Anticancer Activities of Adenine Nucleotides in Tumor Bearing Hosts

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ABSTRACT The utilization of adenosine 5'-triphosphate (ATP) infusions against solid refractory cancers is based on the preclinical findings that low levels of extracellular ATP significantly inhibit the growth of a variety of human and murine tumor cells. The mechanisms of tumor cell killing by extracellular ATP are attributed to effects mediated by its interaction with P2 purine receptors as well as non-receptor-mediated pore formation in the tumor cell membrane. The achievement of elevated extracellular blood plasma pools of ATP is accomplished by the administration of adenine nucleotides which yield elevated liver, red blood cell, and plasma compartment pools of ATP. Administration of AMP or ATP to tumor-bearing murine hosts produced, in addition to the cytotoxic effects against the tumor, a variety of host mediated anticancer activities, including significant inhibition of host weight loss in cachectic tumor models. The inhibition of several adverse events which are among the hallmarks of cancer cachexia is the result of prevention of the significant depletion of visceral energy stores in cachectic animals after the administration of adenine nucleotides, leading to the successful maintenance of hepatic functions, the resumption of normal protein synthesis in the liver, and inhibition of the synthesis of hepatic acute-phase proteins. Thus, the administration of ATP as an anticancer agent in humans at levels (below 0.1 mg/kg min) which do not adversely affect systemic and cardiovascular functions is expected to lead to the following activities resulting from the generation of elevated hepatic, red blood cell, and blood plasma ATP pools: cytolytic effects against the tumor, inhibition of host weight loss, and other adverse effects of cachexia, anti-pain effects, modulation of blood flow to the tumor, positive effects on motor functions and performance status due to increases in regional cerebral blood flow and improvements in several other parameters which are also known to be affected by elevated red blood cell and blood plasma (extracellular) ATP levels.

Key Words: cancer, cachexia, adenosine 5'-triphosphate

Adenosine 5'-triphosphate (ATP) infusions useful against metastatic refractory cancers are entering Phase I of human clinical trials. The two questions which will be answered by these trials are i) is it possible to achieve elevation of liver, red blood cells, and blood plasma compartment pools of ATP after the administration of ATP to patients as was shown extensively in preclinical murine models [Rapaport, 1988; Rapaport and Fontaine, 1989a; Rapaport and Fontaine, 1989b] and ii) can the elevated ATP levels in the human host produce the spectrum of anticancer activities demonstrated in experimental animals [Rapaport, 1990]?

A variety of in vitro and in vivo studies have demonstrated several anticancer activities of extracellular (blood plasma compartment) pools of ATP as well as elevated hepatic and red blood cell pools of ATP. These activities are a) cytostatic and cytotoxic effects on the tumor; b) anti-cachexia effects and improvement of hepatic functions; c) modulation of tumoral blood flow; d) anti-anaemia effects; e) anti-pain activities; f) improvement in motor functions, performance status; g) improvements in oxygen delivery to peripheral sites; and h) enhancement of superoxide anion (O\(^{2-}\)) production by phagocytic cells. Early experiments have demonstrated that low levels of extracellular ATP inhibit the growth of a variety of human tumor cells and subsequently yield substantial cell killing in vitro systems [Rapaport, 1983; Rapaport et
The mechanism of tumor cell killing is attributed to the permeabilization of tumor cell membrane by extracellular ATP [Rapaport, 1983], as well as to other non-receptor-mediated Na⁺ channel opening [Wiley et al., 1990] and P₂-purinergic receptor-mediated opening of plasma membrane Ca²⁺ channels [Fang et al., 1992]. Recent results have also demonstrated that the induced pore in the tumor cell membrane after treatment with extracellular ATP is in the gap junction protein connexin-43 [Beyer and Steinberg, 1991]. Neoplastic cells have been known to be deficient in intercellular communication and therefore have exposed gap junction proteins [Lowenstein, 1987]. ATP administered to murine hosts was shown to possess antitumor activities in vivo in several experimental animal models [Rapaport, 1988; Rapaport and Fontaine, 1989a,b; Rapaport, 1990; Nayak et al., 1990]. The administration of ATP to tumor-bearing murine hosts was also shown to markedly inhibit host weight loss in a cachectic tumor model and, as importantly, the administration of ATP or other adenine nucleotides was shown to elevate extracellular, blood plasma compartment steady state levels (pools) of ATP [Rapaport and Fontaine, 1989a,b]. The generation of elevated blood plasma ATP pools after the administration of adenine nucleotides (ATP, AMP, or other adenine nucleotides) proceeds through the immediate rapid degradation of the adenine nucleotide in the vascular bed followed by the incorporation of adenosine and inorganic phosphate into liver and red blood cell ATP pools. The red blood cells with expanded ATP pools, which are produced by this mechanism, slowly release micromolar levels of ATP into the blood plasma without undergoing hemolysis, thus achieving elevated steady state extracellular ATP levels, in spite of the catabolic enzymatic activities present intravascularly [Rapaport and Fontaine, 1989a]. These elevated levels of ATP inhibit both tumor growth and host weight loss in tumor-bearing murine models. The inhibition of tumor growth proceeds by the receptor-mediated and non-receptor-mediated effects of extracellular ATP on the tumor cell membrane, whereas the inhibition of host weight loss in tumor-bearing hosts is the result of ATP-mediated marked slowdown of hepatic gluconeogenesis and reversal of the depletion of visceral energy stores [Rapaport, 1990]. The inhibition of tumor growth and host weight loss were shown not to exhibit a cause and effect relationship in murine models. Other significant anticancer activities by ATP in animal tumor models were also shown [Nayak, et al., 1990, Pal et al., 1991]. The cytolytic activity of extracellular ATP against tumor cells is now being proposed by five different groups as accounting for the activity of certain cytolytic T lymphocytes [Filippini et al., 1990; Di Virgilio et al., 1990; Zheng et al., 1991; Steinberg and Di Virgilio, 1991; Correale et al., 1992]. These cytolytic T lymphocytes release ATP which is stored in their cellular granules, in response to the target cell interaction with a T cell receptor. The extracellular ATP released in the immediate vicinity of the target tumor cell is proposed to deliver the lethal hit. All of these groups demonstrated tumor cell killing by extracellular ATP in a variety of systems. Several other physiological processes which are related to the presence of P₂-purinoceptors on a variety of tissues as well as on vascular endothelial cells, are induced by the presence of elevated blood plasma ATP pools. Two of these activities which are related to cancer, the enhancement of intratumoral blood flow as shown in malignant gliomas after intra-arterial administration of ATP [Natori et al., 1992] and the remarkable anti-pain activities of intravenously administered ATP [Fukunaga et al., 1990], were recently shown in humans. The analgesic and sedative effects of ATP in humans and experimental animals [Kikuta et al., 1990; Gomaa, 1987; Gomaa et al., 1989] after intravenous administration of ATP persist long after the injections or infusions had stopped and were shown to be intrinsic to the interactions of ATP with P₂ purine receptors. Increases in regional blood flow after administration of ATP can be exploited for enhanced delivery of anticancer agents to a tumor without additional toxic effects on extratumoral sites which are susceptible to the drug's toxicity, as is suggested in the case of malignant gliomas. Since the intravenous infusions of low levels of ATP in humans (below 0.1 mg/kg min) are known to produce an increase in cardiac output or pulmonary artery flow without inducing systemic hypotension or increasing heart rate, combinations of ATP, and mainstay anticancer regimens could constitute a treatment modality in the case of lung cancers whereby all the activities of ATP will be utilized.

Animal studies underestimate the efficacies of the activities of extracellular ATP since it is much easier to achieve elevated blood plasma levels of ATP in humans than in experimental animals. The reason lies in the much higher catabolic soluble blood plasma and ectoenzymatic activities that catalyze the degradation of extracellular ATP in animals as compared to the same activities in humans. These enzymatic activities are related to the higher basal metabolic rates in animals and their need for increased supply of "salvage" precursors for the biosynthesis of nucleotides. Therefore much lower levels of ATP are required for intravenous or intraperitoneal administrations in humans as compared to animals, for sustaining elevated blood plasma ATP pools without
systemic toxicity [Rapaport and Fontaine, 1989a; Ho and Frei, 1970; LePage et al., 1972].

Significant anti-cachexia effects were observed in mice carrying the murine colonic adenocarcinoma CT26 (also referred to as colon-26) after the administration of AMP or ATP. These effects included not only inhibition of host weight loss in tumor-bearing hosts but also alterations in hepatic protein synthesis to proteins existing in non-tumor-bearing normal hosts but also alterations in hepatic protein synthesis to proteins existing in non-tumor-bearing normal mice [Rapaport, 1990]. More recently this murine tumor was demonstrated to be a true model of experimental cachexia [Tanaka et al., 1990]. This murine tumor was also shown to produce interleukin-6 (IL-6) in vivo [Strassmann et al., 1992], and IL-6 is currently proposed as a mediator of the acute-phase response often seen in cachectic cancer patients. Interleukin-6 synthesis and/or release in a non-cancer rat trauma-hemorrhage model was markedly inhibited by the administration of ATP-MgCl₂ in vivo [Wang et al., 1992].

Anti-anæmia effects of AMP were demonstrated both in humans where intramuscular injections of AMP were effective in increasing the viability of red blood cells in a patient with a hemolytic disease [Teitel et al., 1965], and in rabbits rendered anaemic by lead poisoning [Gajdos et al., 1963]. Low levels of adenine nucleotides have been known to stimulate regional cerebral blood flow (rCBF) in experimental animals [Forrester et al., 1979; Molnár et al., 1991]. Striking improvements in the motor functions of Parkinson’s disease patients were recently obtained by Birkmayer after infusions of low levels of reduced nicotine-adenine dinucleotide (NADH) [Birkmayer and Birkmayer, 1989]. The CNS activity of low levels of NADH in Parkinson’s patients and similar types of CNS activities reported for AMP administered to multiple sclerosis patients [Lowry et al., 1953] originate as the result of the stimulation of regional cerebral blood flow (rCBF) by elevated (extracellular) blood plasma ATP levels produced after the rapid degradation of the nucleotides to adenosine and inorganic phosphate. Stimulation of rCBF is expected to increase regional metabolism and since metabolism has been closely linked to neuronal function in the brain, the result of increases in rCBF would tend to lead to short-term improvements in neuronal functions. Elevated red blood cell ATP pools are known to produce significant increases in the red blood cell 2,3-diphosphoglycerate (2,3-DPG) [Brewer and Eaton, 1971; Salari et al., 1991] which in turn cause a shift in the hemoglobin oxygen affinity curves to the right, leading to enhanced oxygen delivery under normal non-hypoxic conditions [Brewer and Eaton, 1971]. This improvement in oxygen delivery to peripheral sites after the expansion of red blood cell ATP pools is expected to lead to improvements in the performance status of the patient suffering from advanced cancers [Ganz et al., 1988]. Recently, significant declines in superoxide anion (O⁻₂) production in patients with advanced cancers was documented and proposed to comprise part of the host deficiencies in defending against tumors [Hara et al., 1992]. There are several studies demonstrating that extracellular ATP stimulates superoxide anion production by phagocytic cells and that extracellular ATP can achieve this physiological function in vivo [Ward et al., 1990].

Thus, the many anticancer activities of extracellular ATP and of elevated hepatic and red blood cell ATP pools in vivo are expected to yield direct antitumor effects as well as improvements in several quality of life parameters once the administration of ATP against advanced refractory cancers is applied to clinical settings.

REFERENCES


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