBSS resulted in relatively small decreases in RBC ATP pools, with the expanded pools being maintained well above normal levels (Table 2). The expansions of RBC ATP pools after i.p. administration of AMP or ATP *in vivo* is presumably the result of their dephosphorylation followed by the RBC uptake of the adenosine that is generated *in situ*. The *in vitro* treatment of whole mouse blood with AMP or ATP resulted in marginal increases in RBC ATP pools (Table 3). The *in vitro* degradation of ATP proceeded faster in heparinized blood as compared with citrated blood, reaffirming the role of plasma phosphodiesterase(s) in catalyzing this catabolic reaction (12). Citrate has been shown to chelate metal cations required for this catalytic activity (12). Thus, it is concluded that the *in vivo* generation of adenosine by the *in situ* dephosphorylation of AMP or ATP requires ecto-enzymatic activities that are present in the vascular bed. It is important to note that the degradation of ATP by human blood *in vitro* proceeded at a slower rate than the degradation of ATP by mouse blood under identical conditions (Table 3). The implications of this finding for the favorable potential of adenine nucleotides in human cancer therapy as compared to the murine models have been discussed (5).

DISCUSSION

Under certain pathophysiological conditions, several sources of extracellular ATP in blood have been identified. These include endothelial cells that release ATP intraluminally during ischemia and hypoxia (13) and blood platelets that release ATP during intravascular platelet aggregation (10). Reports by Born *et al.* have suggested that under conditions of hemodynamic stress, RBCs can release ATP into the extracellular compartment (14, 15). Data presented in this manuscript show that adenine nucleotides can be utilized to expand the total cellular ATP pools (steady-state levels) of pathologically normal RBCs and that these RBCs slowly release their expanded ATP pools into the extracellular blood plasma compartment. All the extracellular ATP in the blood of mice injected i.p. with either AMP or ATP can be attributed to ATP released from the RBCs (Tables 1 and 2). Uptake of [^{3}H]adenosine *in vitro* by either whole blood (Table 1) or isolated washed RBCs (Table 2) results in the immediate release of [^{3}H]ATP into either the blood plasma (Table 1) or the extracellular medium (HBSS) in which the RBCs are suspended at the original hematocrit (Table 2). The radioactive labeling of RBC ATP pools *in vitro* by [^{3}H]adenosine of high specific radioactivity results in the incorporation of the radioactive label into these pools without any effect on the size of the ATP pools. The specificity of the release process is suggested by the following findings. The amounts of the extracellular ATP levels are similar whether they are determined by bioluminometry or by the correlation of the total radioactivity of extracellular [^{3}H]ATP with the specific radioactivity of intracellular [^{3}H]ATP. Thus, all of the extracellular ATP is metabolically labeled within a few seconds by the exposure of RBCs to [^{3}H]adenosine *in vitro* after blood withdrawal. The ratio of extracellular ATP to total RBC ATP increases in the following order of treatment, saline < adenosine < AMP < ATP (Table 1). Therefore, the release of RBC ATP pools is likely to originate in mechanism(s) other than (or in addition to) hemolysis, which would yield similar ratios of extracellular ATP levels to total RBC ATP pools for all treatment groups. The finding that all of the extracellular ATP in mouse blood can be metabolically labeled during a short period of uptake of [^{3}H]adenosine into RBCs *in vitro* suggests that endothelial cells or blood platelets are not the *in vivo* source of extracellular, blood plasma ATP in the studies reported here. The expansion of total blood ATP pools after i.p. injections of AMP or ATP and the accompanying increases in blood plasma ATP levels were previously suggested to mediate the anticancer activities of adenine nucleotides in murine models (5).

Cellular ATP pools were shown to have a role in the regulation of chromosomal DNA replication in mammalian cells (16, 17) in addition to their well-known role in bioenergetics and other metabolic functions. Extracellular ATP pools either can alter the intracellular balance of nucleotide pools and lead to arrest of cells in the S phase of their cycle followed by cell killing (3, 4) or can induce the formation of hydrophilic channels, allowing passage of nucleotides and other phosphate esters in transformed cells (1, 2). The results outlined here demonstrate the clinical utility of adenine nucleotide treatments by achieving significant growth-inhibitory activities after daily i.p. injections of either AMP or ATP starting at a point when the aggressive fast-growing CT26 tumor was palpable. Adenosine was less active than either AMP or ATP against this murine colon adenocarcinoma. The correlations of the magnitude of expansion of RBC total ATP pools with the degree of anticancer activity produced by any of the compounds utilized here is another indication of the relationship of the two phenomena.

The inactivity of adenosine in achieving either significant expansion of RBC ATP pools or inhibition of tumor growth is attributed to certain features of adenosine versus adenine nucleotide metabolism in the vascular bed. Several studies of adenosine kinase and adenosine deaminase, the two enzymes that play a central role in catalyzing adenosine metabolism, showed that the K_m for RBC adenosine deaminase activities is 25–40 μM (18), whereas the K_m for RBC adenosine kinase activities is 1–2 μM (18, 19). Thus, the metabolism of adenosine by intact RBCs is determined by its concentrations. At physiological levels (1–2 μM) (20), >90% of the adenosine will be phosphorylated by adenosine kinase action and converted to adenine nucleotides (by the further action

Table 3. Rates of degradation of 1 mM ATP by mouse or human blood *in vitro* in the presence of heparin or citrate as anticoagulants; lack of expansion of RBC ATP pools under the same conditions

Added substance, 1 mM	Rate of extracellular ATP degradation, pmol·μl^{-1}·min^{-1}: Mouse blood, Citrate	Mouse blood, Heparin	Human blood, Citrate	Human blood, Heparin	RBC ATP pools* (after 30-min incubations in heparinized mouse blood), mM
ATP	6.87	10.21	1.75	4.07	0.76 ± 0.05
AMP					0.73 ± 0.10
Adenosine					0.55 ± 0.17
Saline					0.67 ± 0.10

Blood (450 μl) was withdrawn into a syringe containing either 50 μl of citrate/dextrose or 50 μl of heparin (100 units/ml). Whole blood was then added to 1 mM [^{3}H]ATP in saline (25 μl), and at various time points blood aliquots (90 μl) were removed and centrifuged. Plasma (20 μl) was withdrawn and added to 1 ml of ice-cold trichloroacetic acid. Thin-layer chromatography on poly(ethylene)imine cellulose was performed by published procedures (11). The rate of degradation of 1 mM [^{3}H]ATP was linear for mouse or human blood during the initial 30-min incubation at 37°C.

*RBC ATP pools were determined in isolated washed RBCs after incubations of whole blood with 1 mM of the specified compound by bioluminometry. Data represent means ± SD of three separate experiments.

of adenylate kinase and the glycolytic enzymes). At higher adenosine concentrations (>7 μM), deamination catalyzed by adenosine deaminase becomes the major pathway of adenosine metabolism (19). Adenosine-promoted expansion of ATP pools in RBCs *in vivo* is not associated with increases in the cellular pools of AMP and ADP (20). The expanded RBC ATP pools do not lead to the depletion of pyrimidine nucleotides (20), as was shown to be the case for fibroblasts and lymphoid cells in culture (21). Therefore, the regulatory aspects of adenosine uptake and metabolism in RBCs (22) are commensurable with the slow *in situ* generation of adenosine by the catabolism of adenine nucleotides in being capable of promoting the expansion of RBC ATP pools. Introduction of adenosine itself into the systemic circulation (at levels comparable to those of adenine nucleotides) results in deamination to inosine, which is excreted. Data outlined in Table 3 suggest that the involvement of the vascular bed in the catabolism of adenine nucleotides (6) is required for the efficient *in situ* generation of adenosine *in vivo*.

Another aspect of the potential clinical utility of the anticancer activities of adenine nucleotides is their inhibition of host weight loss when the tumors become progressively larger (Figs. 1 and 2). This activity is not related to the inhibition of tumor growth, and the mechanism of this phenomenon is currently being studied. Although the supply of purines to the host is a contributing factor because adenosine is effective in inhibiting host weight loss, the reduction in the rate of host weight loss by adenosine is smaller than that achieved by AMP or ATP. However, the expanded RBC ATP pools could produce a more effective distribution of purines to host tissues other than the liver, thus accounting for the additional effects of AMP and ATP.

In conclusion, whereas the anticancer activities of adenine nucleotides have previously been shown in murine models (5), this report identifies the physiological mechanisms that generate these activities. The clinical utility of anticancer treatment with adenine nucleotides is expected to be enhanced by their demonstrated inhibition of host weight loss in tumor-bearing animals.

1. Heppel, L. A., Weisman, G. A. & Friedberg, I. (1985) *J. Membr. Biol.* **86**, 189–196.
2. Weisman, G. A., Barun, K. D., Friedberg, I., Pritchard, R. S. & Heppel, L. A. (1984) *J. Cell. Physiol.* **119**, 211–219.
3. Rapaport, E. (1983) *J. Cell. Physiol.* **114**, 279–283.
4. Rapaport, E., Fishman, R. F. & Gercel, C. (1983) *Cancer Res.* **43**, 4402–4406.
5. Rapaport, E. (1988) *Eur. J. Cancer Clin. Oncol.* **24**, 1491–1497.
6. Gordon, J. L. (1986) *Biochem. J.* **233**, 309–319.
7. Costa, G. (1977) *Cancer Res.* **37**, 2327–2335.
8. Bochner, B. R. & Ames, B. N. (1982) *J. Biol. Chem.* **257**, 9759–9769.
9. Torres, I. J., Litterst, C. L. & Guarino, A. M. (1978) *Pharmacology* **17**, 330–340.
10. Holmsen, H. (1985) *Semin. Hematol.* **22**, 219–240.
11. Rapaport, E. & Zamecnik, P. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1145–1147.
12. Lüthje, J. & Ogilvie, A. (1985) *Eur. J. Biochem.* **149**, 119–127.
13. Burnstock, G. & Kennedy, C. (1986) *Circ. Res.* **58**, 319–330.
14. Born, G. V. R., Bergquist, D. & Arfors, K.-E. (1976) *Nature (London)* **259**, 233–235.
15. Born, G. V. R. & Wehmeier, A. (1979) *Nature (London)* **282**, 212–213.
16. Rapaport, E., Garcia-Blanco, M. A. & Zamecnik, P. C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1643–1647.
17. Rapaport, E. (1980) *J. Cell. Physiol.* **105**, 267–274.
18. Meyskens, F. L. & Williams, H. E. (1971) *Biochim. Biophys. Acta* **240**, 170–179.
19. Perrett, D. & Dean, B. (1977) *Biochem. Biophys. Res. Commun.* **77**, 374–378.
20. Mills, G. C., Schmalstieg, F. C., Trimmer, K. B., Goldman, A. S. & Goldblum, R. M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2867–2871.
21. Green, H. & Chan, T.-S. (1973) *Science* **182**, 836–837.
22. Plagemann, P. G. W. & Wohlhueter, R. M. (1980) *Curr. Top. Membr. Transp.* **14**, 225–330.