ADENOSINE TRIPHOSPHATE IN ADVANCED LUNG CANCER

A RANDOMIZED CLINICAL TRIAL

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A RANDOMIZED CLINICAL TRIAL

ADENOSINETRIFOSFAAT BIJ LONGKANKER

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Aan mijn ouders

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1

Introduction & Outline

LUNG CANCER

Lung cancer is one of the leading causes of death in the world. It is the most frequent type of cancer among men and ranks third among women. Worldwide, more than 500,000 new cases are diagnosed each year.[331] In 1994, in the southeastern part of the Netherlands the incidence was 70 per 100,000 person-years in men and 13 per 100,000 person-years in women.[207] The prevalence was 219 per 100,000 in men and 33 per 100,000 in women.[84] The major risk factor of lung cancer is smoking.[126] The prognosis of patients with locally advanced (stage III) or metastatic (stage IV) non-small-cell lung cancer remains poor despite the continuing exploration of new cytotoxic drugs. The 1- and 5-year survival rates in these patients are 25 to 40%, and less than 5%, respectively.[153] The high morbidity and mortality are not only due to progressive tumor growth but also to the high frequency of the cachexia syndrome in this patient population.[94,116,204,440]

CACHEXIA

Cancer-associated cachexia is characterized by involuntary weight loss, fatigue, impaired physical performance, and general malaise. The word cachexia is derived from the Greek words *kakos* and *hexis* meaning 'bad condition'. Cachexia is associated with extensive wasting of energy stores of fat, skeletal muscle and liver tissues;[374] with elevated lipolysis,[128,391] proteolysis,[266,306] and gluconeogenesis.[103,267,389] In newly diagnosed non-small-cell lung cancer patients approximately 35% had more then 5% weight loss, and this was associated with decreased mean survival.[116]

Dietary counseling,[76,317] use of enteral supplements[76] and administration of parenteral nutrition[282] have failed to reverse cachexia. Corticosteroids[56,114,336] and the anti-seritonergic drug cyproheptadine[215] induced an increase in appetite without positive effects on body weight. The phosphoenolpyruvate-carboxykinase inhibitor hydrazine sulfate had no effect on appetite or body weight.[215,259] Anabolic steroids also failed to influence weight in non-small-cell lung cancer patients.[75] Progestagens

induced an increase in appetite,[55,137] reduction of weight loss[137] or weight gain.[396] However, this was attributed mainly to fat gain.[261,396]

ADENOSINE 5'-TRIPHOSPHATE

Adenosine 5'-triphosphate (ATP) is a purine nucleotide found in every cell of the human body. In addition to its well-established intracellular energy-transferring role, ATP is involved extracellularly in biological processes including neurotransmission, muscle contraction, cardiac function, platelet function, vasodilatation, and liver glycogen metabolism. Extracellular ATP has been known to exert its effects through P2 receptors on the surface of many cells. In recent years several clinical applications of ATP have been reported. Intravenously administered ATP has been found to have potential in cardiology, anesthesia, pulmonology, and also oncology. In oncology, preclinical studies showed that administration of ATP induced growth inhibition of various human tumor cell lines[3,36,92,135,149,224,343,344,405,443] and animal-implanted tumors.[134,161,239,301,309,327,344,345,347,348] Furthermore, intraperitoneal ATP administration resulted in inhibition of weight loss[348] and significantly prolonged survival in these animals.[134,239,309]

Recently, in the United States in a small uncontrolled phase II study 15 patients with advanced non-small-cell lung cancer were treated with one to seven intravenous ATP infusions of 96 hours at 4-week intervals. After treatment a mean maintenance of weight, quality of life, and performance status together with inhibition of tumor growth in 10 out of 15 patients was shown.[187] These results supported earlier findings from a phase I study in eight patients with lung cancer.[188]

AIMS OF THE THESIS

Based on these promising preliminary reports, the aim of the present project was to assess the effects of intravenous ATP infusions on weight loss, body composition, performance score, quality of life, tumor response, and survival in patients with advanced non-small-cell lung cancer (stage IIIB or IV). In addition, we performed

supportive studies in order to examine underlying mechanisms contributing to clinical effects of ATP.

OUTLINE OF THE THESIS

In **Chapter 2**, established and potential applications of ATP in clinical practice are reviewed.

In **Chapter 3**, the clinical effects of intravenous ATP infusions in advanced lung cancer patients are reported, including quality of life, muscle strength and body weight (**3.1**), body composition and nutritional status (**3.2**), and tumor response and survival (**3.3**). In addition, we report a pilot study exploring the potential value of intravenous adenosine infusion in the pain treatment of advanced cancer patients (**3.4**).

In **Chapter 4**, five satellite studies are reported. These studies involve the pharmacokinetics of ATP infusion (**4.1**), effects of ATP on liver energy status (**4.2**), effects of ATP on glucose turnover and gluconeogenesis (**4.3**), and a cell line study on ATP-induced growth inhibition of human lung tumor cells (**4.4**). Finally, the validity of skinfold anthropometry for measuring longitudinal changes in body composition in patients with advanced lung cancer is assessed using the deuterium dilution technique as a reference (**4.5**).

A general discussion and conclusions are presented in **Chapter 5**.

2

REVIEW

ADENOSINE TRIPHOSPHATE: ESTABLISHED AND POTENTIAL CLINICAL APPLICATIONS

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ABSTRACT

Adenosine 5'-triphosphate (ATP) is a purine nucleotide found in every cell of the human body. In addition to its well-established role in cellular metabolism, extracellular ATP and its breakdown product adenosine, exert pronounced effects in a variety of biological processes including neurotransmission, muscle contraction, cardiac function, platelet function, vasodilatation, and liver glycogen metabolism. These effects are mediated by both P1 and P2 receptors. A cascade of ectonucleotidases plays a role in the effective regulation of these processes and may also have a protective function by keeping extracellular ATP and adenosine levels within physiological limits. In recent years several clinical applications of ATP and adenosine have been reported. In anesthesia, low-dose adenosine reduced neuropathic pain, hyperalgesia, and ischemic pain to a similar degree as morphine or ketamine. Postoperative opioid use was reduced. During surgery, ATP and adenosine have been used to induce hypotension. In patients with hemorrhagic shock, increased survival was observed after ATP treatment. In cardiology, ATP has been shown to be a well-tolerated and effective pulmonary vasodilator in patients with pulmonary hypertension. Bolus injections of ATP and adenosine are useful in the diagnosis and treatment of paroxysmal supraventricular tachycardias. Adenosine also allowed highly accurate diagnosis of coronary artery disease. In pulmonology, nucleotides in combination with a sodium-channel blocker improved mucociliary clearance from the airways to near normal in patients with cystic fibrosis. In oncology, there are indications that ATP may inhibit weight loss and tumor growth in patients with advanced lung cancer. There are also indications of potentiating effects of cytostatics and protective effects against radiation tissue damage. Further controlled clinical trials are warranted to determine the full beneficial potential of ATP, adenosine and uridine 5'-triphosphate.

INTRODUCTION

Adenosine 5'-triphosphate (ATP) is a naturally occurring nucleotide which is present in every cell. It consists of a purine base (adenine), ribose, and three phosphate groups. Nucleotides were first recognized as important substrate molecules in metabolic interconversions, and later as the building blocks of DNA and RNA. More recently, it was found that nucleotides are also present in the extracellular fluid under physiologic circumstances.[152] Extracellular ATP is broken down by a cascade of ectoenzymes and xanthine oxidase to form uric acid, which is excreted in urine (**Figure 2.1**).

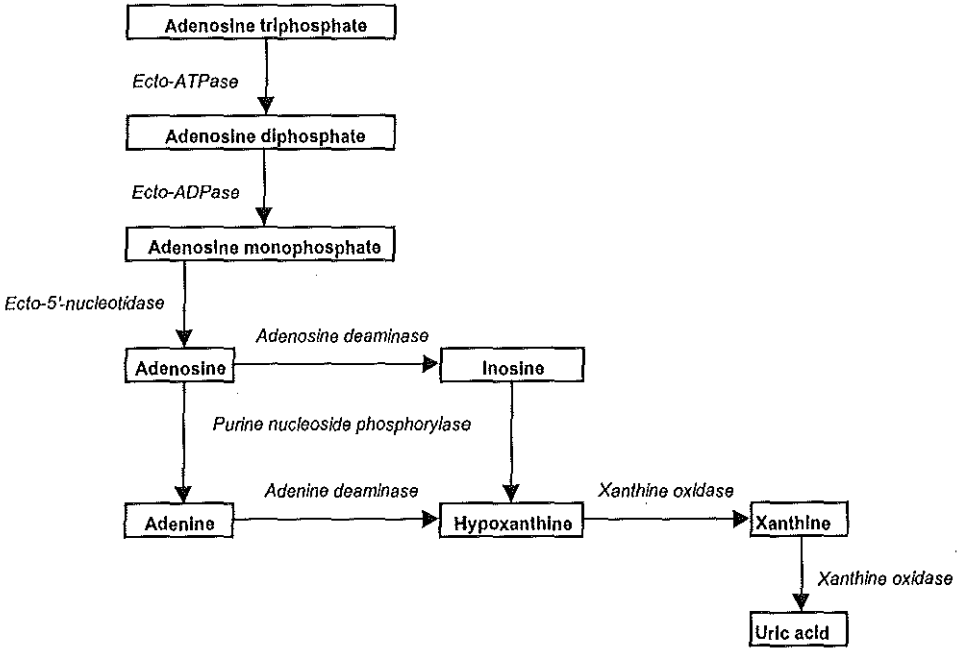

Figure 2.1. Pathway of adenosine 5'-triphosphate (ATP) breakdown to uric acid

Extracellular ATP appears to be involved in the regulation of a variety of biological processes including neurotransmission, muscle contraction, cardiac function, platelet function, vasodilatation, and liver glycogen metabolism. ATP can be released from the

cytoplasm of several cell types and interacts with specific purinergic receptors on the surface of many cells. These receptors play a fundamental role in cell physiology, and are divided in two major classes: P1 and P2 receptors. In general, the effects of adenosine are thought to be mediated through P1 receptors, whereas ATP binds to P2 receptors. P1 receptors are subdivided into A_1, A_{2A}, A_{2B} and A_3 receptors[155] which activate phopholipase C (A_1, A_{2B}, A_3) and adenylate cyclase (A_{2A}, A_{2B}), and modulate ion channels (A_1, A_{2B}, A_3).[138] P2 receptors are subclassified in G-protein-coupled receptors termed P2Y receptors and intrinsic ion channels termed P2X receptors.[1,155] Stimulation of the various receptors evokes diverse biological responses and has been reviewed by Dubyak and el-Moatassim[130] and Conigrave and Jiang.[89]

In recent years the possible pharmacological uses of ATP have received attention, following reports of its potential benefit in pain, vascular diseases and cancer (**Table 2.1**). In this review, current and potential clinical applications, and proposed mechanisms of action of extracellular ATP and its breakdown product adenosine (**Figure 2.2**), are discussed together with their pharmacokinetic properties and adverse effects.

2.1 PHARMACOKINETIC PROPERTIES

Physiological levels

The average physiological concentration of ATP within mammalian cells is 3152 ± 1698 (SD) μM. The ATP content in tissue cells is somewhat higher than in blood cells.[432] In human erythrocytes ATP concentrations of 1500 to 1900 μM are detected.[112,429,463] Only three authors have reported ATP concentrations in human plasma. Forrester and Lind[152] described concentrations of 1.2 ± 0.5 μM, while Harkness et al.[185] measured 3.9 ± 1.5 μM ATP, and Ryan et al.[364] reported a range of 0.15 to 0.65 μM ATP. These concentrations are in the same order of magnitude as the physiological concentrations of adenosine in plasma, that is 0.1 to 1 μM.[314]

Cellular uptake and transport

Using suspensions of washed intact human erythrocytes and labeled purines, Parker et al.[329] found that ATP is metabolized outside the cell via adenosine diphosphate (ADP)

and adenosine monophosphate (AMP) to adenosine. Adenosine rapidly entered the erythrocytes where it was incorporated in adenine nucleotides. At extracellular adenosine concentrations below 3 μM, most intracellular adenosine was phosphorylated to adenine nucleotides. At higher extracellular adenosine concentrations, adenosine was degraded in the erythrocytes to inosine and hypoxanthine.[375] Intravenous administration of ATP is followed by rapid uptake by erythrocytes.[431] In a study using patients with cancer infusion of ATP 50 μg/kg.min induced a 63% increase in whole blood ATP levels; a higher rate of ATP infusion of 75 μg/kg.min gave only a slightly greater increase (67%).[188]

After intraperitoneal bolus injection of adenine nucleotides in mice,[348] an increase in erythrocyte ATP concentrations (from 600 to 1700 μM) was preceded by an increase in liver ATP (from 3000 to 7000 μM). The intraperitoneal mode of administration of adenine nucleotides seems to favor uptake by the liver, presumably because they enter the portal circulation.[348] Lerner and Lowy[250] showed that rabbit liver perfused with [^{3}H]adenine released [^{3}H]adenosine. They suggested that adenosine may be released from liver cells into the interstitial fluid and then the hepatic sinusoids, where it would be rapidly taken up by the circulating erythrocytes and incorporated into adenine nucleotides. Within seconds after intraperitoneal administration of unlabelled ATP and [^{3}H]adenosine, [^{3}H]ATP was found in plasma.[347] Rapaport[344,345] suggested that following rapid degradation of administered ATP, ATP is resynthesized in the liver, and then taken up by erythrocytes from which it is subsequently slowly released into plasma resulting in micromolar plasma levels of ATP.

Metabolism

Degradation of ATP in whole blood *in vitro* is considerably slower than under *in vivo* conditions. *In vitro*, five minutes after incubation of rabbit whole blood with ATP at 37°C, 93.2% of the ATP remained.[388] In contrast, in rabbits 40 seconds after an intravenous bolus injection of ATP only 1% of injected ATP was detected in whole blood.[388] Similarly, a bolus of ATP is almost completely cleared during a single passage through either perfused dog lung[35] or perfused guinea-pig heart.[323] Ryan and Smith[363] showed a half-life of ATP of less than 0.2 seconds in perfused rat lung. At physiological concentrations, the half-life of [^{3}H]adenosine in human plasma *in vivo* is 0.6 to 1.5 seconds.[294]

Table 2.1. Effects of adenosine 5'-triphosphate (ATP) and adenosine with potential or demonstrated clinical implications

Topic	Cells/Organs	Animals	Patients
Anesthesia and analgesia			
blood pressure during surgery			reduced
anesthetic requirement		reduced	reduced
opioid requirement after surgery			reduced
pain		reduced	reduced
Pulmonary hypertension		reduced	reduced
Supraventricular tachycardias			inhibited
SA + AV node conduction	inhibited		inhibited
Mechanism wide QRS complex			diagn. assessment
Coronary artery disease			diagn. assessment
Shock			
organ function	improved	improved	
survival rate		increased	increased
Airway mucosa function			
surfactant secretion	increased		
chloride secretion*	increased		increased
ciliary beat frequency	increased		
mucus secretion	increased		
water secretion	increased		
mucociliary clearance†			increased
Metabolism			
gluconeogenesis	reduced/increased		
glycogenolysis	increased		
Cancer treatment			
weight loss		inhibited	inhibited
tumor growth	inhibited	inhibited	inhibited
chemotherapy efficacy	increased	increased	
radiotherapy damage	reduced	reduced	
radiotherapy efficacy	increased	increased	
radiotherapy survival rate		increased	

*Observed in epithelia of healthy patients and cystic fibrosis patients
† Epithelia of cystic fibrosis patients
AV = atrioventricular; SA = sinoatrial

A cascade of ectonucleotidases on the endothelial cells is thought to be responsible for the hydrolysis of ATP in blood *in vivo*.[178,333,363,365] There are three types of ectonucleotidases: ecto-ATPases, ecto-ADPases and ecto-5'-nucleotidases.[431] These enzymes have also been detected on a variety of other cell types including erythrocytes,[431] leukocytes,[83] B-lymphocytes,[18] both helper and cytotoxic T-lymphocytes,[143] and hepatocytes.[376] The ectonucleotidase system could be of importance in the regulation of neurotransmission, blood-platelet function and vasodilatation.[333] It has also been suggested that ectonucleotidases on the surface of cell membranes may have a protective function by keeping extracellular ATP and adenosine levels within physiological limits.[431]

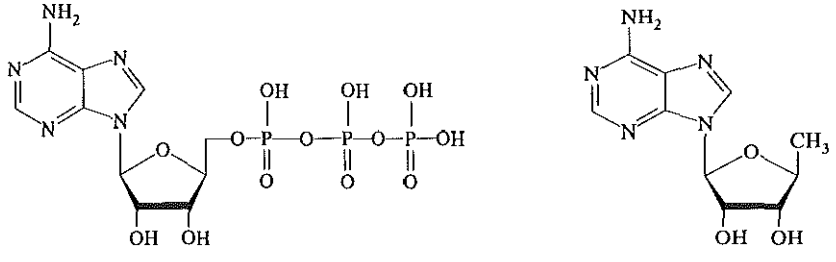

Figure 2.2. Structural formulae of adenosine 5'-triphosphate (ATP) and adenosine

2.2 PHYSIOLOGICAL EFFECTS AND MECHANISMS OF ACTION

Nervous system

ATP acts as neurotransmitter in both the central and peripheral nervous system and can also modulate the release of other neurotransmitters.[177] There is evidence that ATP plays a role as sensory neurotransmitter and adenosine as neuromodulator when both are released from non-nociceptive large diameter primary afferent neurons and capsaicin-sensitive small diameter primary afferent nerve terminals in the dorsal horn, and

that adenosine in particular inhibits pain transmission.[371,372] It also seems likely that the effects of ATP on pain are dependent on ATP being broken down to adenosine.[367,371]

The antinociceptive effects are mediated through activation of A_1 and A_2 receptors.[371,372] These receptors are localized in the substantia gelatinosa of the spinal dorsal horn.[77,78,170] Based on the observation that the adenosine blockers theophylline and caffeine also partly inhibit the analgesic effects of morphine, it has been suggested that adenosine may be one of the intermediary substances involved in morphine-induced spinal antinociception.[371]

Respiratory system

Several cell types in both the upper and lower respiratory system respond to ATP and also to uridine 5'-triphosphate (UTP). In type II alveolar epithelial cells of newborn rats, ATP and UTP stimulate P2Y receptor-coupled surfactant secretion.[173,354] In airway epithelia of healthy individuals and patients with cystic fibrosis (CF), ATP and UTP stimulate transepithelial chloride secretion. Chloride is secreted through the cystic fibrosis transmembrane regulator (CFTR)[81,82,412] as well as the non-CFTR.[60,350,353] In this way, in nasal epithelial cells from CF patients, Knowles et al.[226] observed an increase in transepithelial potential difference. The magnitude of potential difference correlates with the rate of chloride secretion and sodium absorption. Several studies have demonstrated that the effects of ATP and UTP on chloride transport are mediated by a separate class of P2 receptors,[226,279] named $P2Y_2$ receptors.[88,269,332] Stimulation of these receptors activates inositol phospholipid hydrolysis and calcium mobilization.[279]

ATP export from the mucosal cell has also been related to the CFTR gene product. It has been suggested that the CFTR functions directly as an ATP-channel,[2,3,60] but this finding was recently rejected: Reddy and Quinton[350] and Reddy et al.[351] demonstrated that the CFTR is a regulator of an associated ATP channel.

Immune system

Lymphokine-activated killer (LAK) cells and cytotoxic T lymphocytes (CTLs) are subtypes of effector lymphocytes which play an important role in immune defense against tumor cells and virus-infected cells. The mechanism by which LAK cells and CTLs lyse their target cells is a subject of intense interest. Since the early 1990s, ATP

has been proposed as a possible mediator of cytotoxic cell-dependent lysis. ATP could either be secreted via exocytosis, or directly released from the cytoplasm of LAK cells and CTLs by a yet unknown process.[119] Zanovello et al.[474] observed an ATP-induced calcium influx in classical natural killer target cells (YAC-1), and DNA-fragmentation in P815 target cells. Both EL-4 lymphoma cells and multidrug resistant human colon carcinoma cells (LoVo-Dx) were sensitive to ATP, as well as to CTLs and LAK cells.[15,92,93] Filippini et al.[143,144] suggested that the lytic effects of ATP on CTL target cells may be the result of ATP on purinergic receptor or ectoprotein kinase stimulation. CTLs and LAK cells, as putative sources of ATP, have been shown to be resistant to the lytic effects of ATP,[92,117,142,474] possibly because of the absence of purinergic receptors[92] and a high expression of ecto-ATPases.[92,142] These ecto-ATPases would effectively eliminate self-generated ATP from the LAK cell membrane.[117]

Blood vessels

Both P1 and P2 receptors are present in blood vessel walls. Two subtypes of the P2 receptor have been described: P2X and P2Y receptors.[58,200] P2X receptors are located on vascular smooth muscle cells, and mediate vasoconstriction. P2Y and P1 receptors are located on endothelial cells, and mediate a vasodilator response,[39,217] and this vasodilatation is probably responsible for a variety of the physiological effects of ATP.

When given intravenously, low-dose ATP and adenosine have mainly pulmonary rather than systemic effects, because they are rapidly metabolized during their passage through the lung.[436] In the pulmonary circulation, the predominant types of purinergic receptors are the P2Y and P1 receptors.[40,254] ATP binds to the P2Y receptor on the pulmonary endothelial cell and stimulates the formation of nitric oxide (NO). NO increases the concentration of the intracellular messenger involved in smooth muscle relaxation, cyclic guanosine monophosphate (cGMP).[147,203] Adenosine binds to the P1 receptor on pulmonary endothelial cells, increasing endothelial-cell adenylate cyclase activity and cellular cyclic AMP (cAMP) level and causing vascular smooth muscle relaxation.[147,177]

Heart

In the sinoatrial (SA) and atrioventricular (AV) nodal cells, extracellular ATP and adenosine stimulate the release of potassium which induces an electric current and results

in depression of the sinoatrial node as well as slowing down the AV node conduction.[22,23,111,157,475] ATP is thought to induce the effects after degradation to adenosine.[83] Their electrophysiological effects are mediated by activation of extracellular A_1 receptors which are coupled with guanosine triphosphate-binding inhibitory proteins. Dipyridamole, an inhibitor of adenosine transport across cell membranes, potentiates these effects, while methylxanthines, such as theophylline and caffeine antagonize the activities of the adenosine receptors. Stimulation of the A_1 receptor influences both the potassium channels and cAMP production.[233]

Some authors have postulated that the nodal effects of ATP are modulated by atropine, suggesting that these effects may partly be mediated through muscarine receptors of the vagal nerve.[298,458] Recently, Tai et al.[424] demonstrated that during use of atropine, patients required a higher dose of adenosine to terminate tachycardia. However, in other studies atropine did not influence the cardiac effects of ATP and adenosine.[123,136]

Liver

Extracellular ATP induces phosphatidylinositol hydrolysis and Ca^{2+}-mobilization and influx in isolated hepatocytes.[48,64,311] This has been ascribed to stimulation of P2 receptors.[62,64,124,311] Koike et al.[229] showed that the rise in intracellular calcium plays an important role in triggering gluconeogenesis. Depending on the concentration, extracellular ATP can either stimulate or inhibit gluconeogenesis in isolated hepatocytes. Maximal stimulation of gluconeogenesis was shown at 40 μM[366] and 100 μM ATP.[62,133,229] Maximal inhibition was shown at 1000 μM ATP[13] and 500 μM adenosine.[264]

The increase in hepatocyte calcium concentrations is also associated with increased glycogenolysis.[64,97,218,219] Studies in perfused rat livers have been confirmed the stimulating effects of ATP on glycogenolysis.[59]

Tumor

ATP has cytostatic and cytotoxic effects in many types of transformed and tumor cells. Several mechanisms have been proposed:

1. Exposure of human adenocarcinoma cells to extracellular ATP has been reported to cause intracellular accumulation of ATP and arrest of tumor cells in the S-

phase of cell replication, followed by cell death.[343] A similar ATP-induced growth inhibition, caused by prolonging of the S-phase, is found in rat ureter carcinoma cells[357] and in human breast cancer cells.[405] Weisman et al.[461] showed that extracellular ATP was hydrolyzed to extracellular adenosine which was transported into the cells by adenosine translocators.

2. Intracellular adenosine induced growth inhibition by elevating intracellular ATP and ADP and reducing intracellular UTP levels. These changes induced inhibition of pyrimidine nucleotide biosynthesis. Based on this, it was suggested that the inhibition of tumor growth after exposure to extracellular ATP is caused by an adenosine-dependent pyrimidine starvation effect.[134,239,461]

3. ATP-induced tumor growth inhibition is associated with lower rates of protein synthesis and lower γ-glutamylcysteine synthase activity.[239] The latter leads to a decrease in glutathione content of the tumor, but not of normal tissues.[134,239,309]

4. Protein phosphorylation catalyzed by a number of cell surface protein kinases is known to occupy a key role in transmembrane signal transduction. Friedberg and Kuebler[159] and Friedberg et al.[158] showed a positive correlation between the activity of ectoprotein kinase and the ability of ATP to induce cell growth inhibition. Removal of this enzyme prevented the ATP-induced growth inhibition. A growth inhibitor, a protein with an apparent molecular mass of 13 kDa, is produced on exposure to extracellular ATP. Transformed cells have a higher activity of ectoprotein kinase compared with nontransformed cells.[158,159]

5. After ATP administration, increased membrane permeability has been demonstrated in various transformed cells, including fibroblasts,[120,159,193,224,361] ovary cells,[223] melanoma cells,[224,299] neuroblastoma cells,[118] hepatoma cells,[21] hepatocytes,[300,478] erythroleukemia cells,[63] mastocytoma cells,[143] lymphoma cells,[117,119,474,476] and leukemic lymphocytes.[465-467] In contrast, this increase in permeability was not observed in untransformed cells.[159,222,224,299,361,466] Although in these studies no systematic search for receptors was undertaken, the evidence suggests that increased cell permeability after exposure to extracellular ATP may be due to activation of $P2X_7$ (formerly P2Z) receptors.[154,413] These receptors are highly selective for the tetrabasic ATP^{4-} form.[404] The tumor cell types that express these receptors on their cell surfaces include various leukemia cells[36,52,378,404] and neuroblastoma cells.[214] Activation of the $P2X_7$ receptors causes opening of ion channels

which leads to an increase of intracellular Ca^{2+}, loss of K^+, entry of Na^+ and a decrease in the membrane potential. Activation of $P2X_7$ receptors also results in formation of nonselective pores, which induces an increase in nonselective membrane permeability for aqueous solutes that ordinarily do not cross the cell membrane, including nucleotides and small hydrophylic molecules of MW up to 900 Da.[73] In transformed 3T6 mouse fibroblasts, Saribas et al.[369] found that ATP increases the permeability to molecules as large as 20 kDa. Opening of these ATP-sensitive channels or ATP-induced pores will lead to cell death either by cell swelling, which is characteristic for necrosis,[73,193] or DNA fragmentation, which is characteristic for apoptosis.[474,476]

Beyer and Steinberg[32] showed that pore formation in macrophages is the result of damage to the gap junction protein connexin-43. Loewenstein[255] confirmed these results and reported that connexin-43 is more exposed in neoplastic cells than in normal cells. Neoplastic cells are known to be deficient in intercellular communication and therefore have gap junction proteins which are exposed to the extracellular environment.[32,255] It has been suggested that connexin-43 may has a function as a 'suicide receptor'.[255] However, Alves et al.[9] recently were unable to show an effect of extracellular ATP on connexin-43 hemigap-junction channels, thus contradicting the findings of Beyer and Steinberg.[32]

Tissue protection

Under conditions of metabolic stress, such as ischemia, a rapid and massive depletion of intracellular ATP occurs. As a consequence of ATP breakdown, adenosine, inosine and hypoxanthine accumulate in the ischemic tissue.[302,477] Bouma et al.[43] proposed that adenosine may exert protective effects during the reperfusion period by binding to A_2 and A_3 receptors. In this context, Newby et al.[303] introduced the term 'retaliatory metabolite' and Bouma et al.[46] described adenosine as part of a 'natural defense system'. The protective action of adenosine or ATP can be explained by cardiovascular, metabolic and anti-inflammatory activities, as studied under *in vitro*, *in vivo* and clinical conditions.

The cardiovascular effects of ATP seem important in improving flow after ischemia. In animal models of shock, intravenous ATP-$MgCl_2$ enhances the renal[454] and hepatic microcirculation,[71,195,196] portal and total hepatic blood flow,[452] and cardiac output.[451]

ATP-induced metabolic effects, including improved mitochondrial function and electrolyte transport, increased intracellular ATP,[68,69,310] reduced O_2 consumption,[470] enhancement of P2 receptor binding capacity,[276] and normalization of impaired second messengers cAMP and inositol 1,4,5-triphosphate (IP_3).[275]

The inhibitory effects of adenosine extends to different processes related to inflammation:

- inhibition of effectors – neutrophil superoxide production, neutrophil degranulation,[43] and anti-oxidants activation;[273,340]
- inhibition of mediators – tumor necrosis facot (TNF), interleukin (IL)-6,[44,453] IL-8,[44,45] eicosanoids[232] and complement;[238]
- inhibition adhesion of neutrophils,[98] inhibition of adhesion molecule expression.[45]

Furthermore, Cronstein et al.[99] demonstrated that the antiphlogistic action of the potent anti-inflammatory agent methotrexate is caused by increased adenosine release at inflamed sites. The increase in extracellular adenosine diminishes both the accumulation and function of leukocytes in inflamed sites.

2.3 CLINICAL APPLICATIONS

2.3.1 ANESTHESIA

Pain Reduction

During the last decade, studies in both animal models and patients have shown that ATP and particularly adenosine at low doses may modulate pain. Intravenous ATP in mice was found to have a dose-dependent analgesic activity on hot plate and phenylquinone-induced stretching assays.[175,371,372] In dogs, when adenosine was used in combination with halothane, halothane requirement was reduced by 49%.[385]

Several double-blind, placebo-controlled, cross-over studies in healthy volunteers showed pain-reducing effects of intravenous adenosine infusion 50-70 μg/kg.min. Segerdahl et al.[380] observed a reduction in size of the tactile allodynic area by approximately 50%. They also reported ischemic pain-reducing effects of intravenous adenosine (70 μg/kg.min during 30 min) comparable with morphine (20 μg/kg.min during 5

min) or ketamine (20 μg/kg.min during 5 min). Furthermore, adenosine given in combination with morphine or ketamine had an additive effect on pain reduction.[381]

In two randomized double-blind studies in patients undergoing breast surgery (75 patients)[379] and gynecological abdominal surgery (43 patients),[382] a systemic intravenous adenosine infusion (80 μg/kg.min) significantly reduced perioperative isofluorane requirements and postoperative pain. In addition, in both studies the need for opioids was reduced by approximately 25% in the adenosine group during the first 24 postoperative hours.[379,382]

Several reports have provided evidence that low dosages of adenosine (50 μg/kg.min) alleviate neuropathic pain, hyperalgesia and allodynia without inducing other pain symptoms.[24,400] Intravenous adenosine infusion for 45 to 60 minutes led to improvement of spontaneous or evoked pain in six out of seven patients with peripheral neuropathic pain, which lasted from six hours to four days.[24] This positive finding was unexpected since it is known that adenosine is rapidly eliminated from the blood. It was speculated that the effects of adenosine on central hyperexcitability persist longer than the direct action of adenosine on the receptors.[400]

Shock

A common feature of shock is an inadequate circulation with diminished perfusion of tissues, resulting in hypoxia and injury to various tissues. The rescuscitation period after shock is also associated with development of tissue injury and loss of organ function.[46]

Several *in vivo* animal studies show that following hemorrhagic shock, infusion of ATP-$MgCl_2$ has a positive effect on the survival.[65,66,68,79,145,195,196,310,473] Various studies indicate that ATP and adenosine have protective effects on tissue injury following reperfusion after a preceding period of ischemia. ATP-$MgCl_2$ improves rat kidney,[70,315] rat liver,[67,70] dog heart,[231,283] rabbit lung[209] and rat gut function[398] after a period of ischemia. The use of intramuscular ATP-$MgCl_2$ is also protective in rats with burns.[473]

In patients with acute renal failure or multiple organ failure, beneficial effects of ATP-$MgCl_2$ were observed in a single study. Thirty-two patients were randomly divided into two groups. One received intravenous ATP-$MgCl_2$ (40-50 μM/kg) and the

other served as a control. The survival rate was 100% in the ATP group and 73.3% in the control group.[197]

Control of blood pressure

ATP and adenosine have been used to cause hypotension during anesthesia and surgery in patients. Already in 1951, Davies et al.[106] showed in patients that an intravenous or intra-arterial injection of 40 mg ATP induced a moderate fall in blood pressure without change in heart rate. Controlled hypotension may be employed to reduce intraoperative haemorrhage. The hemodynamic effects of ATP and adenosine have been investigated in over 150 patients undergoing oral,[163] orthopedic,[86] abdominal aortic aneurysm,[319,321] cerebral aneurysm[235,318,320,402] and unspecified surgery.[162,164] Intravenous ATP or adenosine infusion (50 to 350 μg/kg.min) induced significant reductions in blood pressure (20 to 43%) with a major decrease in systemic vascular resistance (36 to 67%) and increase in cardiac output (14 to 42%), and only a small increase in heart rate (3 to 16%). The hemodynamic parameters returned to their baseline values immediately after stopping the infusion.[162,164] Tachyphylaxis and rebound-hypertension were not observed.[87,162,164,318-320,402] In contrast, Segerdahl et al.[379,383] showed no change in blood pressure during treatment with 80 μg/kg.min adenosine in patients with abdominal, breast and shoulder surgery. The reason for this discrepancy is not clear.

ATP and adenosine (150 to 300 μg/kg.min intravenously) have been used successfully during surgery for pheochromocytoma to reduce systemic blood pressure.[14,34,181] ATP has been used to antagonize the vasoconstrictive activities of noradrenaline (norepinephrine) and/or sympathetic nerve stimulation.[14] Low-dose adenosine infusion (30 to 50 μg/kg.min) in patients undergoing bypass surgery evoked coronary vasodilatation with only minor effects on the systemic circulation, and, therefore, may possibly be useful in the prevention of early occlusion of coronary artery bypass grafts.[430]

2.3.2 CARDIOLOGY

Pulmonary hypertension

Pulmonary hypertension can be a serious problem after thoracic surgery in patients with chronic obstructive pulmonary disease (COPD), and in children with congenital heart defects. Vasodilators have often been unsuccessful, because they act simultaneously on pulmonary and systemic vessels with predominant systemic effects.[362] However, low-dose intravenous ATP or adenosine has been shown to exert predominant pulmonary vasodilating effects.[165,168]

In newborn lamb models, intravenous administration of ATP was effective against pulmonary hypertension.[146,230,325] Several studies showed a predominant decrease in pulmonary arterial pressure during intravenous infusion of about 100 μg/kg.min ATP, while systemic arterial pressure and vascular resistance decreased at higher levels.[146,168,213,326]

Studies in healthy individuals,[352,436] patients with COPD,[166-168] and children with pulmonary hypertension,[53] showed significant decreases in mean pulmonary arterial pressure and pulmonary vascular resistance during intravenous ATP infusion (to 100 μg/kg.min), without a change in mean systemic arterial pressure and systemic vascular resistance. In seven children with pulmonary hypertension after surgical repair of congenital heart defects, ATP induced a decrease in pulmonary arterial pressure; three children showed complete disappearance of pulmonary hypertensive crises.[53] The children did not have rebound pulmonary hypertension,[53] although in some studies rebound pulmonary hypertension was observed after discontinuation of ATP.[167,213] Recently, the effectiveness of intravenous ATP treatment against pulmonary hypertension has also been shown in 20 patients with septal defects during cardiac surgery.[171]

Fullerton et al.[165] reported two patients with acute life-threatening pulmonary vasoconstriction after thorax surgery. In both, when standard treatment had failed, adenosine (25 to 50 μg/kg.min intravenously) achieved lowering of pulmonary arterial pressure without lowering of systemic arterial pressure.

Supraventricular tachycardias

The anti-arrythmic effects of purines have been known for decades. In 1929, Drury and Szent-Gyorgi[129] described the effect of adenosine on myocardial conduction. The use of intravenous adenosine to terminate supraventricular arrhythmias was first described in 1933 by Jezer et al.[210] In 1955, Somlo[403] reported the first successful clinical trial with ATP in paroxysmal tachycardias.

There has been renewed interest in potential cardiac applications of ATP and adenosine in recent years. Several studies have demonstrated efficacy of intravenous ATP and adenosine in paroxysmal supraventricular tachycardias (PSVT) in both children and adults. In a double-blind study in 39 patients during 68 episodes of supraventricular tachycardias, there was no difference in the clinical efficacy of ATP and adenosine.[341] The actions of ATP may be primarily mediated by its breakdown product, adenosine.[83,401] In newborn children, intravenous ATP[113,176] and adenosine administration[80,328] successfully stopped supraventricular tachycardias. In patients with adult PSVT, bolus injections of 5 to 20 mg ATP or 6 to 12 mg adenosine induced normalization of heart rate in over 90% of them.[25,122,358,387,410,446,475]

Most authors described no difference in efficacy between adenosine and the standard therapy for PSVT, verapamil,[49,122,140,172,272] whereas some authors showed a significantly greater overall efficacy of adenosine than of verapamil: 89 versus 61%[387] or 100 versus 80%.[25] Moreover, adenosine elicited a much more rapid antiarrhythmic response than verapamil. Adenosine terminated tachycardia within only 30 seconds,[50,80,140,297,328,387] whereas in two studies in which verapamil was given, it took 142[387] and 248 seconds,[140] respectively, to terminate tachycardia. Thus, the efficacy of adenosine would appear to be at least equivalent to verapamil, with a considerably more rapid onset of action.

Adenosine has also been used as a diagnostic tool of wide QRS complex tachycardias where the mechanism was uncertain. Wide complex ventricular tachycardia is often misdiagnosed as wide complex supraventricular tachycardia with abberancy. Use of verapamil in these misdiagnosed patients can result in severe hypotension and cardiac arrest.[342,390] Because of its specific action on atrioventricular nodal conduction, adenosine can help to differentiate between supraventricular tachycardia with aberrant conduction and ventricular tachycardia. In

facilitating this diagnosis, a positive response to adenosine had a sensitivity of 90% and a specificity of 94%, based on electrophysiological studies.[180,342,390]

Pharmacological stress test

Assessment of myocardial ischemia and coronary artery disease is used in the evaluation of risk before major surgery, and to select patients for coronary angiography, percutaneous transluminal coronary angioplasty or coronary bypass graft surgery after acute myocardial infarction, in addition to assessment of atypical chest pain. Because many patients are unable to perform an adequate exercise stress test, noninvasive methods to evaluate coronary artery disease have been developed.[136] For several years, thallium-201 or technetium-99m sestamibi myocardial perfusion imaging by single photon emission computed tomography (SPECT) has been used. Because maximal dilatation is needed to produce optimal SPECT images, these imaging techniques are often performed during infusion of a vasodilator.

Several studies clearly demonstrate the potential diagnostic use of the vasodilators ATP and adenosine for detection of coronary artery disease, using coronary angiography as gold standard. The pharmacological stress test of ATP or adenosine (to a maximum of 140 μg/kg.min intravenously for 6 min) in combination with thallium-201 scintigraphy has an overall sensitivity of 83 to 88% and a specificity of 78 to 100%.[95,139,445] Technetium 99m-sestamibi SPECT with adenosine had a sensitivity of 91 to 95% and a specificity of 70 to 75% in detecting coronary artery disease.[10,450]

Echocardiography in conjunction with adenosine-induced coronary vasodilatation also produced accurate results with a sensitivity of 75 to 81% and a specificity of 86 to 100%.[125,438] The sensitivity and specificity values of the adenosine stress tests are approximately similar to those currently obtained during exercise stress tests.[274,450]

2.3.3 PULMONOLOGY

Cystic fibrosis

CF is an autosomal recessive disease characterized by an excessive production of airway secretions, resulting in bronchial obstruction and recurrent episodes of respiratory tract infections. Electrolyte transport across the airway epithelia is abnormal:

excessive sodium absorption and defective regulation of the apical membrane chloride channel result in decreased water secretion and in increased reabsorption of periciliary fluid caused by decreased mucociliary transport. Furthermore, chloride secretion through the CFTR-chloride channel is absent.[42,338]

Because therapeutic agents are not available to improve chloride secretion in airways of CF patients, nucleotides have been tested. In normal as well in CF airway epithelia, ATP induced chloride secretion.[81,279,412] ATP and also uridine 5'-triphosphate (UTP) evoked stimulation of ciliary beat frequency[237,338] and mucus secretion by goblet cells.[107,221,251,287] ATP and UTP also induced net water secretion across excised airway tissues.[29,211] It is possible that the endogenous triphosphate nucleotides may serve as coordinating factors of the airway clearance system.[313]

Knowles et al.[226,227] studied the effects of inhaled aerosolized ATP and UTP in nine healthy individuals and 12 CF patients. These authors showed an ATP- and UTP-induced chloride secretion, with a maximal effective concentration at 100 μM. The efficacy of both ATP and UTP was greater in CF patients than in healthy individuals.[227] Extracellular UTP may be a potentially better agonist than ATP, because the nucleoside breakdown product of ATP, adenosine, is a bronchoconstrictor of human airways.[100,313] In a study in 14 CF patients, the administration of an UTP/amiloride combination was also demonstrated to improve mucociliary clearance from the peripheral airways of CF lungs to near normal basal rates.[30] The sodium channel blocker amiloride, has been shown to effectively inhibit sodium absorption by respiratory epithelium.[42,225] These data support the concept for the use of UTP in combination with amiloride as a therapy to improve clearance of secretions from the bronchi of patients with CF.

2.3.4 ONCOLOGY

Cancer cachexia

Cancer cachexia is a syndrome of progressive weight loss associated with depletion of liver and skeletal muscle energy stores.[374] This depletion is caused by elevated lipolysis,[128,391] protein breakdown,[266,306] and gluconeogenesis.[103,267,389] Dietary[76,317] and enteral supplements[76] fail to reverse the cachexia. In liver tissue[101,374,434] and in

skeletal muscle[374] of tumor bearing rats, significantly lower ATP levels have been demonstrated, and this is associated with an increased gluconeogenesis.[434]

Administration of ATP and adenosine to a suspension of isolated hepatocytes inhibited gluconeogenesis from lactate and pyruvate, but not from glycerol or fructose. *In vivo*, daily intraperitoneal injections of 25 mM ATP, AMP or adenosine for 10 consecutive days into mice bearing colon tumors, induced a significant inhibition of host weight-loss.[347,348] This inhibition was associated with expansion of hepatic ATP pools.[348] Rapaport et al.[345] suggested ATP may inhibit Cori cycle activity (i.e., the gluconeogenesis from lactate followed by reconversion of glucose to lactate in peripheral tissues), which is a potential means of inhibiting weight loss.

In the United States, a phase I/II trial was recently carried out in stage IIIB/IV non-small-cell lung cancer patients (n=8). After treatment with two to three intravenous ATP courses of 96 hours at 4-week intervals, stabilization of body weight was observed.[188] In a subsequent open-ended phase II trial (n=15), an average weight gain of 1.3 kg was demonstrated after four ATP courses.[286] Despite the small sample sizes, these data suggest that ATP may have modulating effects on weight loss in cancer patients.

Inhibition of tumor growth

Extracellular ATP can modulate the growth of neoplastic cells *in vitro*. Below 100 μM,[3,343,461] from 100 to 500 μM,[135,299,405,443] from 500 to 1000 μM,[405,476] and above 1000 μM,[189,190,476] extracellular ATP exerted cytostatic and cytotoxic effects on many transformed cell lines. In general, these effects appear to be greater than the effects on nontransformed mother cells.

In animal cell lines, ATP inhibited the growth of transformed fibroblasts,[28,224,461] leukemia cells,[63,189,190] mastocytoma cells,[143] lymphoma cells,[117] thymocytes,[476] melanoma cells,[299] and ureter carcinoma cells.[357] In human cell lines, ATP inhibited the growth of pancreatic carcinoma cells, colon adenocarcinoma cells,[343,344] melanoma cells,[224,343] androgen-independent prostate carcinoma cells,[135] breast cancer cells,[3,343,405,443] myeloid and monocytic leukemia cells,[36] and multidrug resistant colon carcinoma cells.[92] Similar exposure of nontransformed cell lines to ATP produced relatively less inhibition of cellular growth compared with transformed cells, or no inhibition at all.[189,190,224,299,343]

Hatta et al.[190] revealed the possibility of *ex vivo* purging of leukemia cells by ATP in autologous bone marrow transplantation. Mice injected with an untreated mixture of normal marrow cells and L1210 leukemia cells (10:1) died of leukemia within 18 days. In contrast, 85% of the recipients given ATP-treated mixture cells survived for more than 70 days.

In mice with CT26 colon tumors, daily intraperitoneal bolus injections of ATP (25 to 50 mM) significantly inhibited tumor growth.[344,345,347,348] ATP-induced growth inhibition was also demonstrated in rats with ureter carcinomas,[356] mice with lymphomas,[301] fibrosarcomas,[161] Ehrlich ascites tumors,[239,327] and breast tumors[3] Furthermore, intraperitoneal ATP administration resulted in a significantly prolonged survival of these animals.[134,239,309]

Recently, in an open-labeled phase II study, 15 untreated patients with advanced non-small-cell lung cancer (stage IIIB/IV) received one to four intravenous ATP courses (50 to 65 μg/kg.min for 96 hours), administered at 4-week intervals. No complete or partial tumor responses to ATP were reported, although stable disease was found in about two-thirds of the patients during the courses of treatment.[286] No further studies on ATP as a single anticancer agent in humans are available.

Chemotherapy

In cancer cell lines, ATP may enhance the efficacy of several chemotherapeutic agents. Addition of 200 to 500 μM ATP to doxorubicin in cultures of human ovarian carcinoma cells doubled the cell mortality, when compared with doxorubicin alone. Thirty to 50% more doxorubicin accumulated in the cancer cells when given together with ATP, whereas in healthy human fibroblasts practically no effect of ATP on doxorubicin uptake was observed.[281] Furthermore, in transformed Chinese hamster ovary cells (CHO-K1), the presence of 100 to 500 μM ATP and vinblastine or vincristine induced passive permeability of deoxy[^{3}H]glucose, while the drugs alone did not induce this change.[222] In mouse melanoma cell lines (clone-M3), 500 μg/kg.min ATP administration markedly increased the passive permeability for chemotherapeutic agents such as fluorouracil, doxorubicin, mitomicin and nimustine. The cytotoxic effects of these chemotherapeutic agents were additively potentiated by treatment with ATP. Vincristine combined with ATP showed even a synergistic cytotoxic effect; the effective concentration of vincristine was lowered 10- to 50-fold by ATP-treatment.[299]

Recently, a tendency of potentiating effects of cytostatic agents was also observed in one study *in vivo* after administration of adenosine. In mice inoculated with B-16 melanoma cells, adenosine (5 mM) was injected five days before administration of cyclophosphamide (50 mg/kg). This combined treatment reduced the number of melanoma foci by 60%, while the chemotherapy alone only reduced them by 45%. Moreover, a protective effect of adenosine against chemotherapy-induced decrease of leucocyte counts was seen in this study.[148] The use of ATP in combination with other agents for patients has been proposed, but no studies have been published.

In the treatment of acute myeloid leukemia, a combination of interferon-γ and ATP might provide a potential chemotherapeutic regimen. In bone marrow blast cells obtained from patients with acute myeloid leukemia, a dose-dependent lysis of malignant cells by ATP was demonstrated.[36] Furthermore, it was suggested that ATP might provide substantial benefit for chemotherapeutic treatment of brain tumors. In patients with malignant glioma selective enhancement of intratumoral blood flow after intercarotid administration of 0.5 to 1.3 μg/kg.min ATP was reported. It was hypothesized that in this way, greater amounts of cytostatic drugs might be transported into the brain tumor, without harming healthy brain tissue.[16]

Radiotherapy

Radiation causes DNA-bond breakage and rearrangement. Because of radiation damage, tissue levels of the lytic enzyme acid phosphatase and the neurotransmission enzyme cholinesterase are increased. In contrast, the activities of the glycolytic key enzymes hexokinase and lactate dehydrogenase are drastically decreased.[422] Stress such as by radiation in a living organism, may cause an increased demand for energy and glucose to repair damaged tissues.[419]

It has been suggested that extracellular ATP may provide energy for cellular repair processes.[420] Several studies have shown protection of ATP against damage caused by irradiation. Administration of ATP decreased the activities of acid phosphatase[421] and cholinesterase,[423] and augmented the activities of hexokinase and lactate dehydrogenase.[421] Moreover, it has been demonstrated that exogenous ATP may stimulate glycogenolysis[59] and glucose production in perfused liver of non-irradiated rats.[244]

In various animals, including mice and monkeys, the protecting effects of ATP against radiation damage enhanced survival rates from 5 to 50%,[304] 4 to 40%,[427] 40 to

85%,[422] and from 26 to 86%.[421] In addition, Senagore et al.[386] demonstrated that intravenous ATP-$MgCl_2$ infusion (60 μM/kg) in pigs offered significant cytoprotection from pelvic radiotherapy. ATP infusion lead to diminished colorectal seromuscular ischaemia, decreased skin and subcutaneous tissue injury, and significantly decreased perianastomotic inflammatory reaction.[386]

Furthermore, when radiation was given in combination with ATP, the frequency of formation of aberrant mitoses in epithelial cells of the mouse cornea was lower than when no ATP was given.[448] Moreover, this combination reduced the tumor growth rate of transplanted fibrosarcomas in mice[161] and led to significant regression of Ehrlich ascites tumors in mice.[134]

2.4 ADVERSE EFFECTS

In general, ATP and adenosine, when given as either continuous intravenous infusion or by intravenous bolus administration, induce similar adverse effects. Adverse effects of ATP and adenosine include general discomfort,[140] breathing deeper of more frequently,[33,106,122,212,334,456] headache,[90,122,445] flushing, chest pressure or chest pain, and nausea.[122,127,334,341,342,387,417,445,446] Sinus bradycardia[122,342] and atrial fibrillation[26,410] have been observed in patients receiving a bolus of adenosine. The respiratory stimulation started before chest discomfort and showed characteristics of adenosine receptor mediated responses.[212] The chemoreceptors of the carotic artery wall are described as the most likely site of these responses.[33,457] Studies to characterize chest pain demonstrated that higher concentrations of adenosine increased the intensity of the pain.[417] The chest pain provoked by adenosine is angina-like, but electrocardiogram signs of myocardial ischemia are absent. The algogenic effect of adenosine is related to activation of peripheral nociceptive afferents.[96,236,414-416,418] In angina pectoris, adenosine may be involved as an early messenger between myocardial ischemia and pain.[416] Methylxanthines reduce this pain significantly,[96,416,417] while dipyridamole just increases the intensity of pain.[416,417]

The adverse effects with bolus administration of ATP and adenosine are generally mild and transient because of the short plasma half-life (0.6 to 1.5 sec).[294] Frequent dosage titration is possible because of the rapid adenosine clearance in plasma.[136]

Clinical studies with continuous incremental adenosine infusion, used for the provocation of pain and for the diagnosis of myocardial ischemia, revealed that an adenosine infusion above approximately 75 μg/kg.min is associated with angina-like pain symptoms.[96,236,308,409] During intravenous ATP infusion in a phase I study in patients with advanced cancer, Haskell et al.[188] observed a cardiopulmonary reaction, typically characterized as a combination of a feeling of chest tightness without frank pain, and a sensation of 'needing to take a deep breath'. No significant hematological toxicity was noted. The most appropriate dose of ATP in patients with advanced cancer was 50 μg/kg.min. Adverse effects during intravenous infusion resolved within seconds after discontinuing the ATP infusion.

As reviewed by Faulds et al.,[136] rapid bolus administration of intravenous adenosine induced transient hemodynamic effects, which were usually mild at doses at which ATP induced electrophysiological activity. Intravenous adenosine infusion usually induced a small but significant increase in heart rate, small variations in systolic blood pressure, and a small but significant decrease in diastolic blood pressure and mean arterial pressure in conscious patients and volunteers.

2.5 CONCLUSION

ATP, adenosine and UTP have been shown to have a wide variety of beneficial effects in various clinical situations. The effects are probably mediated by P1 and P2 receptors. ATP, adenosine and UTP have been found to have potential in the management of pain, cancer and some cardiovascular and pulmonary diseases. Much work still needs to be done to define the full range of indication for their compounds, their interactions with other drugs, and ideal dosage schemes. P1 and P2 receptors are also potential targets for novel agonists and antagonists.

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3.1

RANDOMIZED CLINICAL TRIAL OF ADENOSINE 5'-TRIPHOSPHATE IN PATIENTS WITH ADVANCED NON-SMALL-CELL LUNG CANCER

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ABSTRACT

Background: Extracellular adenosine 5'-triphosphate (ATP) is involved in the regulation of a variety of biological processes, including neurotransmission, muscle contraction, and liver glucose metabolism, via purinergic receptors. In nonrandomized studies involving patients with different tumor types including non-small-cell lung cancer (NSCLC), ATP infusion appeared to inhibit loss of weight and deterioration of quality of life (QOL) and performance status. We conducted a randomized clinical trial to evaluate the effects of ATP in patients with advanced NSCLC (stage IIIB or IV).

Methods: Fifty-eight patients were randomly assigned to receive either 10 intravenous 30-hour ATP infusions, with the infusions given at 2- to 4-week intervals, or no ATP. Outcome parameters were assessed every 4 weeks until 28 weeks. Between-group differences were tested for statistical significance by use of repeated-measures analysis, and reported *P* values are two-sided.

Results: Twenty-eight patients were allocated to receive ATP treatment and 30 received no ATP. Mean weight changes per 4-week period were -1.0 kg (95% confidence interval [CI] = -1.5 to -0.5) in the control group and 0.2 kg (95% CI = -0.2 to +0.6) in the ATP group (P=0.002). Serum albumin concentration declined by -1.2 g/l (95% CI = -2.0 to -0.4) per 4 weeks in the control group but remained stable (0.0 g/l; 95% CI = -0.3 to +0.3) in the ATP group (P=0.006). Elbow flexor muscle strength declined by -5.5% (95% CI = -9.6% to -1.4%) per 4 weeks in the control group but remained stable (0.0%; 95% CI = -1.4% to +1.4%) in the ATP group (P=0.01). A similar pattern was observed in knee extensor muscles (P=0.02). The effects of ATP on body weight, muscle strength, and albumin concentration were especially marked in cachectic patients (P=0.0002, P=0.0001, P=0.0001, respectively, for ATP versus no ATP). QOL score changes per 4-week period in the ATP group showed overall less deterioration than in the control group—physical scores (-0.2% versus -2.4%; P=0.0002); functional scores (+0.4% versus -5.5%; P=0.02), psychological scores (-0.7% versus -2.4%; P=0.11); overall QOL score (+0.1% versus -3.5%; P=0.0001).

Conclusions: This randomized trial demonstrates that ATP has beneficial effects on weight, muscle strength, and QOL in patients with advanced NSCLC.

INTRODUCTION

Cachexia contributes significantly to the high morbidity and mortality in patients with advanced non-small-cell lung cancer (NSCLC).[116,440] Maintenance of weight and of quality of life (QOL) is therefore an important issue in the palliative treatment of patients with advanced NSCLC. Dietary counseling[76,317] and the use of enteral supplements[76] have failed to reverse weight loss. Drugs, including corticosteroids,[56] cyproheptadine,[215] hydrazine sulfate,[259] pentoxifylline,[174] and anabolic steroids,[75] were also shown to be ineffective. Prostagens reduced weight loss or increased weight, mainly as fat gain.[261,395] Although QOL was not influenced by prostagens in all studies,[108,395,426] in some studies it was also improved.[27,54]

Adenosine 5'-triphosphate (ATP) is a naturally occurring nucleotide that plays a central role as an energy source in every cell of the human body. In addition, extracellular ATP is involved in the regulation of a variety of biological processes, including neurotransmission, muscle contraction, and liver glucose metabolism, by stimulation of purinergic receptors.[130] Intravenous ATP may offer a novel palliative approach in the treatment of NSCLC. ATP given by daily intraperitoneal injection for 10 days to mice with colon tumors significantly inhibited weight loss.[348] Recently, in the United States, a small open phase II trial in 15 patients with NSCLC (stage IIIB or IV)[187] demonstrated maintenance of weight, QOL, and performance status after treatment with two to four intravenous infusions of ATP (50-65 μg/kg.min) for 96 hours given at 4-week intervals. These results supported earlier findings from a phase I study in 14 cancer patients.[188] On the basis of these promising preliminary reports, we conducted a randomized clinical trial to evaluate the effect of ATP infusion on body weight, muscle strength, and QOL in patients with advanced NSCLC (stage IIIB or IV).

PATIENTS AND METHODS

Patient eligibility, randomization, and study design

From January 1996 through November 1998, 58 patients were entered in the study. Eligible subjects were patients with histologically or cytologically proven NSCLC of

stage IIIB or IV,[295] and a Karnofsky index of 60% or higher.[216] Exclusion criteria were as follows: eligibility for curative treatment, liver failure, renal failure (defined as patients needing limitation of fluid intake), respiratory failure (defined as O_2 dependence), heart failure, angina pectoris, cognitive dysfunction, or psychiatric illness. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. Written informed consent was obtained from all patients prior to the study.

A randomization list was prepared by the Medical Oncology Trial Office of the Erasmus University Medical Center Rotterdam with the use of block randomization in permutation blocks of four. After baseline measurements, patients were stratified according to tumor stage (IIIB versus IV), previous treatment (chemotherapy versus no chemotherapy), and performance status (Karnofsky index >70% versus ≤70%); they were then randomly assigned to receive either supportive care and ATP (ATP group) or supportive care alone (control group). Supportive care, provided by the patients' attending physicians, included analgesics, antibiotics, anticough medication, antiemetics, bisphosphonates, corticosteroids, and palliative radiotherapy for local control of the primary tumor or metastases.

Patients in the ATP-treatment arm were admitted to the Clinical Research Unit of the Erasmus University Medical Center Rotterdam to receive a maximum of 10 ATP courses. To keep hospitalization at a minimum for ethical reasons, we chose not to use the schedule used by Haskell et al.[187,188] with ATP infusions for 96 hours once every 4 weeks; instead, we gave 30 hours infusions at shorter time intervals, i.e., the first seven ATP courses at 2-week intervals, followed by three ATP courses at 4-week intervals. ATP infusions (6.1 mg in 1 ml 0.9% NaCl) were started beginning at a dose of 20 μg/kg.min, and were increased by increments of 10 μg/kg.min every 30 minutes until a maximum dose of 75 μg/kg.min was reached or until the maximally tolerated dose, if this was lower, had been reached. Thereafter, ATP was infused at a continuous rate. If any side effects occurred, the dose was reduced to the last lower dose or further reduced until side effects disappeared. Follow-up was continued until 28 weeks, i.e., 4 weeks after the last ATP course that was given at 24 weeks. Patients in the control arm were followed up at the outpatient department of the Erasmus University Medical Center Rotterdam for 28 weeks. In both groups, anthropometry, blood serum albumin levels, muscle strength, and QOL were assessed

before treatment randomization and after randomization at 4-, 8-, 12-, 16-, 20-, 24- and 28-week periods.

Anthropometry and muscle strength

Body height was determined to the nearest centimeter. Body weight was measured with an electronic scale (Seca Ltd, Birmingham, UK) to the nearest 0.1 kg.

Strengths of two muscle groups (i.e., elbow flexor muscles and knee extensor muscles) were assessed by use of a hand-held Microfet2^{R} dynamometer (Biometrics Europe, Amersfoort, The Netherlands). This technique has been validated in several patient groups, mostly in those patients who were limited in muscle strength.[41,234] The patient while sitting exerted a maximal force with the 90° flexed right elbow, while the examiner pushed with the dynamometer against the patient's thumb pad until the muscle strength was overcome (break test). Similarly, the patient exerted a maximal force with the 90° flexed right knee, while the examiner pushed with the dynamometer against the patient's ankle until the muscle strength was overcome. The strength of both muscle groups was measured three times at an interval of approximately 10 seconds. Muscle strength was expressed in Newtonmeter (Nm) units and was calculated by dividing the measured mean strength (dynamometer reading) by the distance from dynamometer position point to the humeral epicondylus medialis (elbow) and the femural epicondylus medialis (knee). The same observer (H.J. Agteresch) carried out all measurements.

Quality of life (QOL)

QOL was assessed with the use of The Rotterdam Symptom Checklist (RSCL), a 39-item self-report questionnaire.[110] The reliability and validity of the RSCL in cancer patients have been confirmed in both cross-sectional[199] and longitudinal[109,349] studies in patients with a wide range of cancers. This questionnaire, which assesses symptoms during the preceding week, was filled out by the patients. The RSCL measures scores on four domains: 1) a physical score (23 items), 2) a psychological score (seven items), 3) a functional score (eight items), and 4) an overall score (one item). Each item is rated on a 4-point scale. Results per item were transformed into standardized scores, ranging from 0 to 100 with the use of the following formula: 100 - [100 * (item scale score - minimum item score) / (maximum score - minimum score)]. A lower score

represents a greater level of distress.[110]

Statistical analysis

Differences over time between body weight, serum albumin levels, muscle strength, and QOL scores in the two groups were analyzed according to the "intention-to-treat" principle by repeated-measures analysis of covariance with the use of the linear regression model. To account for the within-patient correlation in the measurements of the dependent variable and, simultaneously, for possible non-normality of the dependent variable, the Generalized Estimating Equations[121] approach was followed. These analyses were performed with the SAS procedure Proc Mixed (version 6.12-Windows; SAS Inc., Cary, NC, USA), with the use of the independence working correlation structure. Independent variables in the model were the treatment indicator variable, baseline measurement, measurement time, and interaction between time and treatment. This model assumes a linear relation between measurement and time in both treatment groups, which was checked by adding quadratic time terms. Statistical significance of the treatment effect was assessed by testing the null-hypothesis that the coefficients of the treatment indicator and its interaction with time are simultaneously equal to zero. Survival was compared between groups by means of the log-rank test with the use of SPSS (version 6.1.3-Windows; SPSS Inc., Chicago, USA). Two-sided *P*-values less than 0.05 were considered statistically significant.

RESULTS

Data on patients

Twenty-eight patients were allocated to ATP treatment, and 30 were assigned to no ATP. The trial design is summarized in **Figure 3.1.1**. Age, stage, performance status, previous treatment, and outcome parameters were similar at baseline between the ATP and control arms. Assessable patients in the ATP group weighed more and had lost more weight than patients in the control group. In both groups, the majority were male (**Table 3.1.1**). However, when the statistical analyses for all outcome parameters were adjusted for age, sex, body weight, weight loss, and histologic type, this did not alter the results in both groups.

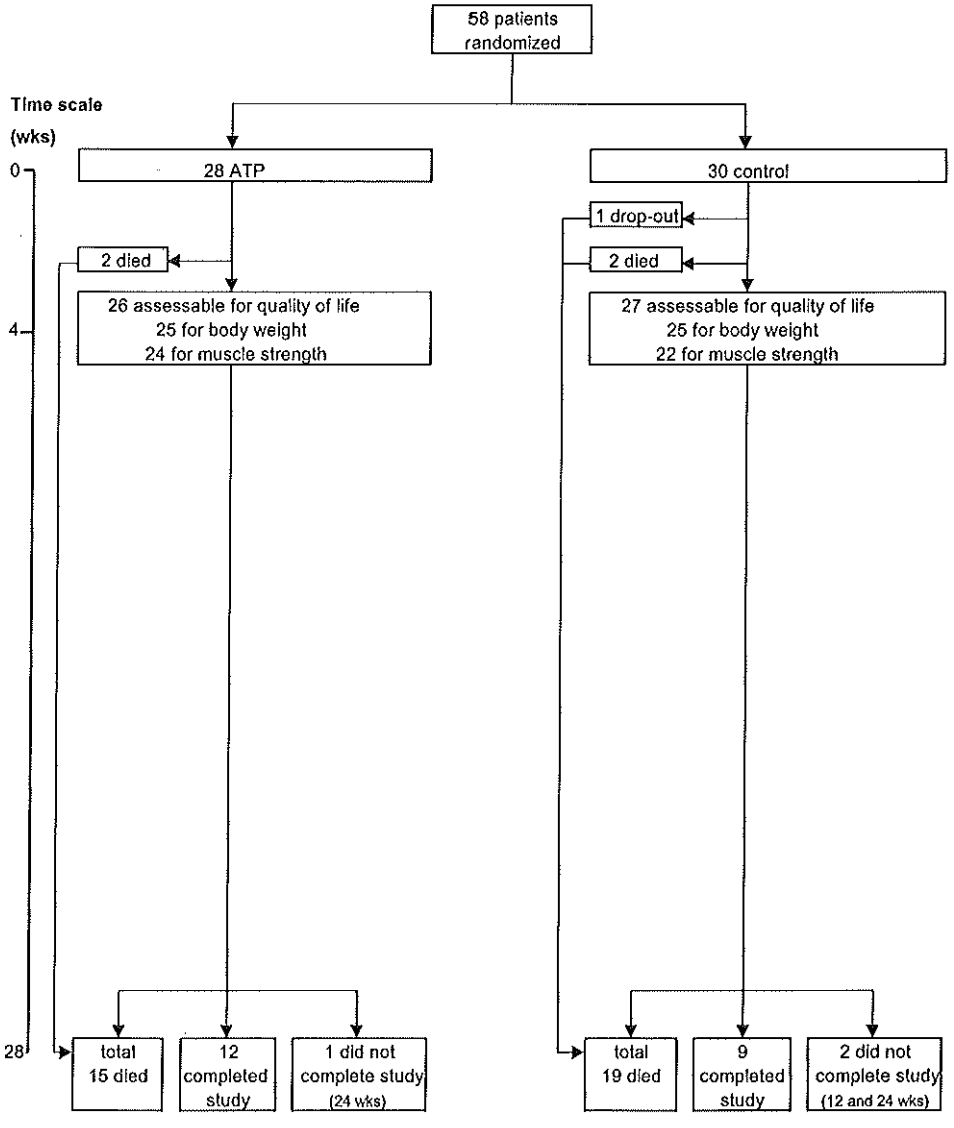


Figure 3.1.1. Flow diagram for the randomized trial of adenosine 5'-triphosphate (ATP) in the treatment of advanced non-small-cell lung cancer. Body weight was not assessable because of hospitalization of one patient in the ATP group and two patients in the control group; muscle strength was not assessable because of hospitalization of two patients in the ATP group and three patients in the control group, and two additional patients in the control group were not assessable because of a technical defect in the dynamometer before treatment randomization.

Table 3.1.1. Baseline patient characteristics of the randomly assigned and assessable patients in the adenosine 5'-triphosphate (ATP) trial

	ATP*		Control*	
	Randomly assigned (n = 28)	Assessable† (n = 26)‡	Randomly assigned (n = 30)	Assessable† (n = 27)‡
Sex				
male	20 (71%)	19 (73%)	18 (60%)	16 (59%)
female	8 (29%)	7 (27%)	12 (40%)	11 (41%)
Age (y)	64 ± 13	64 ± 13	61 ± 10	62 ± 11
Tumor histology				
adenocarcinoma	11 (39%)	11 (42%)	6 (20%)	6 (22%)
squamous cell carcinoma	10 (36%)	10 (38%)	11(37%)	10 (37%)
undifferentiated large-cell carcinoma	4 (14%)	4 (15%)	9 (30%)	7 (26%)
unspecified	3 (11%)	1 (4%)	4 (13%)	4 (15%)
Chemotherapy as pretreatment				
yes	12 (43%)	11 (42%)	14 (47%)	13 (48%)
no	16 (57%)	15 (58%)	16 (53%)	14 (52%)
Stage§				
IIIB	13 (46%)	11 (42%)	14 (47%)	13 (48%)
IV	15 (54%)	15 (58%)	16 (53%)	14 (52%)
Karnofsky score (%)				
≤ 70	12 (43%)	11 (42%)	14 (47%)	13 (48%)
> 70	16 (57%)	15 (58%)	16 (53%)	14 (52%)
Prior weight loss (kg)‖	5.8 ± 6.5	6.2 ± 6.8	4.9 ± 6.7	4.1 ± 6.8
Prior weight loss (%)‖	6.7 ± 7.1	7.1 ± 7.3	6.8 ± 9.7	5.6 ± 9.6
Weight (kg)	75.0 ± 16.4	75.1 ± 17.1	68.2 ± 12.3	69.2 ± 12.1
Body mass index (kg/m^2)	25.3 ± 5.5	25.5 ± 5.9	23.8 ± 4.0	24.3 ± 4.0
Albumin (g/l)	40.7 ± 4.5	40.6 ± 4.6	40.0 ± 4.5	40.5 ± 4.2
Elbow flexor strength (Nm)	533 ± 110	541 ± 114	512 ± 116	527 ± 121
Knee extensor strength (Nm)	828 ± 177	839 ± 151	797 ± 219	838 ± 207
Physical QOL score¶	78.5 ± 13.7.	77.9 ± 13.9	77.2 ± 12.2	78.1 ± 12.3
Psychologic QOL score¶	69.0 ± 23.0	68.8 ± 23.8	70.3 ± 20.2	71.4 ± 19.5
Functional QOL score¶	80.1 ± 19.4	79.9 ± 19.6	77.7 ± 21.6	79.3 ± 22.2
Overall QOL score¶	62.3 ± 21.5	60.7 ± 24.5	57.2 ± 18.4	59.3 ± 18.1

* Scores were expressed as mean ± standard deviation or number (%)

† At least one follow-up analysis was performed

‡ Characteristics for weight and muscle strength, n = 25 and 24, respectively, in the ATP group, and n = 25 and 22, respectively, in the control group

§ See reference 295

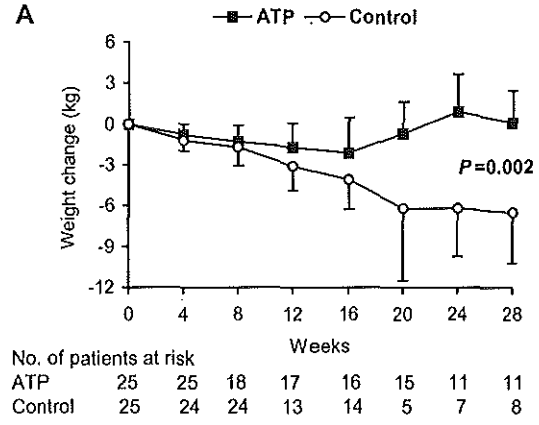
‖ Weight loss in relation to weight before illness

¶ Quality of life (QOL) scores ranging from 0 to 100; a lower score represents a greater degree of symptoms

Twenty-eight patients in the ATP group received a total of 176 ATP courses. Eleven patients received one to three ATP courses, five received four to six courses, and 12 received seven to 10 courses. Fifty-two infusions of ATP were given as low-dose infusions of 25-40 μg/kg.min, 47 as middle-dose infusions of 45-60 μg/kg.min, and 77 as high-dose infusions of 65-75 μg/kg.min. Sixty-seven percent of the ATP courses were without side effects (36% and 73% of first and subsequent courses, respectively). Side effects were monitored according to Common Toxicity Criteria (National Cancer Institute) graded on a 4-points scale according to seriousness. During ATP infusions, the most frequently reported side effects (expressed as percent of total number of infusions [n = 176] resulting in the side effects) were of type 1 (chest discomfort, 15%; urge to take a deep breath, 10%; flushing, 5%; nausea. 5%; lightheadedness, 3%; headache, 2%; sweating, 2%; mood alteration-anxiety, 2%; and palpitations, 1%). Few side effects were of type 2 (injection side reaction, 3%), and none were of type 3. Although mild, one side effect was of type 4 (dyspnea, 3%). In patients with chest discomfort during ATP infusion, electrocardiography (ECG) was performed. In no case were ECG changes suggestive of myocardial ischemia detected. The side effects always resolved within minutes of lowering the ATP dose.

Body weight

In control patients, the mean weight loss over the 28-week study period (expressed as change per 4 weeks) was -1.0 kg (95% CI = -1.5 to -0.5) per 4 weeks, whereas ATP-treated patients experienced no weight loss (0.2 kg; 95% CI = -0.2 to +0.6; *P* for between-group difference = 0.002; **Figure 3.1.2, A**). For further analysis, the participants were stratified according to the presence of cachexia prior to the study (≥5% versus <5% weight loss in relation to weight before the illness; 16 patients in the ATP group and 13 patients in the control group versus 9 patients in the ATP group and 12 patients in the control group). In cachectic patients, progressive weight loss of -1.6 kg (95% CI = -2.1 to -1.1) per 4 weeks was observed in the control group, while no additional weight loss occurred in the ATP group (+0.3 kg; 95% CI = -0.5 to +1.1; *P* for between-group difference = 0.0002). In non-cachectic patients statistical significance between the study groups was not reached ($P=0.22$).



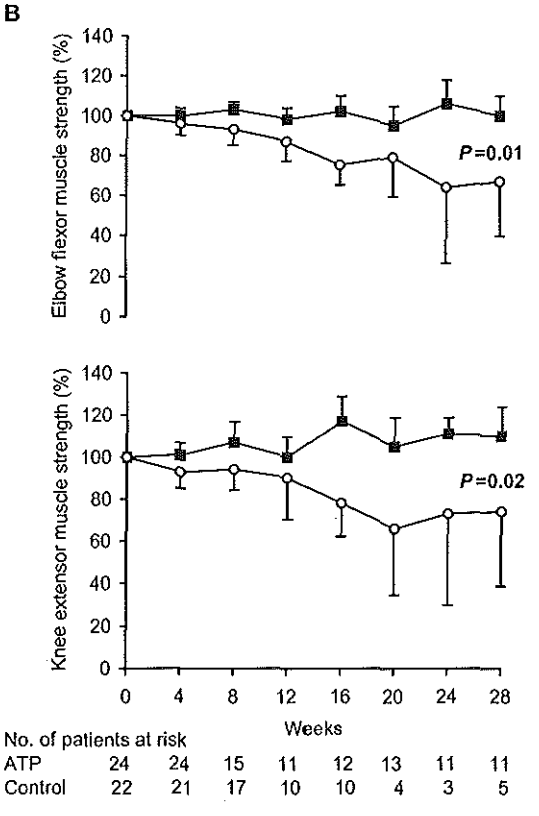


Figure 3.1.2. Changes in A) body weight and B) elbow flexor (**top**) and knee extensor (**bottom**) muscle strength. Graphs represent mean values, and bars represent 95% confidence intervals. Two-sided *P*-values for differences between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined with the use of repeated-measures analysis.

Serum albumin levels

Serum albumin concentration (g/l) declined by -1.2 (95% CI = -2.0 to -0.4) per 4 weeks in the control group, whereas no change was detected in the ATP group (0.0 g/l; 95% CI = –0.3 to +0.3; *P* for between-group difference = 0.006; **Figure 3.1.3**). The effect of ATP on albumin was highly significant in patients who were already cachectic at the beginning of the study (*P*=0.0001) but not in non-cachectic patients (*P*=0.37).

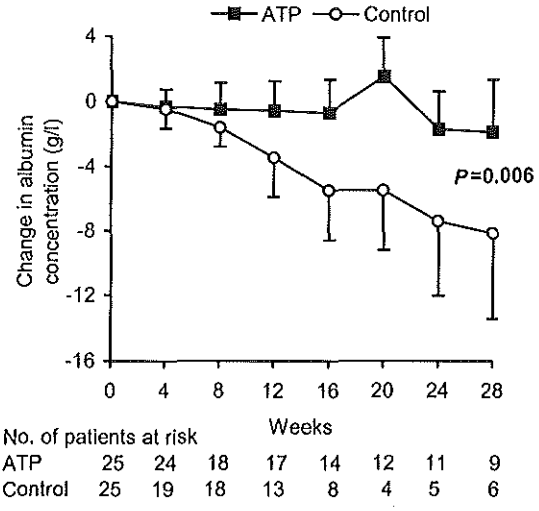


Figure 3.1.3. Changes in serum albumin concentration (mean ± 95% confidence interval). Two-sided *P*-values for difference between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined with the use of repeated-measures analysis.

Muscle strength

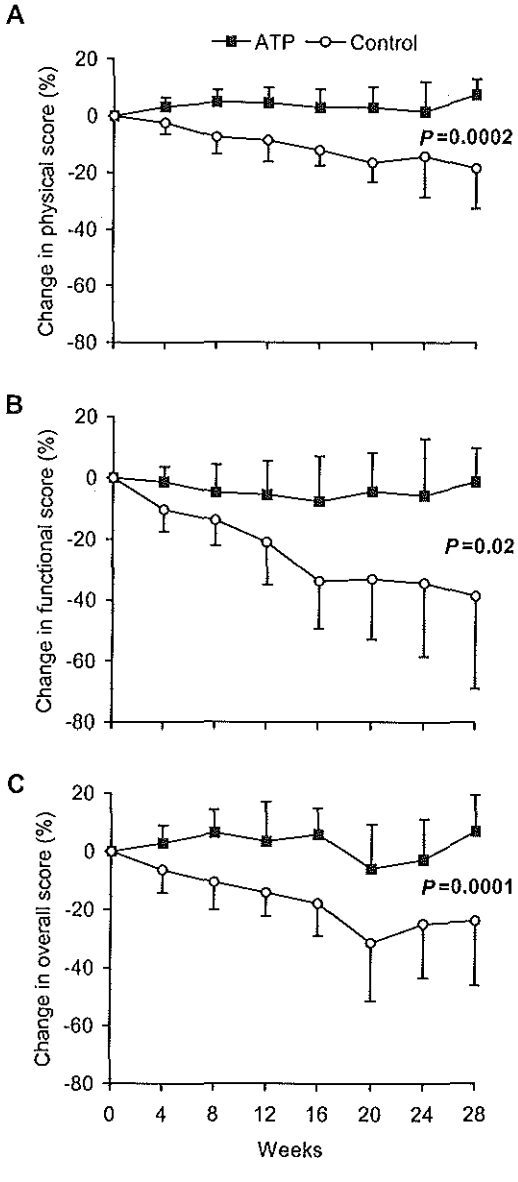
The strength of elbow flexor muscles declined by -5.5% (95% CI = -9.6% to -1.4%) per 4 weeks in the control group, whereas values remained stable in the ATP group (0.0%; 95% CI = -1.4% to +1.4%; *P* for between-group difference = 0.01; **Figure 3.1.2, B, top**). A similar pattern was observed for knee extensor muscles. Muscle strength in control patients decreased by -4.5% (95% CI = -9.7% to -0.7%) per 4 weeks, whereas muscle strength in ATP-treated patients showed no significant change

with time (+1.4%; 95% CI = -0.2% to +3.1%; P=0.02; **Figure 3.1.2, B, bottom**). The effect of ATP on the strength of elbow flexor and knee extensor muscles was highly significant in patients who were already cachectic at the beginning of the study (both P=0.0001) but not in noncachectic patients (P=0.38 and P=0.44, respectively).

Quality of life

During follow-up, patients in the control group showed an impairment in their physical score of -2.4% (95% CI = -4.1% to -0.6%) per 4 weeks, whereas patients in the ATP group showed no change (-0.2%; 95% CI = -1.1% to +0.7%; P for between-group difference = 0.0002, **Figure 3.1.4, A**). The functional score deteriorated by -5.5% (95% CI = -9.6% to -1.4%) per 4 weeks in patients in the control group, whereas patients in the ATP group showed no significant change (+0.4%; 95% CI = -1.8% to +2.6%; P=0.02, **Figure 3.1.4, B**). The psychological score deteriorated by -2.4% (95% CI = -5.9% to +1.1%) per 4 weeks in patients in the control group but showed no significant change in the ATP group (-0.7%; 95% CI = -2.0% to +0.6%; P=0.11). With regard to the overall score of QOL, patients in the control group showed a deterioration in QOL by -3.5% (95% CI = -6.5% to -0.5%) per 4 weeks, whereas patients in the ATP group showed no change (+0.1%; 95% CI = -1.8% to +2.0%; P=0.0001, **Figure 3.1.4, C**). The positive effects of ATP on QOL were similar in both cachectic and noncachectic patients.

Comparison of single items contributing to significant differences in physical scores between the two groups showed beneficial effects of ATP on tiredness (P=0.0001), lack of energy (P=0.001), appetite (P=0.0004), shortness of breath (P=0.001), sore muscles (P=0.0007), shivering (P=0.006), constipation (P=0.003), and difficulties in concentrating (P=0.0005). Items contributing to the significant difference in functional deterioration between the ATP and the control groups included self-care (P=0.009), doing light housework (P=0.031), doing heavy housework (P=0.002), climbing stairs (P=0.016), and walking outdoors (P=0.011). Of the items contributing to the psychological scores, only the score for irritability (P=0.047) was significantly different between the two groups.



No. of patients at risk

ATP	26	25	18	19	17	14	12	12
Control	27	26	22	13	14	9	8	7

Figure 3.1.4. Changes in The Rotterdam Symptom Checklist quality of life (QOL) scores (mean ± 95% confidence interval). A) Physical score, B) functional score, and C) overall score. Two-sided *P*-values for difference between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined with the use of repeated-measures analysis.

Concomitant supportive treatment

During the 28-week follow-up period, none of the patients underwent surgery. One patient in the control group was treated with chemotherapy, which consisted of five courses of a combination of cisplatin and etoposide given at 3-week intervals, followed by radiotherapy involving all lesions. Palliative radiotherapy was given to seven patients in the control group and to six patients in the ATP group. Thirteen patients in the control group and nine patients in the ATP group used corticosteroids to treat symptoms of brain edema (four patients in the ATP group and one patient in the control group), dyspnea (four patients in the ATP group and 12 patients in the control group), and nausea (one patient in the ATP group and no patients in the control group).

Survival

Twenty-eight weeks after randomization, the median survival time was 5.6 months (95% CI = 1.1 - 10.1 months) for patients in the ATP group and 4.7 months (95% CI = 2.5 - 6.8 months) for patients in the control group (*P* for between-group difference = 0.51).

DISCUSSION

Traditional outcome variables in clinical oncology have been survival and tumor response. Although evaluation of functional status, the QOL, and the body weight of cancer patients is increasingly perceived as a major aspect of cancer treatment,[292,349] the number of studies investigating these parameters remains limited.

The aim of our study was to investigate the effect of ATP on body weight and QOL in patients with advanced-stage NSCLC as well as its effect on arm and leg muscle strength as functional and patient-relevant parameters. Since the trial was performed in patients with advanced lung cancer, hand-held dynamometry was chosen because this technique is noninvasive. Clearly, the ideal method for assessing treatment effects regarding QOL would have been a double-blind, placebo-controlled study. However, blinding was not feasible because of the complexity of ATP administration. Some side effects are relatively specific to ATP so that both patients and investigators would have

easily recognized that ATP was being given. In addition, there were ethical reasons for not giving 10 placebo infusions to patients with advanced lung cancer with a short life expectation (three to six months). Despite these limitations, the remarkable consistent positive effects of ATP on QOL and on objective parameters, such as body weight and serum albumin levels, seem to underline the validity of our results.

During treatment and follow-up, a significant difference in mean weight loss was observed between patients in the ATP group and those in the control group. Patients in the control group lost approximately 1 kg per 4 weeks or 6.5 kg over a 6-month period, whereas patients in the ATP group had an average stable weight during the 6-month period. In cancer patients, loss of body weight is mainly due to loss of fat and of skeletal muscle mass.[194] In our study, control patients lost approximately one third of their muscle strength in both arms and legs over a 6-month period, whereas ATP-treated patients lost no muscle strength. The significant difference between patients in the ATP group and those in the control group in concentration of the nutritional parameter albumin provides a biochemical confirmation of the beneficial effects of ATP on the nutritional and functional status of patients with advanced lung cancer. It is noteworthy that the effect of ATP on weight loss, muscle strength, and serum albumin concentration was especially marked in patients who were already cachectic at the start of the study, whereas no statistical significance was reached in noncachectic patients.

Furthermore, a statistically significant difference in overall scores on the QOL instrument evolved between patients in the ATP group and those in the control group. In the control group, the deterioration expected in patients with progressive cancer was seen, whereas this deterioration was not observed in the ATP group. The QOL of the patients in the control group deteriorated significantly both at the physical (2% per 4 weeks) and functional (5% per 4 weeks) levels, whereas these domains remained practically unchanged in patients in the ATP group. The physical and functional scores were significantly different between patients in the ATP group and those in the control group, whereas the psychological scores were not different. Furthermore, patients in the ATP group showed a significantly better general activity level, including doing housework, climbing stairs, and walking outdoors, which supports the validity of the beneficial effects of ATP on muscle strength. Items contributing to the better physical scores in patients in the ATP group included lung cancer-related (e.g. shortness of breath) as well as general symptoms. It is

noteworthy that the items include two of the most common cancer-related symptoms: tiredness and lack of energy.[11] Scores for chest pain and coughing did not significantly differ between the two groups (P=0.05 and P=0.21, respectively).

In both groups of patients, palliative radiotherapy treatment was given to approximately the same number of patients, whereas more patients in the control group than in the ATP group used corticosteroids. None of the patients used corticosteroids as appetite-stimulating drugs or other appetite stimulators, such as cyproheptadine and megestrol acetate. One ATP-treated patient used marihuana tea for nausea; however, in a recent placebo-controlled study in patients with acquired immunodeficiency disease syndrome,[20] no significant effect of dronabinol (the active ingredient of marihuana) on body weight was shown. This finding indicates that differences between the patients in the ATP group and those in the control group were not due to confounding effects of concomitant supportive treatment.

ATP was administered as a constant intravenous infusion without side effects in the majority of courses; if side effects occurred, they disappeared rapidly when the infusion rate was lowered. The reported side effects were mostly of type 1 and sometimes of type 2. Although during the courses some patients showed dyspnea (type 4), this side effect was mild in nature. Since, in addition, no side effects were observed between the ATP infusions, ATP treatment appears to be a nontoxic therapy in patients with advanced lung cancer.

The mechanisms contributing to the effects of ATP on body weight, muscle strength and QOL are not well understood. It is unlikely that the beneficial effects of ATP on body weight and QOL would be caused simply by appetite-stimulating effects, since appetite stimulators, such as cyproheptadine[215] and corticosteroids[336] did not influence the body weight of cancer patients. Rather, our results would suggest that ATP may also influence specific metabolic pathways. In addition to the well-established role of ATP in cellular metabolism, extracellular ATP appears to be involved in the regulation of a variety of biological processes, including neurotransmission, muscle contraction, cardiac function, vasodilatation, and liver glycogen metabolism.[130] ATP can be released from the cytoplasm of several cell types and interacts with purinergic P1 and, particularly, P2 receptors on the surface of many cells.[130] Significantly lower ATP levels have been found in liver and in skeletal muscle of tumor-bearing rats.[374] Rapaport and Fontaine[348] showed that

intraperitoneal ATP administration doubled hepatic ATP pools and inhibited weight loss in tumor-bearing mice. These authors suggested that ATP may inhibit Cori cycle activity (i.e., conversion of glucose to lactate in peripheral tissues followed by gluconeogenesis from lactate followed in the liver). Studies in isolated hepatocytes showed that extracellular ATP induced Ca^{2+} mobilization and influx by stimulation of surface P2 receptors[311] that are involved in the control of gluconeogenesis[13] and glycogenolysis.[218] Furthermore, since albumin levels are affected by both nutrition and acute-phase response, it is possible that the stabilization of serum albumin levels by ATP may be caused by inhibition of the acute-phase response. In animals, ATP has been shown to decrease the production of the proinflammatory cytokines interleukin 1 and interleukin 6.[453] This observation is noteworthy, since increased serum levels of these interleukines[19,291] have been found in patients with advanced cancer and were suggested to play a prominent role in progressive weight loss in cancer.[316] In addition to the effects described above, ATP may have other benefits in oncology. Preclinical studies showed that ATP administration may potentiate the antitumor effects of cytostatics[281] and radiotherapy,[134] and may also have protective effects against radiation tissue damage.[386]

In conclusion, our results demonstrate that ATP infusion has marked beneficial effects on the QOL of patients with advanced NSCLC. In patients who are losing weight, ATP prevents further weight loss and maintains muscle strength of both the upper and the lower extremities. ATP can be administered as a constant intravenous infusion without side effects in the majority of courses; if side effects occur, they are mild and transient. In contrast to earlier pharmacological attempts to reverse cancer cachexia, it appears that ATP might be the first agent with beneficial effects on skeletal muscles of cachectic cancer patients. We conclude that ATP has potential in the palliative management of lung cancer. Further clinical trials using an appropriate placebo control are warranted.

Notes

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3.2

BENEFICIAL EFFECTS OF ADENOSINE TRIPHOSPHATE ON NUTRITIONAL STATUS IN ADVANCED LUNG CANCER PATIENTS: A RANDOMIZED CLINICAL TRIAL

Submitted for publication

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ABSTRACT

Purpose: Adenosine 5'-triphosphate (ATP) has been demonstrated to inhibit loss of body weight and quality of life in non-small-cell lung cancer (NSCLC).[7] In a randomized clinical trial we aimed to investigate the effects of ATP infusion on body composition, energy intake and energy expenditure of NSCLC patients.

Patients and methods: Patients with NSCLC, stage IIIB or IV were randomized to receive either 10 intravenous 30-hour ATP infusions every 2 to 4 weeks, or no ATP. Fat mass (FM), fat-free mass (FFM), and arm muscle area were assessed at 4-week intervals during 28 weeks. Food intake, body cell mass (BCM) and resting energy expenditure (REE) were assessed before randomization, and at 8 and 16 weeks. Between-group differences were tested for statistical significance by repeated-measures analysis.

Results: Fifty-eight patients were randomized (28 ATP, 30 control). No change in body composition over the 28-week follow-up period was found in the ATP group, whereas the control group lost -0.6 kg (95% confidence interval [CI]= -0.9 to -0.3) of FM (between-group difference: P=0.004), -0.5 kg (95% CI = -0.85 to -0.1) of FFM (P=0.02), -1.8% (95% CI = -4.0% to +0.4%) of arm muscle area (P=0.02), and -0.6% (95% CI = -1.0% to -0.2%) of BCM / kg body weight per 4 weeks (P=0.054). Appetite, as assessed by a validated questionnaire, decreased significantly in the control group, but remained stable in the ATP group (P=0.0004). Expressed as change per 4 weeks, control patients showed a significant decrease in energy intake of -568 KJ/day (95% CI = -793 to -343), whereas ATP patients demonstrated a non-significant increase of 110 KJ/day (95% CI = -106 to +326; between-group difference: P=0.0001). No significant differences in REE between the ATP and control group were observed.

Conclusion: The inhibition of weight loss by ATP infusions in patients with advanced NSCLC is due to counteracting the loss of both metabolically active (skeletal muscle and BCM as parts of FFM) and inactive (FM) tissues. These effects are partly ascribed to maintenance of energy intake.

INTRODUCTION

Cancer-associated cachexia is a syndrome of progressive weight loss associated with extensive wasting of energy stores of fat, skeletal muscle and liver tissues;[374] and with elevated lipolysis,[128,391] protein breakdown,[266,306] and gluconeogenesis.[103,267,389] Cachexia in cancer patients is associated with increased morbidity and mortality.[94,116,204,407,440] In clinical practice, dietary counseling,[76,317] use of enteral supplements,[76] and pharmacological approaches[56,75,174,215,259] have failed to reverse cancer-associated cachexia. Treatment with progestagens resulted in attenuation of weight loss or in weight gain, but this was attributed mainly to gain of fat mass.[396]

In uncontrolled phase I and II studies in patients with non-small-cell lung cancer (NSCLC), adenosine 5'-triphosphate (ATP) was shown to inhibit weight loss.[187,188] We have recently confirmed these effects in a randomized clinical trial in patients with advanced NSCLC. ATP infusions induced maintenance of not only body weight, but also muscle strength, quality of life, and serum albumin levels over a 6-month period.[7] In the present paper, effects of ATP on body composition, energy intake, and energy expenditure in the same patients are reported.

PATIENTS AND METHODS

Patients

Patients with histologically or cytologically proven NSCLC, stage IIIB or IV without curative options,[295] and a Karnofsky index of 60% or higher[216] were eligible for the study. Patients with cognitive dysfunction or liver, renal, respiratory, or heart failure, and patients undergoing surgery, concurrent chemotherapy, or radiotherapy involving all lesions were excluded. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. Written informed consent was obtained from all patients prior to the study.

Treatment allocation

Randomization was performed by the Medical Oncology Trial Office of the Erasmus University Medical Center Rotterdam, using block randomization in permutation blocks of four. Following baseline measurements patients were stratified for tumor stage (IIIB versus IV), prior treatment (chemotherapy versus no chemotherapy), and performance status (Karnofsky index >70% versus ≤70%), and were then randomly assigned to receive either supportive care and ATP (ATP group), or supportive care alone (control group).

Treatment schedule

Patients in the ATP-treatment arm received a maximum of 10 ATP courses of 30 hours each: i.e., seven courses at 2-week intervals, followed by three ATP courses at 4-week intervals. ATP infusions (6.1 mg ATP-$Na_2 \cdot 3H_2O$ in 1 ml 0.9% NaCl) were started at a dose of 20 μg/kg.min and increased by steps of 10 μg/kg.min every 30 minutes until a maximum dose of 75 μg/kg.min, or the maximally tolerated dose, had been reached. Thereafter, ATP was infused at a continuous rate. If any side effects occurred, the dose was reduced to the last given dose or further until side effects disappeared. Follow-up was continued until 28 weeks, i.e. four weeks after termination of the last ATP course which was given at 24 weeks. Patients in the control arm were followed up in the outpatient department at 4-week intervals until 28 weeks after randomization. Because of possible confounding effects on metabolism and appetite,[258,435] patients who started corticosteroid treatment during the study were excluded from evaluation of appetite, food intake, body cell mass and energy expenditure from the time point of starting the medication. Since at 28 weeks, 28 out of 30 control patients had died, were hospitalized, or used corticosteroids, statistical analysis for body cell mass, food intake and energy expenditure was only performed before randomization and after randomization at 8- and 16-week periods.

Anthropometry

Body height was determined to the nearest centimeter. Body weight was measured with an electronic scale (Seca Ltd, Birmingham, UK) to the nearest 0.1 kg.

Skinfold thicknesses were measured in triplicate to the nearest 0.2 mm with a Holtain^R skinfold caliper (CMS weighing equipment LTD, London, UK), and the

median used for further calculations. Total body fat mass (FM) was estimated from the sum of median skinfold thicknesses at four sites (triceps, biceps, subscapula, and supra-iliac) using the age- and gender-specific tables from Durnin and Womersley[132] Fat-free mass (FFM) was calculated by subtracting FM from body weight.

Mid-upper arm circumference of the left arm was measured with a flexible measuring tape. Arm muscle area was derived using the equation: (arm circumference - π x triceps skinfold)^2 / 4π.[182]

Throughout the study anthropometric measurements were performed by one trained observer (H.J. Agteresch).

Deuterium oxide and bromide dilution

After an overnight fast of approximately 12 hours, patients received a oral dose of 15 or 20 grams deuterium-labeled water (99.9%; Isotec inc., Miamisburg, USA) at about 9.30 hours a.m. Following D_2O administration, patients had to abstain from eating and drinking until blood sampling completed. Venous blood samples were drawn into heparin tubes from the forearm at baseline and after 2½, 3, and 3½ hours. Blood samples were immediately placed on ice and then centrifuged for 10 min 1300 x *g* at 4° C. Deuterium oxide in plasma was measured by infrared spectrophotometry (Miran 1FF, Foxboro, South Norwalk, USA). Total body water (TBW) volume was calculated by dividing D_2O dose by D_2O concentration in plasma. FFM was calculated using a constant water percentage of 73.[322] FM was calculated by subtracting FFM from total body weight.

Extracellular water (ECW) was estimated by bromide dilution. Following the same protocol as to the deuterium oxide dilution method, patients drank a known dose of sodium bromide solved in water. Bromide concentration in plasma ultrafiltrate was determined with high-performance liquid chromatography according to the anion-exchange chromatographic method.[288]

Body cell mass (BCM) was defined as (TBW - ECW) / 0.73, which is the same as intracellular water / 0.73.[392]

Energy intake

Before randomization, all participants kept a 7-day food record. Three-day food records were repeated at 8 and 16 weeks. Intake of energy, protein, fat and

carbohydrate was calculated using the software program Komeet (version 2.0, VBS Nutrition Software, Arnhem, The Netherlands).

Appetite was evaluated as a part of the Rotterdam Symptom Checklist (RSCL), a 39-item validated self-report quality of life questionnaire.[110] The item 'lack of appetite' was rated on a 4-point scale (not at all=4, a little=3, quite a bit=2, very much=1). The RSCL, which assesses symptoms over the preceding week, was filled out by the patients before randomization, and at 4-week intervals until 28 weeks.

Resting energy expenditure

Before randomization and at 8 and 16 weeks, resting energy expenditure (REE) was assessed by indirect calorimetry using a ventilated hood system (Deltatrac™ MBM-100, Datex Instrumentarium Corp, Helsinki, Finland). After an overnight fast and a resting period of approximately half an hour, CO_2 production and O_2 consumption were measured during a period of 30 minutes between 9 and 11 a.m. lying down at complete rest. REE was calculated by using the abbreviated Weir formula.[459] Measured REE was compared to predicted REE using the Harris-Benedict equations[186] which are age-, height-, weight-, and gender-specific. The equipment was calibrated at the start of each experiment.

Statistical analysis

The differences over time between FM, FFM, arm muscle area, BCM, appetite, energy intake, and energy expenditure in the two groups were analyzed by repeated-measures analysis of covariance using the linear regression model. To account for the within-patient correlation in the measurements of the dependent variable, and simultaneously for possible non-normality of the dependent variable, the Generalized Estimating Equations[121] approach was followed. These analyses were performed with the SAS procedure Proc Mixed (version 6.12-Windows; SAS Inc., Cary, NC, USA), using the independence working correlation structure. Independent variables in the model were the treatment indicator variable, baseline measurement, measurement time and interaction between time and treatment. This model assumes a linear relation between measurement and time in both treatment groups, which was checked by adding quadratic time terms. Statistical significance of the treatment effect was assessed by testing the null hypothesis that the coefficients of the treatment indicator

and its interaction with time are simultaneously equal to zero. Two sided *P*-values below 0.05 were considered statistically significant.

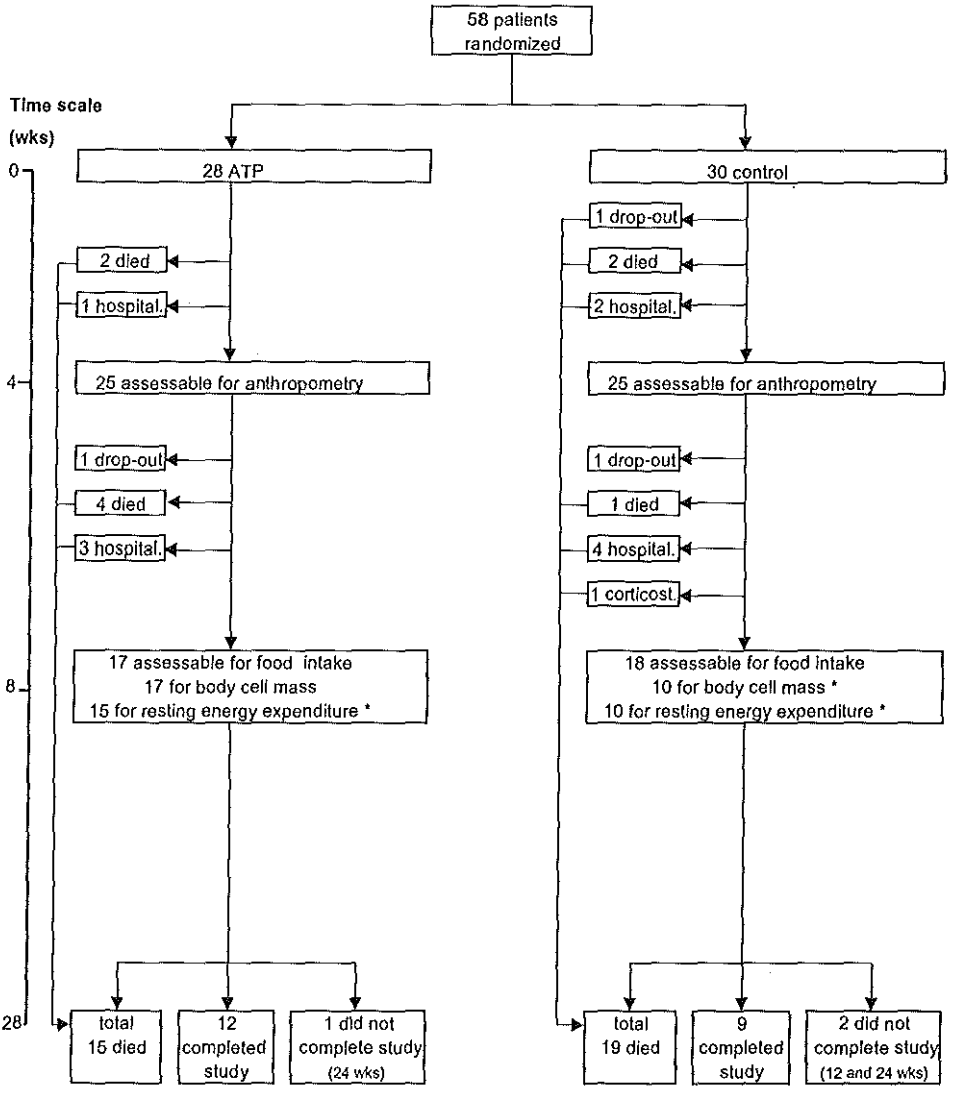

Figure 3.2.1. Flow diagram. * Body cell mass was not assessable because of a technical reason (1 control) or burden (7 control); Resting energy expenditure was not assessable because of technical reason (2 ATP, 1 control) or burden (7 control).

RESULTS

Patient characteristics and treatment

Fifty-eight patients were randomized to the ATP (n=28) or control group (n=30). The trial profile is summarized in **Figure 3.2.1**. General baseline characteristics including age, stage, performance status, and prior treatment were similar in the ATP and control group, both for randomized and assessable patients. In both groups the majority were male (ATP 71%, control 60%). Baseline anthropometric parameters of assessable patients are listed in **Table 3.2.1**. Compared to control patients, assessable patients in the ATP group weighed more, but had a higher degree of weight loss at time of randomization.

Table 3.2.1. Baseline anthropometric parameters of assessable patients

	ATP	Control
Prior weight loss (%)*	7.1 ± 7.3	5.6 ± 9.6
Weight (kg)	75.2 ± 17.0	69.2 ± 12.1
Body mass index (kg/m^2)	25.5 ± 5.9	24.3 ± 4.0
Fat mass (%)	28.0 ± 7.0	29.3 ± 8.4
Fat-free mass (%)	72.0 ± 6.7	70.7 ± 8.4
Arm muscle area (cm^2)	70.4 ± 25.7	64.0 ± 15.7
Body cell mass (kg/kg body weight)†	0.37 ± 0.03	0.37 ± 0.04
'Lack of appetite' score‡	3.2 ± 0.8	3.4 ± 0.9

At least one follow-up analysis performed (25 ATP, 25 control)
Scores expressed as mean ± standard deviation
* Weight loss relative to pre-illness weight
† Characteristics for 17 ATP patients and 10 control patients
‡ Four-point scale for presence of 'lack of appetite': 4) not at all, 3) a little, 2) quite a bit, 1) very much

Twenty-eight patients in the ATP group received a total of 176 ATP courses. Eleven patients received one to three ATP courses, five received four to six courses, and 12 received seven to 10 courses. Fifty-two infusions of ATP were given as low-dose infusions of 25-40 μg/kg.min, 47 as middle-dose infusions of 45-60 μg/kg.min, and 77 as high-dose infusions of 65-75 μg/kg.min. As previously reported,[7] 67% of ATP courses were without side effects (36% and 73% of first and subsequent courses, respectively). Side effects occurring in the remaining courses were mainly

cardiopulmonary reactions such as chest discomfort which were mild and transient, resolving within minutes after lowering the ATP dose.

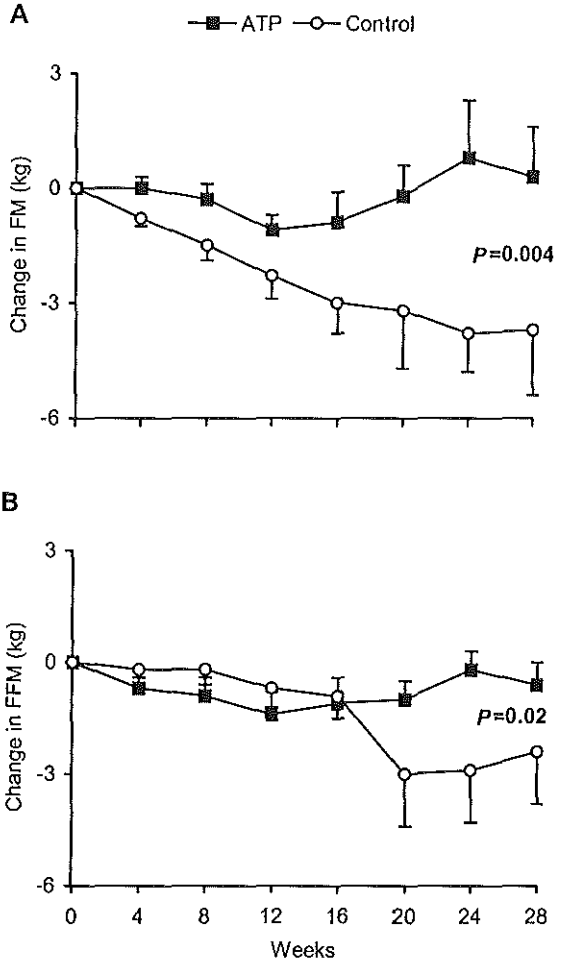

Figure 3.2.2. Changes in A) fat mass (FM), and B) fat-free mass (FFM). Graphs represent mean values, and bars represent standard error of the mean (SEM). *P*-values shown are differences between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group as determined by repeated-measures analysis.

Body composition

As previously reported,[7] weight loss over the 28-week study period, expressed as change per 4 weeks, was -1.0 kg (95% CI = -1.5 to -0.5) in control patients (P=0.0001), whereas in ATP treated patients no weight loss (0.2 kg; 95% CI = -0.2 to +0.6; between-group difference: P=0.002) was observed. Changes in FM and FFM for both study groups during the 28-week follow-up period are plotted in **Figure 3.2.2.** In the control group a significant loss of -0.6 kg (95% CI -0.9 to -0.3) FM per 4 weeks was observed (P=0.0002) which was not seen in the ATP group (+0.1 kg; 95% CI = -0.3 to +0.5; between-group difference: P=0.004). FFM of patients in the control group remained relatively stable for the first 16 weeks, but then showed a marked drop; during the overall 28-week study period FFM decreased by -0.5 kg (95% CI = -0.9 to -0.1) per 4 weeks (P=0.03), whereas no significant change was observed in ATP-treated patients (+0.1 kg; 95% CI = 0.0 to 0.2; between-group difference: P=0.02).

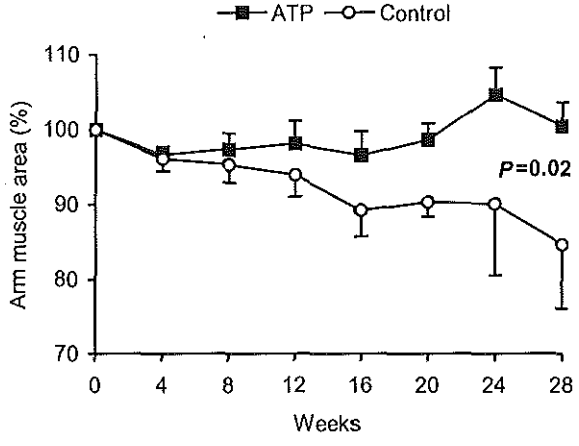

Figure 3.2.3. Changes in arm muscle area expressed as percentage of baseline values. Graphs represent mean values, and bars represent SEM. Two-sided P-values for differences between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined by repeated-measures analysis.

Mid-upper arm muscle area (**Figure 3.2.3**) showed a non-significant decline of -1.8% (95% CI = -4.0% to + 0.4%) per 4 weeks in the control group, but a significant

increase of 1.1% in the ATP group (95% CI = +0.3% to 1.9%; P=0.02; between-group difference: P=0.02).

Changes of BCM per kg body weight for a subgroup of control patients and ATP-treated patients at 8- and 16-week periods are plotted in **Figure 3.2.4**. As described in the legend of **Figure 3.2.1**, in eight control patients BCM was not assessable because of burden (n=7) and a technical reason (n=1). The control group showed a significant decline in BCM of -0.6% (95% CI = -1.0% to -0.2%) of body weight per 4 weeks (P=0.0004), whereas the ATP group showed no change (-0.1%; 95% CI = -0.5 to +0.3; between-group difference: P=0.054).

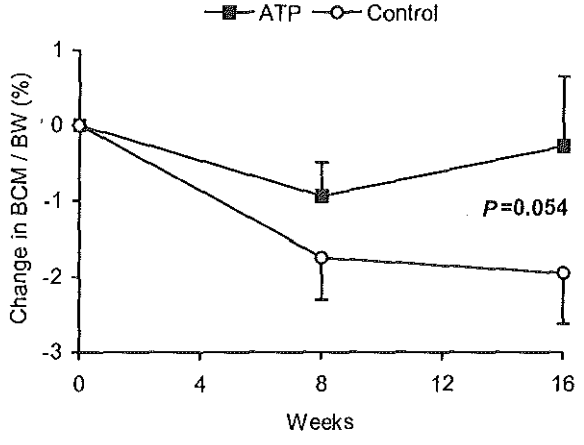

Figure 3.2.4. Changes in body cell mass (BCM) per kg body weight (BW). Graphs represent mean values, and bars represent SEM. Two-sided P-values for differences between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined by repeated-measures analysis.

Energy intake

Intake of energy and selected nutrients is summarized in **Table 3.2.2**. Expressed as change per 4 weeks, the control group showed a highly significant decrease in energy intake of -568 kJ/day (95% CI = -793 to -343; P=0.0001), whereas the ATP group showed a non-significant increase of 110 kJ/day (95% CI = -106 to +326; P=0.32).

The difference in change of energy intake between the two groups was significant (P=0.0001). This difference between the ATP and control groups was the result of significant differences in changes of both protein (P=0.005), carbohydrate (P=0.0002), and fat intake (P=0.002), without changes in the proportions of these nutrients.

Table 3.2.2. Energy intake and energy expenditure at 0, 8, and 16 weeks

	ATP			Control			ATP vs C
	0	8	16	0	8	16	*P*-value*
	n = 17	n = 16	n = 15	n = 17	n = 18	n = 6	
Energy intake (MJ/d)	7.2 ± 2.2	7.9 ± 2.0	7.9 ± 2.2	7.6 ± 1.8	7.0 ± 1.8	5.2 ± 2.1	0.0001
Protein intake (g/d)	62 ± 22	67 ± 23	72 ± 22	72 ± 14	70 ± 21	45 ± 20	0.005
Protein intake (%)	15 ± 18	15 ± 20	16 ± 18	17 ± 14	18 ± 21	16 ± 17	NS
Carbohydrate intake (g/d)	208 ± 73	232 ± 74	213 ± 72	220 ± 57	199 ± 54	147 ± 46	0.0002
Carbohydrate intake (%)	52 ± 61	52 ± 66	48 ± 58	52 ± 58	51 ± 53	51 ± 39	NS
Fat intake (g/d)	63 ± 22	73 ± 23	74 ± 26	70 ± 17	63 ± 18	48 ± 26	0.002
Fat intake (%)	33 ± 39	35 ± 43	36 ± 28	35 ± 15	34 ± 37	35 ± 46	NS
REE (MJ/d)†	6.4 ± 0.9	6.3 ± 0.5	6.4 ± 1.1	6.3 ± 0.6	6.4 ± 0.6	7.0 ± 0.8	NS

Values expressed as mean ± standard deviation
* Based on repeated-measures analysis
† Resting energy expenditure

At baseline, appetite reported by the patients as part of a validated questionnaire was similar in the two groups. During the 28-week study period, reported appetite deteriorated in the control group, whereas the ATP group showed no change (**Figure 3.2.5**; between-group difference P=0.0004).

Energy expenditure

Comparison of total resting energy expenditure (REE) as measured by indirect calorimetry revealed no significant differences between patients in the ATP and the control group at 8 and 16 weeks (P=0.33; **Table 3.2.2**). There was also no difference between the two groups when REE was expressed per kg body weight, per kg FFM, per kg BCM, or as a percentage of the Harris Benedict value.

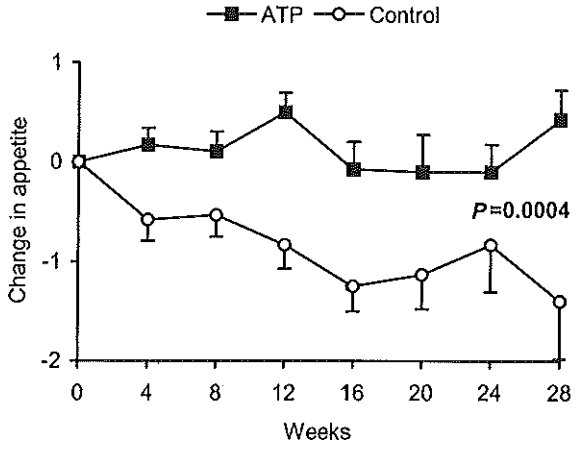

Figure 3.2.5. Changes in the 4-point scaled item 'appetite', as part of the quality of life Rotterdam Symptom Checklist. A lower value shown in this figure implies less appetite. Graphs represent mean values, and bars represent SEM. Two-sided *P*-values for differences between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined by repeated-measures analysis.

DISCUSSION

Weight loss is a frequent phenomenon in cancer patients and particularly in patients with lung cancer.[116] The aim of the present randomized clinical trial was to investigate the effects of ATP on body composition, energy intake, and energy expenditure in patients with NSCLC (stage IIIB or IV).

Weight loss is the result of an imbalance between energy intake and energy expenditure. Fredrix et al.[156] found an elevated REE (>115% of the predicted Harris Benedict value) in two-thirds of newly detected patients with primary lung cancer. Since the elevation was more pronounced in weight-losing patients, these authors suggested that hypermetabolism might contribute to weight loss. However, results of the present study indicate that the inhibitory effects of ATP on weight loss were not caused by a reduction in REE by ATP.

We found that the progressive weight loss in the control group was due to loss in

FM and, to a lesser degree, FFM. These results confirm the findings of Heymsfield et al.[194] who demonstrated that weight loss in cancer patients is primarily caused by loss of FM and muscle mass, whereas total FFM appears to decrease to a smaller extent because of maintenance of visceral organ mass and tumor growth.[194] We found that ATP significantly inhibited the loss in upper arm muscle area, as an indication of muscle mass.[262] This finding is consistent with our recent observation that control patients lost approximately one-third of strength in both elbow flexor and knee extensor muscles over the 28-week study period, whereas muscle strength of ATP-treated patients remained stable.[7]

A key component of weight loss in cancer cachexia is the loss of BCM which is the vital compartment containing the oxygen-exchanging, potassium rich, and metabolically active tissue.[293] Simons et al.[397] demonstrated a correlation between BCM and Karnofsky performance status in weight-losing lung cancer patients, and between decreased BCM and increased systemic inflammatory state. It is noteworthy that ATP treatment appears to counteract BCM wasting in advanced lung cancer patients.

The beneficial effects of ATP not only on FM but also on muscle and BCM as parts of the FFM are noteworthy in view of the lack of positive results from other randomized clinical studies using pharmacological approaches to treat cachectic cancer patients. Corticosteroids[56,114,336] and the anti-seritonergic drug cyproheptadine[215] induced an increase in appetite, however without positive effects on body weight. The phosphoenolpyruvate-carboxykinase inhibitor hydrazine sulfate had no effect on either appetite or body weight.[259] Anabolic steroids also failed to influence weight in NSCLC patients.[75] Administration of medroxyprogesterone acetate or megestrol acetate induced an increase in appetite,[55,137] attenuation of weight loss,[137] and weight gain.[396] However, as assessed by dual-energy X-ray absorptiometry[261] and deuterium oxide dilution,[396] this was attributed to gain in FM, and not in FFM.

The mechanisms underlying the positive effects of ATP on nutritional status are not clear. The scores on the item 'lack of appetite' which was part of the quality of life questionnaire revealed a significant difference in appetite appearing over time between the ATP and control group. This subjective finding was confirmed by food records kept by the patients which showed a significant decline in energy intake in the control group but not in the ATP group. This would suggest that ATP may have appetite-regulatory effects contributing to increased food intake. However, approximate calculations of

differences between the ATP and control group with regard to energy intake (≈10.000 kJ over 4 weeks) and energy expenditure from both fat and fat-free body mass wasting (≈22.000 kJ over 4 weeks) indicate that differences in food intake cannot entirely account for the observed differences in body composition between the two groups. Moreover, the appetite stimulator megestrol acetate was shown only to increase FM but not FFM.[396] It is therefore unlikely that the inhibitory effects of ATP on loss of BCM and skeletal muscles would be solely caused by appetite stimulating effects of ATP. This would suggest that ATP may also influence specific metabolic pathways involved in weight loss.

In experimental cancer models, depletion of ATP levels in liver[101,374,434] and skeletal muscle[374] were reported to be significantly reduced. Recently, reduced liver ATP levels were also demonstrated in advanced lung cancer patients,[104] particularly in weight-losing patients.[246] Intraperitoneal ATP administration doubled hepatic ATP pools and inhibited weight loss in tumour-bearing mice.[348] It is noteworthy that ATP infusion leads to a significant rise in liver ATP pools also in weight-losing lung cancer patients.[245] Rapaport et al.[345] suggested that ATP may inhibit Cori cycle activity (i.e., conversion of glucose to lactate in peripheral tissues followed by gluconeogenesis from lactate in the liver). Studies in isolated hepatocytes showed that extracellular ATP evoked Ca^{2+}-mobilization and influx by stimulation of surface purinergic P2 receptors[311] which are involved in the control of gluconeogenesis[13] and glycogenolysis.[218] It is speculated that these ATP-induced effects might attenuate energy-consuming catabolic processes like gluconeogenesis, which could contribute to maintenance of body fat and cell mass in cancer patients.

In conclusion, our study demonstrates that inhibitory effects of ATP on weight loss are due to counteracting the loss of both metabolically active (skeletal muscle and BCM as parts of FFM) and inactive (FM) tissues in cachectic patients with advanced NSCLC. The beneficial effects of ATP on body composition are associated with maintenance of energy intake but not with reduced REE. Further studies are warranted to elucidate the mechanisms underlying the observed clinical effects of ATP in metabolically active tissues.

Acknowledgment

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Stijnen PhD (Department of Epidemiology & Biostatistics, Erasmus University Medical Center Rotterdam, The Netherlands) for advice in statistical analyses.

3.3

RANDOMIZED TRIAL OF ADENOSINE TRIPHOSPHATE ON TUMOR GROWTH AND SURVIVAL IN ADVANCED LUNG CANCER PATIENTS

Submitted for publication

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ABSTRACT

Purpose: To evaluate the effect of adenosine 5'-triphosphate (ATP) on tumor response and overall survival in a randomized clinical trial in patients with non-small-cell lung cancer (NSCLC).

Patients and methods: Fifty-eight NSCLC patients (stage IIIB or IV) were randomly assigned to receive either 10 intravenous 30-hour ATP infusions every two to four weeks over a 24-week period (n=28), or no ATP (control patients, n=30).

Results: ATP was given for a median of 6.5 infusions. Forty-nine patients were evaluable for tumor response. None of the evaluable patients showed a complete or partial response. Median time to progression was 3.9 months (95% confidence interval [CI] = 2.3 to 5.5) in the ATP group compared to 3.0 months (95% CI = 2.4 to 3.7) in the control group (P=0.71). Median survival was 5.6 months (95% CI = 1.1 to 10.1) for the ATP group, and 4.7 months (95% CI = 2.6 to 6.8) for the control group (P=0.68). ATP treatment was associated with a significant increase in survival in weight-losing patients with stage IIIB NSCLC: median survival was 9.3 months (95% CI = 2.1 to 16.5) for ATP-treated patients versus 3.5 months (95% CI = 2.3 to 4.7) for control patients (between-group difference: P=0.02).

Conclusion: ATP as a single therapy does not lead to tumor regression or increased survival in patients with advanced lung cancer but may prolong survival in weight-losing patients with stage IIIB lung cancer.

INTRODUCTION

The prognosis of patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC) remains poor because most tumors are refractory to chemotherapy. The high morbidity and poor overall survival is not only due to progressive tumor growth but is also aggravated by the high frequency of weight loss in this patient population.[116,440] Adenosine 5'-triphosphate (ATP) may offer a novel approach in the treatment of lung cancer patients. Administration of ATP has been shown to inhibit growth of various human tumor cell lines[36,92,135,224,343,405] and of experimental tumor models *in vivo*.[134,61,239,301,309,327,344,345,347,348] In addition,

intraperitoneal ATP administration inhibited weight loss and significantly prolonged survival in these animals.[134,239,309] In an uncontrolled phase II study, 15 untreated patients with advanced NSCLC (stage IIIB or IV) were treated with one to seven intravenous ATP courses (50-65 μg/kg.min) for 96 hours, administered at 4-week intervals. Although no complete or partial tumor response was seen after the ATP courses, ten patients showed stable disease. In addition, mean body weight and quality of life were maintained at a stable level in the total group.[187] Recently, in a randomized clinical trial,[7] we confirmed that ATP infusions contribute to maintenance of body weight, muscle strength, serum albumin levels, and quality of life in patients with advanced NSCLC during a 6-month period. In the present paper we report on the effect of ATP on tumor growth and survival in the same group of patients.

PATIENTS AND METHODS

The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. Written informed consent was obtained from all patients prior to the study.

Patient selection

Patients were eligible if they had stage IIIB or IV,[295] histologically or cytologically proven NSCLC, and a Karnofsky index of 60% or higher.[216] Exclusion criteria were: eligibility for curative treatment; liver failure; renal failure defined as the need for limited fluid intake; respiratory failure defined as O_2 dependence; heart failure; angina pectoris; cognitive dysfunction, and psychiatric illness.

Randomization and treatment schedule

A randomization list was prepared by the Medical Oncology Trial Office of the Erasmus University Medical Center Rotterdam using block randomization in permutation blocks of four. After baseline measurements, patients were stratified for tumor stage (IIIB versus IV), prior treatment (chemotherapy versus no chemotherapy) and performance status (Karnofsky score $>70\%$ versus $\leq 70\%$); and then randomly

assigned to receive either supportive care and ATP (ATP group), or supportive care alone (control group). Supportive care, provided by the patients' attending physician, included analgesics, antibiotics, anticough medication, antiemetics, bisphosphonates, corticosteroids, and intercurrent palliative radiotherapy for local control of the primary tumor or metastases.

Patients in the ATP-treatment arm were admitted to the Clinical Research Unit of the Erasmus University Medical Center Rotterdam to receive a maximum of 10 ATP courses of 30 hours each: seven courses at 2-week intervals, followed by three ATP courses at 4-week intervals. ATP infusions (6.1 mg ATP-Na_2·$3H_2O$ in 1 ml 0.9% NaCl) were started beginning at a dose of 20 μg/kg.min and were increased by increments of 10 μg/kg.min every 30 minutes until a maximum dose of 75 μg/kg.min, or the maximally tolerated dose if lower, had been reached. Thereafter, ATP was infused at a continuous rate. If any side effects occurred, the dose was reduced to the last given dose or further until side effects disappeared.

In both study arms the minimum evaluation of tumor response for all patients included history and physical examination, chest X-ray, and biochemistry. Patients were evaluated for response at 4-week intervals.

Tumor response

Tumor response was evaluated by standard WHO criteria (WHO, 1979). Partial response required >50% reduction of the product of the perpendicular diameters of all measurable lesions. Stable disease was defined as <50% reduction and <25% increase in measurable or evaluable lesions, whereas progressive disease was defined as >25% increase in the size of tumor lesions or the appearance a new lesion.

Statistics

Differences in time to progression and survival between patients in the ATP group and patients in the control group were tested by means of the log-rank test. Survival curves were fitted according to Kaplan-Meier. Survival analysis was based on the entire study population according to the intention-to-treat principle. Because of demonstrated inhibitory effects of ATP on weight loss,[7] the effect of ATP on survival was also assessed separately in patients who were losing weight prior to the ATP. Cox proportional hazards model was used for survival analysis. Since ATP-treated

patients had higher baseline body weight (Tables 3.1.1 and 3.3.2), baseline body weight was included as a covariate in all survival analyses. Results were expressed as median months (95% confidence interval [CI]). *P*-values below 0.05 were considered statistically significant.

RESULTS

Patients

Fifty-eight patients (38 men and 20 women) entered in the trial from January 1996 through November 1998. Twenty-eight patients were allocated to ATP treatment, and 30 were assigned to no ATP treatment. General baseline characteristics including age, stage, performance status, and treatment before inclusion in this trial were similar in the ATP and control group. Patients in the ATP group weighed more than patients in the control group. Patient characteristics are shown in **Table 3.3.1**. Of the 58 patients, nine were not evaluable for tumor response because of concomitant chemotherapy (one patient in the control group), radiotherapy (two patients in the control group), patient refusal (one patient in the control group), hospitalization elsewhere (one patient in the ATP group), and early death (two patients in the ATP group and two patients in the control group).

Treatment

Twenty-eight patients in the ATP group received a total of 176 ATP courses. ATP was given for a median of 6.5 (range 1-10) infusions. Eleven patients received one to three ATP courses, five received four to six courses, and 12 patients received seven to 10 courses. Fifty-two infusions of ATP were given as low-dose infusions of 25-40 μg/kg.min, 47 as middle-dose infusions of 45-60 μg/kg.min, and 77 as high-dose infusions of 65-75 μg/kg.min. The reasons not completing all ATP cycles were death (n=15), progressive disease (n=2), or patients refusal (n=1).

As previously reported,[7] 67% of ATP courses were without side effects (36% of the first and 73% of the subsequent courses). Side effects occurring in the remaining courses were mainly cardiopulmonary reactions such as chest discomfort and the urge to take a deep breath (both grade 1) which were transient, and resolved within

minutes after lowering the ATP infusion rate. These reactions were most common in patients with a history of cardiovascular dysfunction or chronic obstructive pulmonary disease. None of the patients developed hematological toxicity. Between the ATP courses, no side effects of the ATP treatment were reported.

Table 3.3.1. Baseline patient characteristics of the 58 randomized patients

	ATP	Control
	(n = 28)	(n = 30)
Gender		
male	20 (71%)	18 (60%)
female	8 (29%)	12 (40%)
Age (y)	64 ± 13	61 ± 10
Tumor histology		
adenocarcinoma	11 (39%)	6 (20%)
squamous-cell carcinoma	10 (36%)	11(37%)
undifferentiated large cell carcinoma	4 (14%)	9 (30%)
unspecified	3 (11%)	4 (13%)
Previous chemotherapy		
yes	12 (43%)	14 (47%)
no	16 (57%)	16 (53%)
Stage		
IIIB	13 (46%)	14 (47%)
IV	15 (54%)	16 (53%)
Karnofsky Index (%)		
≤ 70	12 (43%)	14 (47%)
> 70	16 (57%)	16 (53%)
Prior weight loss (kg)*	5.8 ± 6.5	4.9 ± 6.7
Prior weight loss (%)*	6.7 ± 7.1	6.8 ± 9.7
Weight (kg)	75.0 ± 16.4	68.2 ± 12.3
Body mass index (kg/m^2)	25.3 ± 5.5	23.8 ± 4.0

Findings are expressed either as a percentage in brackets or as mean ± standard deviation.
* Weight loss in relation to pre-illness weight.

Tumor response

No complete or partial responses were observed. The median time to progression was 3.9 months (95% CI = 2.3 - 5.5) in the ATP group (n=25) compared to 3.0 months (95% CI = 2.4 - 3.7) in the control group (n=24; between-group difference: P=0.71).

Survival

Kaplan Meier plots of survival in all patients given ATP versus no-ATP are shown in **Figure 3.3.1.** Because patients in the ATP group weighed more at randomization, the survival analysis was adjusted for baseline body weight. The median survival time from randomization was 5.6 months (95% CI = 1.1 to 10.1) for patients in the ATP group, and 4.7 months (95% CI = 2.6 to 6.8) for patients in the control group. There was no statistically significant difference between the two groups (P=0.68). In the ATP group six patients (21%) survived for more than one year, and in the control group five patients (17%). On the census date, four of 58 patients (two patients in the ATP group and two patients in the control group) were still alive.

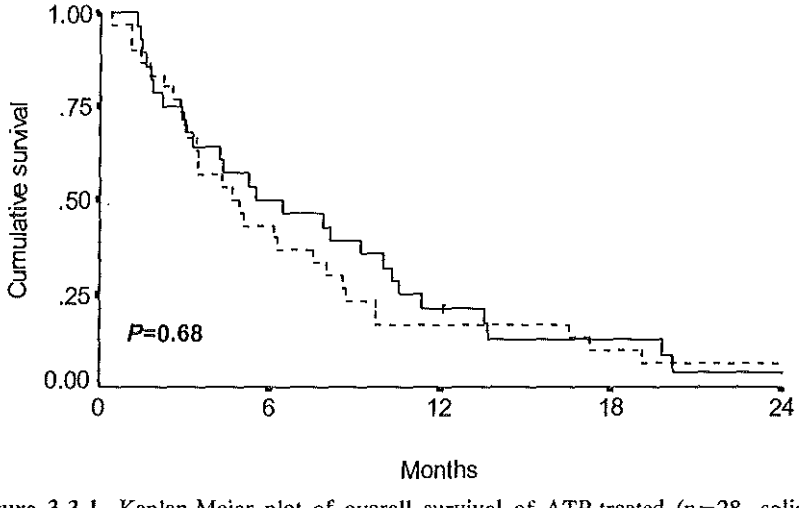

Figure 3.3.1. Kaplan-Meier plot of overall survival of ATP-treated (n=28, solid line) and control (n=30, dotted line) patients with advanced non-small-cell lung cancer (stage IIIB or IV).

In univariate analysis, weight loss was an unfavorable prognostic factor for survival (P=0.03). Subgroup analysis for weight-losing patients stage IIIB and stage IV separately (adjusting for age and body weight at randomization) demonstrated a significant survival benefit of ATP-treatment given to stage IIIB patients with ≥5% weight loss before randomization (**Figure 3.3.2**): median survival in ATP-treated patients (n=8) was 9.3 months (95% CI = 2.1 to 16.5) compared to 3.5 months (95% CI = 2.3 to 4.7) in control patients (n=8; between-group difference: P=0.02). Baseline characteristics of these patients are shown in **Table 3.3.2.** No significant survival difference was seen in weight-losing patients with stage IV: ATP-treated patients (n=9) died after a median of 3.1 months (95% CI = 2.6 to 3.6), and control patients (n=10) after 2.3 months (95% CI = 0.0 to 6.8).

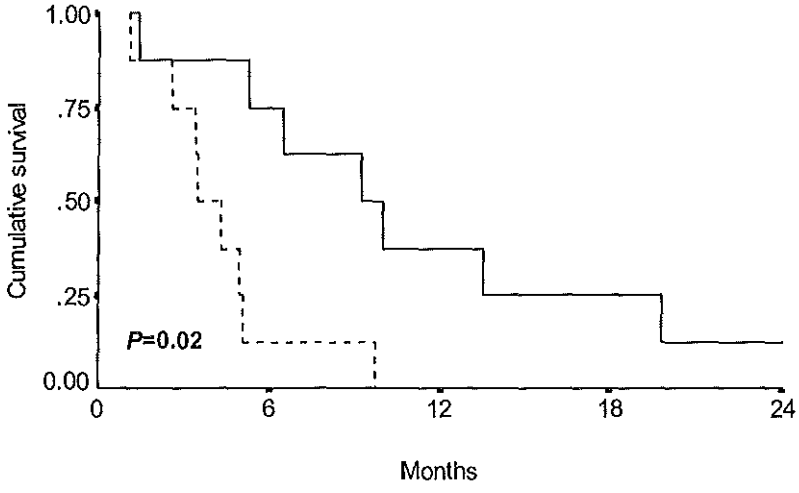

Figure 3.3.2. Kaplan-Meier survival plot for a subgroup of eight ATP-treated (solid line) and eight control (dotted line) patients with stage IIIB non-small-cell lung cancer and 5% weight loss at randomization.

Treatment as cointervention

Three months after randomization one patient in the control group (stage IIIB, no weight loss) was treated with combination chemotherapy, which consisted of five

courses of a combination of cisplatin and etoposide, given at 3-week intervals, followed by radiotherapy involving all lesions. One year after randomization one patient in the ATP group was enrolled in a phase I trial with six courses of PNU-159548 (an alkylcycline). Palliative radiotherapy was administered to seven patients in the control group, and to six patients in the ATP group.

Table 3.3.2. Baseline patient characteristics of stage IIIB patients with weight loss

	ATP	Control
	(n = 8)	(n = 8)
Gender		
male	6 (75%)	5 (63%)
female	2 (25%)	3 (37%)
Age (y)	66 ± 14	59 ± 7
Tumor histology		
adenocarcinoma	1 (13%)	0 (0%)
squamous-cell carcinoma	4 (50%)	5 (63%)
undifferentiated large cell carcinoma	1 (13%)	2 (25%)
unspecified	2 (25%)	1 (12%)
Previous chemotherapy		
yes	3 (37%)	3 (37%)
no	5 (63%)	5 (63%)
Karnofsky Index (%)		
≤ 70	4 (50%)	4 (50%)
> 70	4 (50%)	4 (50%)
Prior weight loss (kg)*	9.3 ± 5.2	9.8 ± 3.7
Prior weight loss (%)*	10.4 ± 4.5	14.2 ± 7.0
Weight (kg)	78.4 ± 27.8	63.9 ± 16.2
Body mass index (kg/m^2)	26.3 ± 9.0	22.4 ± 5.0

Findings are expressed either as a percentage in brackets or as mean ± standard deviation.
* Weight loss in relation to pre-illness weight.

DISCUSSION

This is the first randomized clinical trial to examine the effects of intravenous ATP infusions on tumor growth and survival in patients with advanced NSCLC (stage IIIB or IV). In the present study, ATP did not contribute to objective tumor response. Furthermore, no effect of ATP treatment on overall survival of this patient group was demonstrated. Univariate analysis showed that patients with weight loss prior to randomization survived significantly shorter than patients without weight loss. This finding is consistent with earlier reports which show that weight loss is an unfavorable prognostic variable for survival.[116,407,440] We previously reported[7] that ATP inhibited weight loss in these patients, while weight loss continued in patients not receiving ATP infusion. Our data suggest that in the subgroup of weight-losing patients with stage IIIB NSCLC, ATP treatment did have a survival benefit. This effect could not be attributed to differences in baseline characteristics since both treatment arms were well balanced, except for age and body weight for which the survival analysis was adjusted. It is possible that the observed survival benefit in these patients with non-metastatic disease is related with the inhibition of weight loss by ATP.

We conclude that despite its beneficial effect on weight loss and quality of life,[7] ATP as a single therapy did not affect tumor response or increase survival in patients suffering from advanced NSCLC, stage IIIB or IV. Nevertheless, despite the lack of a direct anti-tumor effect in these patients, treatment with ATP may be worth further study. Firstly, our data suggest that ATP infusions may prolong survival in weight-losing stage IIIB lung cancer patients, possibly by reducing weight loss. Secondly, several animal model studies demonstrated ATP-induced inhibition of tumor growth.[134,161,239,301,309,327,344,345,347,348] Thirdly, ATP has been reported to potentiate the cytotoxic effect of chemotherapeutic agents[36,222,281] and radiotherapy,[134,448] and could therefore be examined as an adjuvant to these therapies. We conclude that further phase III studies of ATP in non-metastatic cancer patients with weight loss are warranted.

3.4

PAIN REDUCTION BY ADENOSINE IN ADVANCED CANCER: A PILOT STUDY

Submitted for publication

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ABSTRACT

Intravenous adenosine has been shown to reduce pain in animals, healthy volunteers and several patient groups, but no data are available on the effects of adenosine in cancer pain. The present pilot study was aimed at exploring the potential value of intravenous adenosine infusion for relief of nociceptive pain in advanced cancer patients. Six cancer patients with no satisfactory pain relief from previous therapy were included. After two days of baseline, two four-hour infusions were administered on two successive days, with one day of follow-up. Pain scores were assessed by numerical rating scale (NRS), verbal rating scale (VRS), and pain relief scale. Scores over time were tested by repeated-measures analysis SAS Proc Mixed. Pain scores for both NRS and VRS decreased significantly during adenosine treatment as compared to baseline (P=0.0001). The mean NRS ($\pm$ SD) decreased from 5.5$\pm$10 at baseline to 3.2$\pm$1.8 during the first adenosine infusion, and 2.2$\pm$1.5 during the second infusion. The VRS decreased from 3.8$\pm$0.6 at baseline to 2.7$\pm$0.7 during the first infusion, and 2.2$\pm$0.9 during the second adenosine infusion. At follow-up, pain scores were still reduced, with values of 2.8$\pm$1.7 for NRS and 2.2$\pm$0.6 for VRS. Compared to baseline, three patients decreased >2 points on the NRS (strong-responder) during the two adenosine infusions, one patient >1 point (weak-responder), and one decreased <1 point (non-responder). There was a significant correlation between NRS and pain relief score of r=-0.63 (P<0.001). The mean number of rescue doses was reduced by approximately 50%, i.e., from 2.3$\pm$2.2 per day at baseline to 1.2$\pm$1.2 per day at follow-up (P=0.11). The analgesic effects of adenosine suggested by this exploratory study warrant further study in patients with cancer pain.

INTRODUCTION

Pain is reported by over 70% in advanced cancer[337] and can be a major cause of anxiety, depression, or anger. Pain is treated according to the analgesic ladder of the WHO. When these steps fail, more invasive methods are indicated.

During the last decade, it has been shown that adenosine may relieve pain in both animal models[175,240,371,372,385] and man. Randomized blinded studies show pain-relief effects of intravenous adenosine infusion in healthy volunteers (50 to 70 μg/kg.min),[380,399] patients with neuropathic pain, hyperalgesia and allodynia (50 μg/kg.min),[24,400] and in breast[379] and gynecological surgery (80 μg/kg.min).[382] In one study, adenosine infusion contributed to reduction of perioperative isofluorane and opioids postoperatively.[382] Combination of adenosine with morphine or ketamine may have an additive effect on pain reduction as shown in a double-blind, placebo-controlled, cross-over study in healthy volunteers.[381]

Because no data are available on the effects of adenosine in cancer, we conducted a pilot study aimed at exploring the potential value of intravenous adenosine infusion on nociceptive pain of advanced cancer.

PATIENTS AND METHODS

Patients with localized cancer pain due to radiologically documented tumor, a pain score of ≥4, and requiring opioid therapy were eligible. Patients with failure of either liver (bilirubine >40 μmol/l), kidneys (creatinine >200 μmol/l), heart, or those undergoing chemotherapy or radiotherapy <6 weeks before the study, were excluded. The study was approved by the Protocol Review Board and the Medical Ethical Committee of the Daniel den Hoed Cancer Center. Written informed consent was obtained from all patients prior to the study.

Treatment schedule

Patients were admitted to the Palliative Care Unit or the Outpatients Center of Daniel den Hoed Cancer Center to receive two intravenous adenosine infusions during four hours on two successive days. At approximately nine hours a.m., adenosine infusions (5 mg adenosine per ml NaCl 0.9%) were started at a rate of 20 μg/kg.min and increased by increments of 10 μg/kg.min every 15 minutes until a maximum dose of 50 μg/kg.min, or the maximally tolerated dose if lower, had been reached. Thereafter, adenosine was infused at a continuous rate. If side effects occurred, the dose was reduced to the last given dose or lower until side effects disappeared. During the time

of the study, patients were not allowed to take coffee or tea with caffeine because of potential reversal of pain reduction by adenosine. [371]

Pain assessment

Pain scores were recorded three times per day on two consecutive days at baseline, the two days of adenosine infusion, and the day of follow-up: i.e., in the morning, afternoon, and evening. In addition, patients scored the pain questionnaire every hour during the four hours of adenosine infusion. Pain intensity was assessed using a numerical rating scale ranging from 0 to 10 (NRS),[324] a verbal rating scale (VRS) recorded as either no pain (1), slight (2), mild (3), considerable (4), severe (5), or excruciating (6), and a pain relief scale recorded as either complete (5), considerable (4), moderate (3), slight (2) or no pain relief (1) and pain worse (0).[252,280,442] These scales were recorded in a pain diary. Patients were allowed to continue the use of opioids therapy either orally or intravenously. As rescue medication, patients took 10 or 20 mg morphine sulphate orally, or received intravenous morphine doses. The numbers of rescue medications used were recorded by the patients in the diary, and verified in the patient's medication list which was kept at the ward.

Side effects

Side effects were monitored according to Common Toxicity Criteria version II (National Cancer Institute) scaled on a 4-point scale according to seriousness. In this system, dyspnea is graded as follows: 0, no change; 1, not defined; 2, dyspnea on significant exertion; 3, dyspnea at normal activity; 4, dyspnea at rest. In general, toxicity was graded as: 1, mild; 2, moderate; 3, severe; and 4, life-threatening. During adenosine infusions, electrocardiographics, heart rate, and blood pressure were monitored using a data-scope (Propaq, Protocol Systems Incorporated, Beaverton, US)

Statistical analysis

The mean scores of NRS and VRS were calculated for each patient. The baseline assessment was the mean score of the seven pain assessments before the first adenosine infusion. Treatment effects were calculated from the mean pain score during the first adenosine infusion (four scores), during the in-between infusion period (three scores), during the second adenosine infusion (four scores), after

discontinuation of the second adenosine infusion (two scores), and during one day of follow-up (three scores). The individual response rate was assessed by comparing pain scores during both adenosine infusions to the baseline scores. Patients were classified as non-responder (decreasing <1 point on NRS), weak-responder (decreasing ≥ 1 point but <2 points on NRS), or strong-responder (decreasing ≥ 2 points on NRS). Pain scores over time were tested by repeated-measures analysis using the SAS procedure Proc Mixed (SAS Inc., Cary, N.C. USA). This technique has been recommended to analyze all observations and time-varying covariates data simultaneously because of its relatively great power.[277] In order to compare repeatedly measured pain scores during and after adenosine treatment with their baseline scores, the independent variables in the model were the treatment indicator variable, baseline pain scores, measurement time, and interaction between time and treatment. In addition, the two-tailed Wilcoxon signed rank test was applied for comparing separate time periods with baseline values, using SPSS (SPSS Inc., Chicago, USA). The NRS and the pain relief scale were compared using the partial correlation coefficient, adjusting for patient. Results are expressed as means $\pm$ standard deviation (SD). *P*-values of less than 0.05 indicated significance.

RESULTS

Patient characteristics and treatment

Six cancer patients (four males, two females) with a mean age of 60 ± 10 years and weight of 80 ± 16 kg participated in the study. All patients had previously been treated with non-steroidal anti-inflammatory drugs or morphine without satisfactory pain relief. Clinical details of the remaining five patients are shown in **Table 3.4.1**. Patient no.6 was not evaluable for pain assessment because he developed progressive malaise, headache, chest discomfort, and fever as a result of a radiologically confirmed pneumonia approximately one hour after starting adenosine infusion. The infusion was discontinued and treatment with intravenous amoxicilin/clavulan acid was started, and the patient recovered uneventfully. The five evaluable patients received infusion for four hours on two subsequent days with a mean dose of 33 ± 10 μg/kg/min. Side effects reported during the total of 10 adenosine courses were

presented in **Table 3.4.2.** All side effects resolved within minutes after lowering the adenosine dose. Patient no.1 did not report any side effects. Heart rate, systolic and diastolic blood pressures did not change during adenosine infusions.

Table 3.4.1. Baseline patient characteristics

Patient (no)	Age/sex	Weight (kg)	Type of cancer	Localization of pain	Pain treatment*	Morphine resc† (mg)	Adenosine (μg/kg.min)
1	64 m	60	renal cell carcinoma	lumbar	morphine iv 18 mg/h	20 (iv)	50
2	63 f	75	non-Hodgkin lymphoma	lumbar	naproxen 500 mg tid ms contin 60 mg bid	0	25
3	61 f	103	breast carcinoma	lumbar/sacral	diclophenac 100 mg bid 75/d	0	30
4	69 m	73	chondro sarcoma	sacral	morphine sc 1mg/h	20 (orally)	35
5	59 m	87	aggressive fibromatosis	subscapular	nabumeton 500 mg tid phentanyl patch 50 μg/h amitriptylin 10 mg tid	10 (orally)	25
6	40 m	69	adenoid carcinoma	head	phentanyl patch 75 μg/h amitriptylin 10 mg tid	20 (orally)	0

* Continuous pain treatment prior to and during adenosine infusion; † Rescues need throughout this study

Pain relief

Pain scores for the NRS showed a highly significant overall decrease during adenosine treatment compared to baseline (**Figure 3.4.1**; repeated-measures analysis: $P=0.0001$). The mean NRS ($\pm$SD) decreased from 5.5 ± 10 at baseline to 3.2 ± 1.8 during the first adenosine infusion (Wilcoxon's signed rank test: $P=0.08$), 3.4 ± 2.2 during the between-infusion period ($P=0.08$), and 2.2 ± 1.5 during the second infusion ($P=0.04$). After discontinuation of the adenosine infusion on the second day, patients maintained significant pain reduction compared to baseline with a mean NRS of 2.8 ± 1.9 ($P=0.04$). On follow-up, the pain intensity was still reduced with a NRS of 2.8 ± 1.7 ($P=0.04$). A similar pattern was shown for the VRS which showed a significant reduction during adenosine treatment (repeated-measures analysis:

P=0.0001). The VRS decreased from 3.8±0.6 to 2.7±0.7 during the first adenosine infusion (P=0.08), to 2.8±0.9 between the two successive adenosine infusions (P=0.04), and to 2.2±0.9 during the second adenosine infusion (P=0.04). After discontinuation of adenosine infusion, the VRS pain score was still reduced at 2.6±1.1 (P=0.04), and 2.2±0.6 on the next day (P=0.07).

Table 3.4.2. Side effects ascribed to adenosine during a total of 10 intravenous cycles (25-50 μg/kg.min during 4 hours); CTC grading

Grade:	0	1	2	3	4
Chest discomfort	7	3	0	0	0
Flushing	7	3	0	0	0
Lightheadedness	9	1	0	0	0
Headache	9	1	0	0	0
Dyspnea	9	0	0	0	1
Urge to take a deep breath	5	5	0	0	0

There was a significant correlation between the NRS and the pain relief scale of r=-0.63 (P< 0.001). As shown in **Table 3.4.3**, the mean number of rescue doses was reduced by approximately 50%, i.e., from 2.3±2.2 per day at baseline to 1.2±1.2 per day on follow-up (P=0.11). On the NRS, patients no.2, 4, and 5 were strong-responders, patient no.3 was a weak-responder, and patient no.1 a non-responder.

Table 3.4.3. Number of morphine rescues needed per day

Day*	Morphine rescues†	P-value‡
1 and 2	2.3 ± 2.2	-
3	2.2 ± 2.0	0.28
4	1.2 ± 1.2	0.11
5	1.2 ± 1.2	0.11

* Day 1 and 2 (baseline), day 3 and 4 (4-hour during adenosine infusions), and day 5 (follow-up)
† Mean ± standard deviation
‡ Compared to baseline (Wilcoxon signed-rank test)

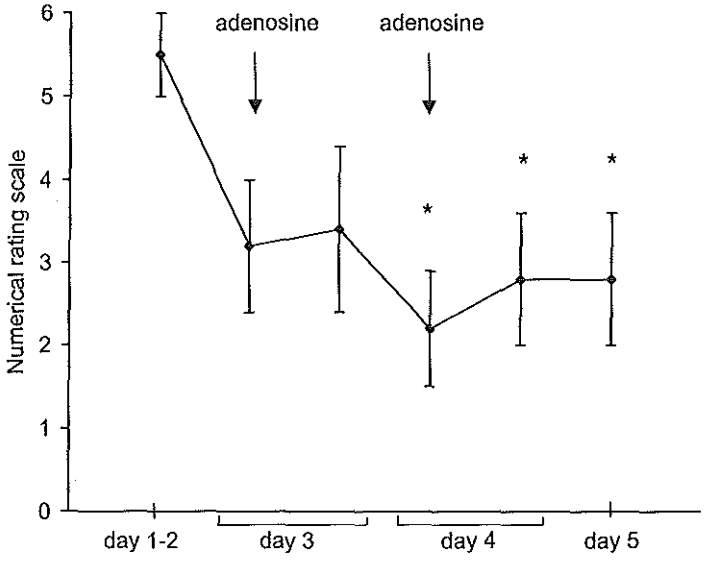

Figure 3.4.1. Plot of mean (±SEM) pain intensity by numerical rating scale (NRS) on day 1-2 (baseline, mean of seven scores), day 3 (during 4 hours of adenosine infusion, mean of four scores, respectively interval, mean of three scores), day 4 (during 4 hours of adenosine infusion, mean of four scores, respectively follow-up, mean of two scores), and day 5 (follow-up, mean of three scores). Scores at days 3 to 5 compared to days 1-2 as tested by repeated-measures analysis: $P=0.0001$. *) $P< 0.05$ (two-tailed Wilcoxon Signed Rank Test).

DISCUSSION

The aim of this pilot study was to explore potential pain reduction by intravenous adenosine infusion in advanced cancer. The rationale for using adenosine was based on studies reporting adenosine-induced pain relief in animals,[175,371,372,385] healthy volunteers[380,381] and patient groups.[24,379,380,382,400] As far as we know, no clinical studies on intravenous adenosine in cancer have been performed to date.

In the present study, five cancer patients suffering from nociceptive pain, without satisfactory pain relief from previous therapy received adenosine infusion for 4 hours on two subsequent days. Results based on the NRS, VRS, and pain relief scale showed

that adjuvant adenosine infusion reduced pain intensity substantially. Pain scores decreased by more then 50% on the second day of adenosine infusion compared to baseline. In spite of the short plasma half-life of adenosine (0.6 to 1.5 seconds after a bolus injection),[294] pain scores were still reduced one day after adenosine administration, suggesting that adenosine may induce prolonged pain relief. This is in line with findings in animals[240] and healthy humans.[24] Adenosine infusion for 45 to 60 minutes reduced evoked pain in six out of seven healthy subjects for periods varying from six hours to four days.[24]

Adenosine was administered as a constant intravenous infusion without any severe side effects. If side effects occurred, they disappeared rapidly on lowering the infusion rate. The reported side effects were mostly of type 1. Although during one patient showed dyspnea (type 4), this was mild of character.

Several mechanisms may explain the pain-reducing effects of adenosine. In the periphery, adenosine A_1 receptor activation induces suppression of pain. Adenosine A_1 receptors are present on the cell body of dorsal root ganglion cells[270] and on the central terminals of primary afferent neurons,[368] so that transport of these receptors to the peripheral aspect of this nerve is likely.[370] Within the dorsal spinal cord, concentrated in the substantia gelatinosa,[77,78,170] the antinociceptive effects are mediated through activation of both A_1 and A_2 receptors.[370-372] Possibly, central receptor stimulation by adenosine contributes to spinal hyperexcitability[240,400] which may explain the prolonged duration of pain reduction. It is also conceivable that adenosine may counteract inflammatory processes contributing to cancer pain. Adenosine has been shown to inhibit neutrophil degranulation,[43] neutrophil superoxide production,[43] anti-oxidant activation,[273,340] adhesion of neutrophils,[98] and expression of adhesion molecules.[45] Adenosine has also been observed to inhibit the production of cytokines,[44,45,453] eicosanoids,[232] and complement.[238]

In conclusion, this pilot study demonstrates a substantial reduction in pain scores during and after intravenous adenosine infusion in patients with cancer. If side effects occur, they are mild and transient, resolving within minutes of lowering the adenosine dose. Further study of analgesic effects of adenosine as a new modality in pain treatment seems warranted.

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4.1

PHARMACOKINETICS OF INTRAVENOUS ATP IN CANCER PATIENTS

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ABSTRACT

Objective: To characterize the pharmacokinetics of adenosine 5'-triphosphate (ATP) in patients with lung cancer after intravenous administration of different ATP dosages.

Methods: Twenty-seven patients received a total of 176 intravenous ATP courses of 30 hours. Fifty-two courses were given as low-dose infusions of 25-40 μg/kg.min, 47 as middle-dose infusions of 45-60 μg/kg.min, and 77 as high-dose infusions of 65-75 μg/kg.min ATP. Kinetic data of ATP concentrations in erythrocytes were available from 124 ATP courses. Results are expressed as means ± SEM.

Results: Most ATP courses in cancer patients were without side effects (67%), and side effects occurring in the remaining courses were mild and transient, resolving within minutes after decreasing the infusion rate. Baseline ATP concentration in erythrocytes was 1554±51 μM. ATP plateau levels at 24 hours were significantly increased by 53±3, 56±3, and 69±2% after low-dose, middle-dose and high-dose ATP infusions, respectively. At the same time, significant increases in plasma uric acid concentrations were observed: 0.06±0.01, 0.11±0.01, and 0.16±0.01 mM, respectively. The mean $t½$ for disappearance of ATP from erythrocytes, measured in five patients, was 5.9±0.5 hours.

Conclusions: During constant intravenous infusion of ATP in lung cancer patients, ATP is taken up by erythrocytes and reaches dose-dependent plateau levels 50 to 70% above basal concentrations at approximately 24 hours.

INTRODUCTION

Extracellular adenosine 5'-triphosphate (ATP) is involved in the regulation of a variety of biological processes including neurotransmission, muscle contraction, cardiac function, platelet function, vasodilatation, and liver glycogen metabolism. ATP can be released from the cytoplasm of several cell types and interacts with P1 and particularly P2 receptors on the surface of many cells. These receptors play a fundamental role in cell physiology and are also a potential target in the treatment of various diseases, including paroxysmal supraventricular tachycardias,[475] pulmonary

hypertension,[168] shock,[46] and pain syndromes.[371] Furthermore, there is evidence that ATP administration may inhibit the growth of tumor cells and implanted tumors.[3,345] The range of physiological ATP concentration within mammalian cells is 1500 to 4800 μM. ATP content in tissue cells is somewhat higher than in blood cells.[432] In human erythrocytes ATP concentrations have been described to be between 1500 and 1900 μM.[112,429,463] Much lower ATP concentrations have been reported for human plasma, in general a factor 1000 below erythrocyte ATP concentrations.[150,184]

When adenine nucleotides are administered intravenously, the nucleotides are taken up by erythrocytes.[431] Using suspensions of washed intact human erythrocytes and labeled purines, Parker et al.[330] found that ATP was metabolized outside the cell via ADP and AMP to adenosine. Adenosine rapidly entered the erythrocytes where it was incorporated into adenine nucleotides. At extracellular adenosine concentrations below 3 μM, most intracellular adenosine was phosphorylated to adenine nucleotides, whereas at higher extracellular adenosine concentrations, adenosine was degraded to inosine and hypoxanthine within the erythrocytes.[375]

There is little information about pharmacokinetics of administered ATP in humans. In a phase I study, Haskell et al.[188] measured whole blood levels of ATP during 96 hours of continuous intravenous ATP infusions in 14 patients with advanced cancer of different tumor types. Continuous ATP infusion of 50 μg/kg.min induced a mean 63% increase in whole blood ATP levels in these patients after 24 hours. A higher dose of ATP infusion of 75 μg/kg.min gave only a marginally greater increase (67%). This study however did not provide information on the time interval until plateau values were reached. Furthermore, no study has attempted to determine washout parameters of ATP from blood cells.

Recently, in a randomized clinical trial in patients with advanced non-small-cell lung cancer, we demonstrated that ATP infusions contribute to maintenance of body weight, muscle strength, and quality of life in these patients.[7] The present pharmacokinetic study was aimed at investigating the ATP increase in erythrocytes during intravenous ATP infusion at different doses in patients participating in this trial. Furthermore, we wanted to determine the half-time ($t½$) of disappearance of ATP from erythrocytes, which is needed to design an optimal dosing schedule. Finally, we attempted to measure changes in ATP concentrations in plasma during ATP infusion.

METHODS

Patients

The study protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam. Written informed consent was obtained from each individual prior to the study. In the ATP group were included 28 patients (20 male and eight female) with locally advanced and metastatic non-small-cell lung cancer (stage IIIB or IV), of mean age 64 (range 30 to 85) years, and weight 75 (range 54 to 133) kg. None had respiratory, cardiac, hepatic or renal failure. Baseline characteristics of the patients are listed in **Table 4.1.1**.

Design

A maximal number of 10 ATP courses of 30 hours were given: the first seven courses at 2-week intervals, and thereafter three ATP courses at 4-week intervals. Eleven patients received one to three courses, five patients four to six, and 11 patients received seven to ten ATP courses.

ATP treatments were started after an overnight fast of 10 to 12 hours, between 9.00 and 11.00 hours. ATP was infused over 30 hours through a peripheral vein using an AVI270CI^{R} infusion pump. ATP infusions were started at 20 μg/kg.min and increased by increments of 10 μg/kg.min every 30 minutes until the maximum dose of 75 μg/kg.min, or until the maximally tolerated dose (MTD), if this was lower, had been reached. Thereafter, ATP was infused at a continuous rate. If any side effects occurred, the dose was reduced to the last given dose or further until side effects disappeared. Occurring side effects were registered systematically.

Sampling

Prior to ATP administration and at 4, 7, 12, 24, 28 and 30 hours after starting ATP infusion, venous blood was sampled into EDTA tubes from the arm opposite to the arm in which ATP was infused. Half of the blood samples was immediately placed into separate cryo-tubes, and then frozen in liquid nitrogen at -80 °C. The other half was immediately centrifuged for 10 min 1300 x *g* at 4 °C. Samples of plasma were also placed into separate cryo-tubes, and then frozen in liquid nitrogen at -80 °C. In order to obtain a more detailed estimate of time-concentration relationship and the

washout rate of ATP, in five patients blood samples were drawn at shorter time intervals during both the ATP course and the 12 hours after termination of the ATP infusion. Uric acid levels were measured at 0, 24, and 30 hours after starting ATP infusion. Information about the rate of renal uric acid clearance (CL_R) was obtained from six patients who collected urine during 24 hours before treatment and during 30 hours while ATP was being infused.

Chemicals

Adenosine 5'-triphosphate (ATP-$Na_2 \cdot 3H_2O$) of >98% purity was obtained from Merck (Darmstadt, Germany) in 50 grams vials; 6.1 grams ATP were solved in 1 liter NaCl 0.9%, sterilized by ultrafiltration (0.2 μM), and supplied in sterile glass bottles. As tested by the method using hexokinase and glucose-6-phosphate dehydrogenase,[31] the obtained ATP solution was stable for at least 5 months at -20 °C, and >5 days at room temperature. Perchloric acid (71%) and other chemicals were also from Merck.

Laboratory analyses

Whole blood and plasma samples were deproteinized by addition of perchloric acid to obtain a final concentration of 4% and centrifuged for 10 min 14000 x *g* at 4 °C. The supernatant was neutralized (pH 6-7) with 2 M K_2CO_3 in 6 M KOH, and the sample was kept cold to precipitate the potassium perchloride. After storage at -20 °C the supernatant was taken for high-performance liquid chromatograph (HPLC) analysis of ATP. Analyses were performed on a Shandon Hypersil ODS (C18) column 150 mm x 4.6 mm 3 U with a flow rate of 1.0 ml/min at room temperature, using a 0.1 M phosphate buffer (pH 6.0) as eluens. Identification and quantification of the samples was performed by comparing peak areas with appropriate standards. Peaks were detected by absorption at 254 nm and were identified by retention time.[377] Because ATP concentrations in erythrocytes have been reported to be a factor 1000 above plasma ATP concentrations,[150,184] ATP concentration levels in the erythrocytes were calculated by dividing measured ATP concentrations in whole blood by individual hematocrit values.

Table 4.1.1. Baseline characteristics of the patients

Patient (no)	Tumor stage	Sex (m/f)	Age (y)	Weight (kg)	Hematocrit (l/l)	Erythrocyte ATP µM	Plasma uric acid mM
1	3B	m	71	79.0	0.31	1261	0.27
2	3B	m	73	55.8	0.31	1535	0.27
3	3B	m	74	70.8	0.43	1698	0.29
4	3B	f	60	61.7	0.40	-	0.31
5	3B	m	76	89.6	0.48	1071	0.37
6	3B	m	48	79.7	0.39	1431	0.31
7	3B	m	41	87.9	0.32	1581	0.26
8	3B	m	46	80.2	0.34	1850	0.48
9	3B	m	85	53.9	0.40	1423	0.28
10	3B	m	71	71.0	0.44	1771	0.34
11	3B	m	54	74.5	0.45	1624	0.40
12	3B	f	65	133.2	0.38	-	0.36
13	3B	m	77	81.6	0.40	-	0.26
14	4	f	62	68.2	0.38	1553	0.34
15	4	m	69	70.8	0.39	1921	0.38
16	4	m	30	71.6	0.32	1981	0.50
17	4	m	82	75.4	0.40	1500	0.33
18	4	m	73	60.2	0.37	1768	0.51
19	4	f	71	74.7	0.41	1385	0.28
20	4	m	77	66.6	0.33	1327	0.25
21	4	f	52	65.0	0.42	1538	0.37
22	4	f	57	57.5	0.31	2069	0.24
23	4	m	66	68.4	0.36	1311	0.46
24	4	f	53	69.0	0.31	1703	0.19
25	4	m	68	114.0	0.47	1117	0.31
26	4	m	68	65.5	0.41	1483	0.38
27	4	f	62	68.8	0.29	1664	0.28
28	4	m	67	73.3	0.34	1285	0.42

-) not determined

Side effects

Side effects were monitored according to Common Toxicity Criteria (National Cancer Institute) scaled on a 4-point scale according to seriousness. In this system, dyspnea is graded as follows: 0, no change; 1, not defined; 2, dyspnea on significant exertion; 3, dyspnea at normal activity; 4, dyspnea at rest. In general, toxicity was graded as: 1, mild; 2, moderate; 3, severe; and 4, life-threatening.

Pharmacokinetic analysis

Results are expressed as means ± standard error of the mean (SEM). Results of independent groups were tested for significance by Student's *t*-test, and changes in time by Student's paired *t*-test. *P*-values of less than 0.05 indicated significance. The correlation between variables was analyzed using partial correlation coefficient controlling for patient. Half-time ($t½$) of ATP disappearance from erythrocytes was calculated for each patient individually by fitting a mono-exponential curve to the washout data, using the software program MicroMath Scientist (Salt Lake City, Utah 84121, USA).

RESULTS

Dosage and side effects

Twenty-eight patients received a total of 176 ATP courses. Seventy-seven infusions were given as high-dose infusions of 65-75 μg/kg.min ATP. Because of lower MTD, 47 infusions were given as middle-dose infusions of 45-60 μg/kg.min ATP, and 52 as low-dose infusions of 25-40 μg/kg.min ATP. Side effects observed during the ATP infusions are reported in **Table 4.1.2**. The most frequent side effects were chest discomfort (15%) and an urge to take a deep breath (10%) which resolved within minutes after lowering the ATP dose. Electrocardiography (ECG) was performed in patients with chest pain/discomfort during ATP infusions. No ECG changes suggestive of myocardial ischemia were detected. The reactions were most common in patients with a history of cardiovascular dysfunction or chronic obstructive pulmonary disease. Heart rate decreased from mean (± SEM) 88±1 at baseline to 85±1 beats/minute at 24 hours of ATP infusion ($P<0.05$). Systolic blood pressure

decreased from 129±2 to 127±2 (n.s.), and diastolic blood pressure from 75±1 to 72±1 mm Hg ($P<0.005$).

Table 4.1.2. Side effects ascribed to ATP during a total of 176 intravenous ATP cycles (25-75 μg/kg.min) in 28 patients; CTC-grading. In some courses more then one side effect was observed

	0	1	2	3	4
Palpitations	174	2	0	0	0
Cardiac-ischemia	176	0	0	0	0
Chest discomfort	150	25	1	0	0
Sweating	171	5	0	0	0
Flushing	168	8	0	0	0
Injection side reaction	172	0	4	0	0
Nausea	168	8	0	0	0
Epistaxis	175	1	0	0	0
Lightheadédness	170	6	0	0	0
Mood alteration-anxiety	173	3	0	0	0
Headache	171	5	0	0	0
Dyspnea	171	0	0	0	5
Take a deep breath	158	18	0	0	0

Erythrocyte ATP levels before and during ATP administration

Kinetic data of ATP concentrations in erythrocytes were available from 124 courses in 23 patients. In erythrocytes, the baseline ATP concentration prior to the first ATP course was 1554±51 μM. Mean baseline ATP concentrations prior to subsequent ATP courses did not show any significant differences from the initial baseline ATP concentrations (data not shown).

We compared the increase in ATP concentrations in erythrocytes during low, middle, and high-dose ATP infusion, respectively. At all time points the mean ATP values were significantly higher compared to the baseline concentration of ATP ($P<0.001$).

As seen in **Figure 4.1.1**, plateau levels of ATP were reached at approximately 24 hours. In a subgroup of patients in whom blood was sampled at 2-hour intervals, a

similar kinetic profile of blood ATP concentrations was observed. Compared to baseline levels, low-dose ATP infusions induced a 53±3% increase in erythrocyte ATP concentrations ($P<0.001$), and middle-dose ATP infusions a 56±3% increase ($P<0.001$), whereas high-dose ATP infusions evoked an increase of 69±2% ($P<0.001$). The rise in erythrocyte ATP concentrations after high-dose ATP infusion was significantly larger compared to middle-dose ($P<0.05$) and low-dose ($P<0.01$) ATP infusions at all times points from 12 to 30 hours. In contrast, erythrocyte ATP concentrations during middle-dose ATP infusions did not differ significantly from those during low-dose ATP infusions. Nevertheless, a moderate overall dose-level relationship was observed at 24 and 30 hours (r=0.56 and r=0.49, respectively, $P<0.005$).

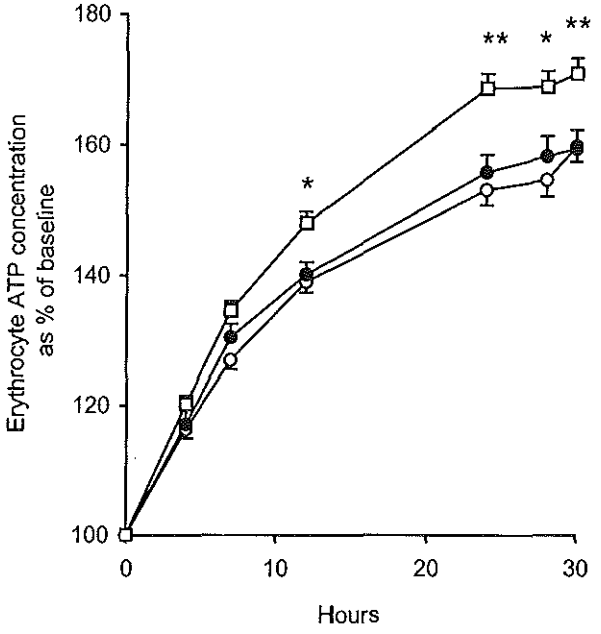

Figure 4.1.1. Erythrocyte ATP concentrations before and during intravenous ATP administration. Values are expressed as a percentage of baseline at ATP dose 25-40 μg/kg.min (O, n=35); 45-60 μg/kg.min (●, n=23); and 65-75 μg/kg.min (□, n= 66). Differences as compared to middle dose: *) $P<0.05$, **) $P<0.01$. Data are presented as mean values ± SEM.

ATP concentrations in erythrocytes from one patient who received 10 successive ATP courses of 75 μg/kg.min are plotted in **Figure 4.1.2.** The standard deviation between erythrocyte ATP levels during the different courses varied from 5 to 9%.

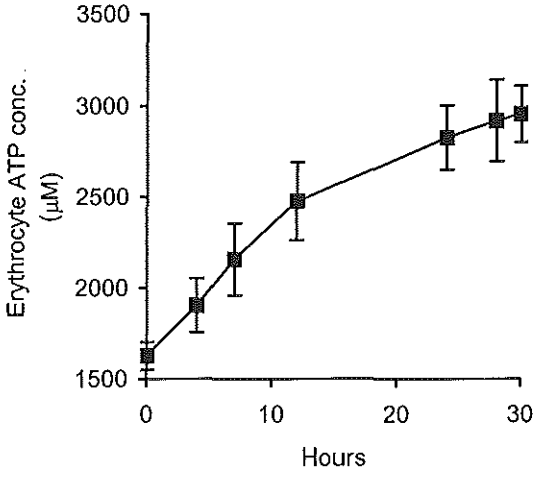

Figure 4.1.2. Erythrocyte ATP concentrations in one patient during 10 subsequent ATP courses of 75 μg/kg.min given as 2- to 4-week intervals (see methods). Data are presented as mean values ± SD.

Uric acid concentrations in plasma

Plasma levels of uric acid as a breakdown product of ATP showed a dose-dependent increase after 24 and 30 hours of ATP infusions (**Figure 4.1.3**). Plasma uric acid concentrations increased by 0.06±0.01 mM after 24 hours during low-dose ATP infusion ($P<0.001$), by 0.11±0.01 mM with middle-dose infusion ($P<0.001$), and by 0.16±0.01 mM with high-dose ATP infusion ($P<0.001$). At all doses of ATP administration mean uric acid concentrations at 30 hours were almost identical to those at 24 hours. A significant ATP dose - uric acid level relationship was observed both at 24 and 30 hours ($r=0.76$ and $r=0.81$, respectively, $P<0.001$). There was a poor relationship between ATP levels in erythrocytes and uric acid levels in plasma, with correlation coefficients of 0.31 at $t=24$ hours ($P=0.07$), and 0.21 at $t=30$ hours ($P=0.08$), respectively.

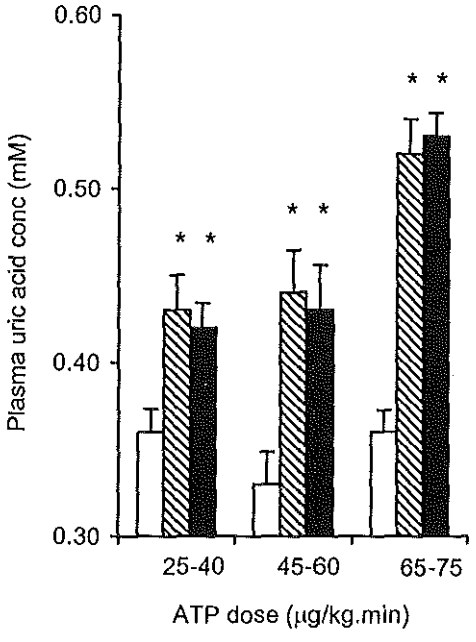

Figure 4.1.3. Plasma uric acid concentrations (mM) at baseline (white bars), 24 hours (hatched bars) and 30 hours (black bars) with ATP doses of 25-40 μg/kg.min, n=35; 45-60 μg/kg.min, n=27; and 65-75 μg/kg.min, n=43. Significantly different from baseline: *) $P<0.001$. Data are presented as mean values ± SEM.

Disappearance of ATP from erythrocytes

Information concerning the rate of disappearance of ATP from erythrocytes after termination of the ATP infusion was obtained in five patients. **Figure 4.1.4** shows that the rates of ATP disappearance in these patients were approximately similar regardless of ATP infusion doses. ATP concentrations during washout followed a mono-exponential pattern, with a $t½$ of 5.9±0.5 hours.

ATP concentrations in plasma

In plasma no increases in ATP concentration were shown during ATP infusions. In several patients the data obtained before and during ATP infusions were highly scattered (range 0.5 to 8.5 μM).

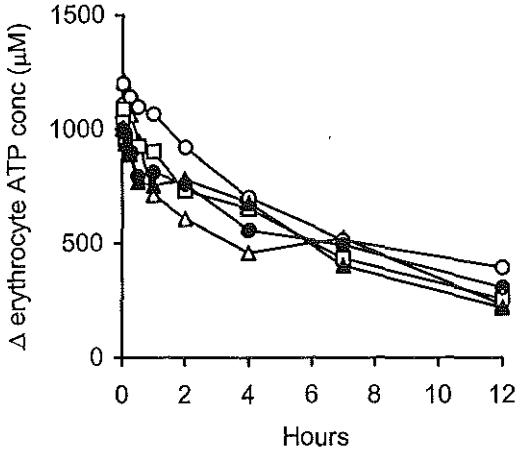

Figure 4.1.4. Erythrocyte ATP concentrations in five patients during washout following intravenous ATP administration. Values are expressed as differences (Δ) from baseline values. Solid symbols: after low-dose ATP infusion. Open symbols: after high-dose ATP infusion.

DISCUSSION

The aim of the study was to investigate pharmacokinetics of ATP in erythrocytes in patients with lung cancer following intravenous ATP administration. In a subgroup of patients the disappearance of ATP from erythrocytes *in vivo* was measured.

The mean baseline concentration of ATP in erythrocytes was 1554±51 μM, and comparable baseline ATP concentrations were observed prior to the subsequent ATP courses. Mean ATP concentrations in lung cancer patients in this study are in agreement with values reported by Stocchi et al.[408] for 10 normal adult controls (1501±25 μM), but not with their finding of lower erythrocyte ATP concentrations in 10 patients with gastrointestinal adenocarcinoma (1099±41 μM). We checked ATP values of erythrocytes in normal healthy subjects (n=9), and observed mean values of 1400±60 μM. Thus Stocchi's finding of lower erythrocyte ATP in patients with gastrointestinal adenocarcinoma do not seem to apply to patients with bronchus carcinoma.

A cascade of ectonucleotidases on the surface of endothelial cells is thought to be responsible for the hydrolysis of ATP.[178,333,363,365] It has been suggested that the ectonucleotidases may regulate plasma nucleotide levels[83] and thus have a protective function by keeping extracellular ATP and adenosine levels within physiogical limits.[431] Three types of ectonucleotidases have been described: ecto-ATPases, ecto-ADPases and ecto-5'-nucleotidases.[431] In blood, these enzymes have also been detected on erythrocytes,[431] leukocytes,[83] B-lymphocytes,[18] and both helper and cytotoxic T-lymphocytes.[143] In the extracellular space ATP is subject to breakdown by ecto-enzymes and xanthine oxidase to form uric acid which is excreted in urine. In the present study, continuous ATP infusions induced significant and dose-dependent increases in erythrocyte ATP concentrations with plateau levels between 24 and 30 hours. This is in line with the findings of Haskell et al.[188] who infused ATP for 96 hours and observed no further rise of ATP in erythrocytes after 24 hours of ATP infusion. Uric acid plasma concentrations at 24 and 30 hours were increased when compared with initial uric acid plasma concentrations.

Low-dose ATP infusions (25-40 μg/kg.min) induced a significant increase in erythrocyte ATP concentrations parallel with increasing plasma uric acid concentrations. At middle-dose ATP infusions (45-60 μg/kg.min) no further rise in erythrocyte ATP concentrations was measured, whereas the degradation of ATP was further increased as demonstrated by a further rise in uric acid concentrations. High-dose ATP infusions (65-75 μg/kg.min) induced not only an even larger increase in uric acid levels, but also showed an additional increase in erythrocyte ATP levels as compared to low and middle-dose ATP infusion. In order to exclude the possibility of ATP-induced inhibition of the CL_R of plasma uric acid, we measured the rate of uric acid clearance in six patients who collected urine before and during ATP infusion. None of these patients who were treated with either low, middle, or high-dose ATP infusions, showed any change in uric acid CL_R (P=0.91; paired t-test).

A strong dose-level relationship was observed between ATP dose and uric acid concentrations in plasma. In the phase I study by Haskell et al.,[188] ATP-infused subjects received allopurinol as a standard therapy because the first two subjects treated with ATP developed asymptomatic hyperuricemia and uricosuria. In the present long-term intervention study, we chose not to give allopurinol since the risks of asymptomatic hyperuricaemia are known to be small, and because allopurinol

treatment may itself induce side effects such as renal insufficiency, gastric irritation, diarrhea, skin rash, and occasionally vasculitis.[183,206]

We found a mono-exponential rate of ATP disappearance from erythrocytes with a *t½* of approximately 6 hours. This is in line with the finding of Rapaport et al.[345] that after uptake of ATP by erythrocytes the subsequent release of ATP into plasma is relatively slow. In animal studies, ATP in plasma is rapidly broken down. In these studies ATP was administered as a bolus, so that erythrocyte ATP levels would increase only slightly. In rabbits, 40 seconds after an intravenous bolus injection of ATP only 1% of ATP was detected in whole blood.[388] Similarly, a bolus of ATP was found to be almost completely cleared during a single passage through either perfused dog lung[35] or perfused guinea pig heart.[323] In a perfused rat lung, Ryan et al.[363] showed a *t½* of labeled ATP of less than 3 seconds.

The few reports on physiological ATP concentrations in human plasma give mean values ranging from 0.02 μM[151] to 10.9 μM.[185] Determination of ATP in plasma is technically difficult as the concentration in plasma is three orders of magnitude lower than within the cells. It is obvious that even limited hemolysis could cause considerable elevation of plasma adenine nucleotides[184] since as little as 0.1% hemolysis during sampling would double the ATP concentration in plasma.[184] In addition, EDTA can induce selective nucleotide release from cells.[83] Other factors influencing plasma ATP measurements may be released from platelets,[364] thrombus formation,[289] partial arterial occlusion, and exercise.[151] Furthermore, the time between venous blood sampling and centrifuging may have a marked influence on plasma ATP concentrations. It is therefore not surprising that we obtained scattered data when attempting to measure ATP in plasma prior to and during ATP infusions.

In conclusion, our study in cancer patients shows that during continuous ATP infusions in a dose range of 25-75 μg/kg.min, ATP is taken up by the erythrocytes and reaches dose-dependent plateau levels within erythrocytes which are 1.5 to 1.7 fold higher than baseline values at approximately 24 hours. After discontinuation of the ATP infusion, *t½* of ATP disappearance from erythrocytes was approximately 6 hours. ATP infusions can be administered without side effects in the majority of courses, and when side effects occur they are mild and transient. Further study of ATP pharmacokinetics is warranted in order to optimize the ATP dosage scheme for further clinical trials in cancer and other diseases. It would be useful to perform a

study with infusions of 25 or 75 μg/kg.min ATP, respectively, in order to show whether there are differences in clinical results between low and high dose ATP. Courses should have a minimal duration of 24 hours to reach a plateau ATP level in erythrocytes. Patients suffering from cardiopulmonary disease may receive low dose ATP infusion in order to avoid side effects. In view of the observation that hyperuricaemia is only transient and asymptomatic, it seems unnecessary to give allopurinol in parallel to ATP infusions.

Acknowledgments

We are grateful to L.M. Hanff, C.T.M. Kerkvliet, M.A.L. Pluim, and A.G. Vulto, pharmacists, for coordinating and supervising preparation of ATP infusions. We would like to thank the Netherlands Organization for Scientific Research (NWO) for financial support.

4.2

ADENOSINE TRIPHOSPHATE INFUSION INCREASES LIVER ENERGY STATUS IN ADVANCED LUNG CANCER PATIENTS: AN *IN VIVO* ^{31}P MAGNETIC RESONANCE SPECTROSCOPY STUDY

Submitted for publication

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ABSTRACT

Decreased liver adenosine 5'-triphosphate (ATP) levels and phosphorylation status have recently been observed in lung cancer patients with weight loss. The aim of the present study was to investigate whether ATP infusion restores liver energy status in advanced lung cancer patients using ^{31}P magnetic resonance spectroscopy (MRS).

Nine patients with advanced non-small-cell lung cancer were studied one week before (baseline) and at 22 to 24 hours of continuous ATP infusion (37 to 75 μg/kg.min). After an overnight fast, localized hepatic ^{31}P MR spectra (repetition time 15 sec) were obtained and analyzed for ATP and P_i content. Ten healthy subjects (without ATP infusion) were studied as a control.

Liver ATP levels in lung cancer patients increased from 8.8±0.7% (of total MR-detectable phosphate; mean ± SEM) at baseline to 12.2±0.9% during ATP infusion ($P<0.05$). These levels were similar to those in healthy subjects (11.9±0.9%). In patients with ≥ 5% weight loss, the increase in ATP levels was most prominent (from 7.9±0.7 to 12.8±1.0%, $P<0.01$).

In conclusion, ATP infusion restores hepatic energy levels in patients with advanced lung cancer, especially in weight-losing patients. These changes could have beneficial effects on the nutritional status of weight-losing lung cancer patients.

INTRODUCTION

Weight loss is a common phenomenon in lung cancer patients and contributes significantly to the high morbidity and mortality in this disease.[116,407] Alterations in intermediary host metabolism have been frequently described, including elevated protein turnover,[191,265] glucose production[228,425] and Cori cycle activity.[198] In an *in vitro* study, gluconeogenesis in isolated hepatocytes from sarcoma-bearing rats was increased during incubation with lactate as compared to hepatocytes from healthy rats.[434] This increased gluconeogenesis resulted in a 42% decrease in adenosine 5'-triphosphate (ATP) levels, whereas in healthy rats no change in ATP was observed.[434] This suggests that elevated rates of gluconeogenesis in the cancer-

bearing host may put an increased demand on the energy stores and may contribute to weight loss.

Alterations in hepatic energy status have been well documented in animal models of various tumors. Decreased liver phosphorylation status, as observed by increased P_i/ATP ratios, was detected in rats bearing prostate tumors[102] or sarcomas[12,51,374] and was correlated with increasing tumor burden.[374] It is noteworthy that these alterations in liver energy status were already detected before the development of weight loss.[169] Decreased liver ATP levels as detected by ^{31}P magnetic resonance spectroscopy (MRS) were reported in patients with various tumor types.[104] Recently, we reported decreased hepatic ATP and phosphorylation status in weight-losing lung cancer patients, when compared with weight-stable patients and healthy controls.[247]

In mice bearing colon tumors daily intraperitoneal ATP injections increased total liver and erythrocyte ATP pools.[348] These increases were associated with a significant inhibition of host weight loss.[348] The present study was aimed at investigating whether continuous ATP infusion increases hepatic ATP levels and phosphorylation status in advanced lung cancer patients.

SUBJECTS AND METHODS

Subjects

The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. All patients signed informed consent. Eligible for the study were patients with histological or cytologically proven non-small-cell lung cancer (NSCLC), stage IIIB or IV (WHO grading system) without curative options, and Karnofsky index of 60% or more. Patients with cognitive dysfunction or liver, renal, respiratory, or heart failure, and patients undergoing surgery, concurrent chemotherapy, or radiotherapy involving all lesions were excluded. Ten healthy subjects (age range 37 to 69 years; body weight 76.4 ± 3.6 kg (mean $\pm$ SEM)) were included as a control.

Study design

In nine patients (seven males, two females) liver energy and phoshorylation status were studied using ^{31}P MRS one week before (baseline) and during continuous ATP

infusion, i.e., 22 to 24 hours after starting ATP infusion. The patients received ATP infusion doses varying from 37 to 75 μg/kg.min. Clinical characteristics of these patients are listed in **Table 4.2.1.** Healthy control subjects were only studied at baseline. ATP infusions (6.1 mg ATP-Na_2·3H_2O per ml NaCl 0.9%) were given with an initial dose of 20 μg/kg.min and increased by increments of 10 μg/kg.min every 30 min until a maximum dose of 75 μg/kg.min, or until the maximally tolerated dose had been reached. If any side effects occurred, the dose was reduced to the last given dose or further until side effects disappeared, usually within minutes after lowering the ATP dose. Thereafter, ATP was infused at a continuous rate. The most frequently occurring side effects were chest discomfort and the urge to take a deep breath.

Table 4.2.1. Characteristic of non-small-cell lung cancer patients

Patient (no)	Gender (m/f)	Age (y)	Tumor stage	Weight (kg)	Weight change* (%)	ATP dose (μg/kg.min)
1	m	85	3B	54.9	-10.2	75
2	m	75	3B	70.8	-1.7	67
3	m	71	3B	71.8	-4.2	75
4	m	49	3B	77.1	-1.0	75
5	m	76	3B	91.1	-11.7	42
6	f	57	4	56.6	-22.5	40
7	f	52	4	64.2	-9.0	75
8	m	68	4	65.1	-0.3	37
9	m	31	4	71.6	-20.4	67
Mean ± SEM		63 ± 6		69.2 ± 3.7	-9.0 ± 2.7	61 ± 6

* Prior to MRS

^{31}P MR spectroscopy of the liver

Subjects were studied after an overnight fast. MRS studies were performed with a whole-body MR system equipped with a Helicon magnet operating at 2 T (Vision Magnetom, Siemens AG, Erlangen, Germany). A 16 cm diameter transmit/receive ^{1}H/^{31}P surface coil was used for MRI localization, shimming and ^{31}P MR

spectroscopy. Elastic bands were used for positioning the coil lateral to the liver in the mid-axillary plane. Field homogeneity achieved in shimming resulted in water peak line widths which were usually less than 40 Hz (≈0.5 ppm). After obtaining an image of the region of interest, a one-dimensional chemical shift imaging (1D-CSI) sequence was applied on a transverse slice of 4 cm centered on the surface coil and the liver (1x4 phase-encoded matrix, field of view 40x40 cm^2), yielding volumes of 40x10x4 cm^3.[394] Spectra were collected with a 640 msec Hanning-sinc shaped radio frequency pulse resulting in a flip angle of 135° in the center of the coil, where a methylene diphosphonate reference sample was positioned, and 60° (weighted average) in the liver volume. Spectra with repetition time of 15 sec (10 acquisitions) were obtained in each patient examination. Time domain data were Fourier transformed after Gaussian multiplication (center: 0 msec, width 30 msec) and phase corrected. Quantification of spectral peak areas was performed using Numaris-3 software package (Siemens AG, Erlangen, Germany) including polynomial baseline correction followed by frequency domain curve fitting.[393] Metabolite concentrations were calculated from peak areas and expressed relative to total MR-detectable phosphate as previously described.[104] ATP infusion did not change total liver phosphate levels (data not shown).

Statistics

Results are presented as means ± standard error of the mean (SEM). Changes of liver ATP concentrations and P_i /ATP ratios during ATP infusion were tested for significance using Student's paired *t*-test. Between-group differences were compared using the Student's *t*-test for independent groups. Pearson's correlation coefficients were calculated to investigate possible relationships between parameters. *P*-values of <0.05 indicated statistical significance.

RESULTS AND DISCUSSION

Baseline ATP levels in the liver of lung cancer patients were significantly lower than in healthy subjects ($P<0.05$, **Table 4.2.2**). When patients were stratified for presence of weight loss, it was shown that ATP levels in patients with ≥5% weight

loss were as much as 34% lower than in healthy subjects ($P<0.05$, **Table 4.2.2**). In contrast, ATP concentrations in patients with <5% weight loss were not significantly different from healthy controls. During ATP infusion, liver ATP levels in lung cancer patients increased by 48±15% ($P<0.05$) reaching levels similar to those in healthy subjects (**Table 4.2.2**). In lung cancer patients with ≥ 5% weight loss, ATP increased by 64±9% ($P<0.01$).

Table 4.2.2. Liver energy and phosphorylation status as observed by ^{31}P MRS in healthy subjects and advanced lung cancer patients before and during infusion of adenosine triphosphate (37 to 75 μg/kg.min)[1]

	Healthy	Lung cancer		
	(n = 10)	Total (n = 9)	<5% weight loss (n = 4)	≥5% weight loss (n = 5)
ATP[2]				
Baseline	11.9 ± 0.9	8.8 ± 0.7*	10.3 ± 1.3	7.9 ± 0.7*
During ATP infusion	-	12.2 ± 0.9†	11.5 ± 1.5	12.8 ± 1.0‡
P_i/ATP				
Baseline	0.73 ± 0.16	0.94 ± 0.15	0.77 ± 0.21	1.07 ± 0.21
During ATP infusion	-	0.68 ± 0.11	0.68 ± 0.11	0.68 ± 0.19

[1]Mean±SEM
[2]Expressed as percentage of total MR-detectable phosphate
Significance of difference from healthy subjects: *) $P<0.05$; significance of difference from baseline: †) $P<0.05$, ‡) $P<0.01$

P_i/ATP ratios during ATP infusion decreased to levels close to those in healthy subjects, although none of these changes were statistically significant. No significant correlations were observed between ATP dose and the change in liver ATP or phosphorylation status (r=0.08 and r=0.28, respectively).

To our knowledge, no earlier studies have addressed the effect of ATP infusion on ATP concentrations in the liver of human subjects. In tumor bearing mice, single intraperitoneal injections of ATP increased total liver ATP pools from 3.2 to 8.3 mM.[348] Following this expansion of liver ATP pools, erythrocyte ATP levels increased from 0.6 to 2.4 mM.[348] In patients in the present study we measured whole blood ATP concentrations of 0.71±0.02 mM at baseline. After 22 to 24 hours of

ATP infusion, blood ATP levels increased to plateau levels of 53±3 to 69±2% above baseline, depending on the ATP dose given.

It could be argued that the observed increase in liver ATP levels in the present study might be caused by contamination of MR spectra by ATP in blood circulating in the liver voxel, due to elevated erythrocyte ATP concentrations and/or vasodilation. Although ATP concentrations in humans are three to four times higher in liver[38,202] than in erythrocytes,[112,429,463] and ATP infusions were reported to even further increase liver ATP levels in mice,[348] no data are available on the contribution of ATP from blood to the ^{31}P MR signal of liver ATP in humans *in vivo*. Therefore, an estimation of ATP contamination from blood was made using in this study measured whole blood ATP concentrations of 0.7 mM, and assuming a liver ATP concentration of 2.5 mM,[202] and a liver blood volume of 0.25 ml/g wet weight.[179] Results showed a relative contribution of blood ATP to total liver ATP in the MRS liver voxel of ≈9%. During ATP infusion, whole blood ATP concentrations increased to 1.1 mM which would give a ≥5% increase in total MRS-observed ATP concentrations in the liver voxel. This is one order of magnitude less than the 50 to 60% increase in liver ATP levels.

Yet another effect of ATP is vasodilation[6,359] mediated by purinergic receptors P1 and P2 which are located on endothelial cells of blood vessel walls.[39,217] Vasodilation in the liver could affect the ^{31}P MRS measurement by increasing blood volume within one liver voxel. Indeed, increased liver ATP levels were observed by ^{31}P MRS in rat liver after dopamine administration, probably due to increased hepatic blood flow.[312] Based on reported maximal changes in hepatic blood volume in humans between -20%[160] and +20%[17] depending on the stimulus used, increased blood volume in the liver could account for an increase in liver ATP levels by 8%. Again, this is one order of magnitude short of the observed effect. If liver ATP values are corrected for the highest possible blood contribution from combined vasodilation and raised erythrocyte ATP levels (18%), the increase in patients with 5 % weight loss still remains highly significant ($P<0.01$). We therefore conclude that the increase in total liver ATP as measured by ^{31}P MRS during ATP infusion does reflect a significant rise in ATP levels in liver tissue and is not caused by altered ATP contamination from blood.

In conclusion, this study shows that intravenous ATP infusion is able to restore depleted liver ATP pools in patients with advanced lung cancer to levels similar to or above those in healthy subjects. This effect is most marked in weight-losing lung cancer patients. The increase in liver ATP pools could have beneficial effects on the nutritional status of weight-losing lung cancer patients.

Acknowledgment

We thank P. Kappert for assistance during the ^{31}P MRS measurements, M. Oudkerk for providing MR-facilities, and J.H.P. Wilson for useful comments on the manuscript.

4.3

EFFECTS OF ADENOSINE TRIPHOSPHATE INFUSION ON GLUCOSE TURNOVER AND GLUCONEOGENESIS IN PATIENTS WITH ADVANCED LUNG CANCER

Clinical Science (in press)

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ABSTRACT

Cancer cachexia is associated with elevated lipolysis, proteolysis, and gluconeogenesis. Recently, adenosine 5'-triphosphate (ATP) infusion was found to significantly inhibit loss of body weight, fat mass and fat-free mass in advanced lung cancer patients. The present study was aimed at exploring the effects of ATP on whole body glucose turnover, alanine turnover, and gluconeogenesis from alanine. Twelve patients with advanced non-small-cell lung cancer (NSCLC) were studied one week before and at 22 to 24 hours of continuous ATP infusion. After an overnight fast, turnover rates of glucose and alanine, and gluconeogenesis from alanine were determined using primed-constant infusions of [6,6-$^{2}H_2$]-glucose and [3-^{13}C]-alanine. Thirteen NSCLC patients and eleven healthy subjects were studied as control groups without ATP infusion. During high-dose ATP infusion (75 μg/kg.min), glucose turnover was 0.62±0.07 mmol/kg.h compared to 0.44±0.13 at baseline (P=0.04). For gluconeogenesis a similar but non-significant trend was observed (baseline, 0.30±0.16; during ATP, 0.37±0.13 mmol/kg.h (P=0.08). At lower ATP doses (37 to 50 μg/kg.min) these effects were not detected. The relative increase of glucose turnover during ATP infusion compared to baseline showed a significant correlation with the ATP dose (r=0.58, P=0.02). No change in alanine turnover was observed at any ATP dose. Results of this study indicate an increase in glucose turnover during high-dose ATP infusion compared to baseline. During high-dose ATP infusion glucose turnover is similar to that during low-dose ATP infusion and to control NSCLC patients. Between ATP infusions, glucose turnover in the high-dose ATP-treated patients is significant lower than in the low-dose and control NSCLC patients (P=0.04 and P=0.03, respectively), and similar to that in healthy subjects. This would suggest that repeated high-dose ATP infusions may inhibit glucose turnover between infusion periods.

INTRODUCTION

Cachexia is a common phenomenon in lung cancer patients, and contributes significantly to morbidity and mortality.[94,116,407] Cancer cachexia is associated with

metabolic alterations including elevated lipolysis,[128,391] protein breakdown,[266,306,355] and increased glucose turnover.[192] In patients with advanced cancer increased glucose production has been shown from lactate,[389] glycerol,[267] and alanine.[248,455]

It has been argued that the liver plays an important role in the metabolic alterations contributing to the development of weight loss in cancer.[51,220] In liver[101,374,434] and skeletal muscle[374] of tumor bearing rats, significantly reduced adenosine 5'-triphosphate (ATP) levels have been demonstrated, which were associated with increased gluconeogenesis[434] and increased gluconeogenic enzyme activity.[307] In mice bearing CT26 colon tumors, daily intraperitoneal injections of ATP, adenosine 5'-monophosphate or adenosine for 10 consecutive days significantly inhibited host weight loss.[348] This inhibition was associated with increased hepatic ATP pools.[347,348]

In a randomized clinical trial in patients with advanced non-small-cell lung cancer (NSCLC), we recently demonstrated beneficial effects of ATP infusions on body weight, muscle strength,[7] skeletal muscle mass, and body cell mass.[4] The present pilot study was aimed at exploring effects of ATP infusion on whole-body glucose turnover, alanine turnover and gluconeogenesis from alanine. Based on the beneficial clinical outcomes we hypothesized that ATP would have an inhibitory effect on these processes. Alanine was selected as a gluconeogenic substrate because this amino acid is the key protein-derived precursor of glucose utilized by the liver,[411] and one major component of muscle protein degradation.

SUBJECTS AND METHODS

Subjects and study design

Patients with histologically or cytologically proven NSCLC (stage IIIB or IV) without curative options, and a Karnofsky index of 60% or more were eligible for the study. Patients with cognitive dysfunction or liver, renal, respiratory, or heart failure, and patients undergoing surgery, concurrent chemotherapy, or radiotherapy involving all lesions were excluded. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. All participants signed informed consent. Eleven healthy subjects and 13 NSCLC cancer patients without ATP

infusion were included as control groups. The control NSCLC patients were matched for the ATP-infused NSCLC patients for age, sex, and weight loss.

In the clinical trial, 28 patients were randomized to ATP treatment, to receive a maximum of 10 ATP courses of 30 hours each: seven courses at 2-week intervals, followed by three ATP courses at 4-week intervals. ATP infusions (6.1 mg ATP-$Na_2 \cdot 3H_2O$ per ml 0.9% saline) were started with an initial dose of 20 μg/kg.min and were increased by increments of 10 μg/kg.min every 30 minutes until a maximum dose of 75 μg/kg.min, or until the maximally tolerated dose had been reached. If any side effects occurred, the dose was reduced to the last given dose or further until side effects disappeared, usually within minutes after lowering the ATP dose. Thereafter, ATP was infused at a continuous rate. The most frequently occurring side effects were chest discomfort and the urge to take a deep breath.[5]

In 12 out of the 28 ATP-allocated patients, glucose turnover and gluconeogenesis from alanine were studied one week before (baseline) and during a ATP course (22 to 24 hours after starting ATP infusion). Seven patients received low-dose infusions of 37-50 μg/kg.min ATP, and five high-dose infusions of 75 μg/kg.min ATP.

The subjects were studied in the morning after an overnight fast. A cannula (0.8x25 mm) was placed in the left cubital vein for the infusion of stable isotope tracers. In the contralateral cubital vein, an identical cannula was positioned for blood sampling. To study gluconeogenesis, a solution was prepared containing [6,6-$^2H_2$]-D-glucose, 98 atom%, and [3-^{13}C]-L-alanine, 99 atom% (Mass Trace, Woburn, USA), in water and this was sterilised by autoclaving in glass vials. A priming dose of 0.03 mmol/kg [6,6-$^2H_2$]-D-glucose was administered followed by a continuous infusion of 0.01 mmol/kg.h [6,6-$^2H_2$]-D-glucose for 90 minutes. Simultaneously, a priming dose of 0.08 mmol/kg [3-^{13}C]-L-alanine was given followed by a continuous infusion of 0.04 mmol/kg.h [3-^{13}C]-L-alanine during 90 minutes. Both tracer solutions were infused using calibrated syringe pumps (Perfusor^R fm, Braun, Germany).

Venous blood samples were drawn immediately before the isotope infusions were started, and at 10 min intervals from 30 to 90 min, i.e., after steady state conditions during the tracer infusions had been achieved.

Analytical methods

Blood samples were collected in tubes containing lithium heparin (Becton Dickinson Vacutainer[R], Meylan Cedex, France) and immediately stored on ice. After centrifugation (10 min., 1200 *g*, 4 °C), the plasma was collected and stored at -20 °C until analyzed. An aliquot of the infusate was analysed to document the actual concentrations of the tracers in each study.

Blood glucose concentrations were determined enzymatically with a glucose-oxidase / peroxidase assay system (Boehringer Mannheim, Mannheim, Germany). Plasma alanine was measured enzymatically as described by Williamson.[468] Isotopic enrichments were determined using the following procedures. Plasma was deproteinized by adding 0.3 M barium hydroxide (Sigma Diagnostics, St.Louis) and 0.3 M zinc sulphate (Merck, Darmstadt, Germany). After centrifugation (8 min,15000 *g*, 4 °C) the supernatant was applied to an ion exchange column (mixed bed: AG50W-X8 and AG1-X8, 200-400 mesh, 0.2 *g* each; BioRad, California). Glucose and alanine were eluted from the column using water and 4 M ammonium hydroxide (Merck, Darmstadt, Germany), respectively, and dried under nitrogen.

A glucose derivative (aldonitril penta acetate) was prepared according to Varma et al.[444] An alanine t-butyldimethylsilyl derivative was prepared as described by Chaves Das Neves et al.[72]

Isotopic enrichments were measured by injecting 1 µl samples with a split ratio of 50:1 on a fused silica capillary column of 25 m x 0.22 mm, coated with 0.11 µm HT5 (SGE, Victoria, Australia). The relative isotopic enrichments of deuterated glucose and carbon-13 alanine were determined using a Carlo Erba GC8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (GC-MS) (Intersience B.V., Breda, The Netherlands) in electron impact ionisation mode. In general, the variation coefficient in enrichment was 0.2 mole% for both [6,6-$^{2}H_2$]-glucose and [3-^{13}C]-alanine measurement, and no concentration effect was observed at this mole% enrichment level. Ions were selectively monitored at mass per unit charge (*m/z*) 187 for natural glucose and 189 for the deuterated molecule. The isotopic enrichment of [3-^{13}C]-alanine was determined at the *m/z* ratios 260 and 261 for carbon-12 and carbon-13 alanine, respectively.[278]

Total enrichment of carbon-13 glucose was measured separately (aldonitril penta-acetate derivation) using a gas chromatograph combustion isotope ratio mass

spectrometer (GC-IRMS) (Optima, Micromass UK, Middlewich, Cheshire, Great Britain). The [^{13}C]-glucose enrichment in atom% excess (APE) was monitored after combustion to CO_2 at mass 44 for carbon-12 and 45 for carbon-13, respectively.

Calculations

Whole body rate of appearance (Ra) of glucose was calculated during steady state following a one-compartment model, using the equation:

$$Ra = F \times ((IE_i / IE_{ecf}) - 1),$$

where F is the isotope infusion rate (mmol/kg.h), IE_i the isotopic enrichment of the infusate (mole% excess), and IE_{ecf} the isotopic enrichment of the extracellular fluid (mole% excess).[471] The percentage glucose produced from alanine equals:

$$IE\ ^{13}C\text{-glucose}_{plasma} / (IE\ ^{13}C\text{-alanine}_{plasma} \times 0.33)$$

The correction factor in formula (2) is applied in order to correct for the number of carbons in both glucose and alanine. Gluconeogenesis from alanine (mmol/kg.h) was then obtained as:

$$\%\ \text{glucose from alanine} \times Ra\ ([^2H_2]\text{-glucose})$$

Finally, the percentage of alanine converted into glucose was calculated by dividing the rate of gluconeogenesis from alanine by the rate of appearance of alanine.[91]

Statistical analysis

Results are presented as means ± standard deviation (SD). Changes in turnover between baseline and ATP infusion were tested for significance by the two-tailed Wilcoxon's signed rank test. Results of independent groups were tested for significance by the Mann Whitney U test. Correlation between variables were calculated as Spearman's rank correlation coefficients. Results were considered statistically significant with a *P*-value <0.05.

RESULTS

Study population

Characteristics of the study population are shown in **Table 4.3.1**. Twelve lung cancer patients (nine males, three females) with a mean (±SD) age of 64±13 years and

weight of 72.7±13.0 kg participated in the study. Mean weight loss was 7.1±9.0 %. Patients had received an average of 2.3±2.1 previous ATP courses. Furthermore, 11 healthy subjects (three males, eight females; age 56±12 years; body weight 79.5±55.0 kg; no weight loss) and 13 NSCLC cancer patients (10 males, three females; age 64±14 years; body weight 67.8±13.1 kg; weight loss 6.3±9.9 kg) were included as control groups without ATP infusion.

Table 4.3.1. Clinical details of non-small-cell lung cancer patients treated with ATP or not treated with ATP (controls) and healthy subjects (mean ± standard deviation or number)

	Healthy subjects	Cancer patients	
	no ATP	no ATP	ATP
Subject (no)	11	13	12
Sex (m/f)	3 / 8	10 / 3	9 / 3
Age (y)	56 ± 12	64 ± 14	64 ± 13
Weight (kg)	79.5 ± 55.0	67.8 ± 13.1	72.7 ± 13.0
Weight loss (%)	0 ± 0	6.3 ± 9.9	7.1 ± 9.0
Previous ATP courses	-	-	2.3 ± 2.2
ATP-dose (μg/kg.min)	-	-	57 ± 16

Glucose and alanine metabolism

Baseline plasma glucose and alanine concentrations were 4.9±0.8 mM and 0.36±0.02 mM, respectively, and did not change during ATP infusion. When data were analyzed for the ATP-treated patient group as a whole, turnover rates of glucose and alanine, and gluconeogenesis from alanine during ATP infusion did not differ significantly from baseline. However, as shown in **Figure 4.3.1**, stratification for ATP dose revealed clear differences according to ATP dose. In patients with low-dose ATP infusion (37-50 μg/kg.min), no change was detected in glucose turnover (baseline, 0.58±0.10 mmol/kg.h; during ATP infusion, 0.56±0.13 mmol/kg.h) or gluconeogenesis from alanine (baseline, 0.34±0.17 mmol/kg.h; during ATP infusion, 0.35±0.24 mmol/kg.h). In contrast, in patients with high-dose ATP infusion (75 μg/kg.min), glucose turnover was 0.44±0.13 mmol/kg.h at baseline and

0.62±0.07 mmol/kg.h during ATP infusion (change: P=0.04). Gluconeogenesis from alanine amounted to 0.30±0.16 mmol/kg.h at baseline and to 0.37±0.13 mmol/kg.h during ATP infusion (P=0.08). The change of glucose turnover from baseline was significantly correlated with the ATP-dose [r=0.58, P=0.02]. No such dose-effect relation was demonstrated for gluconeogenesis from alanine.

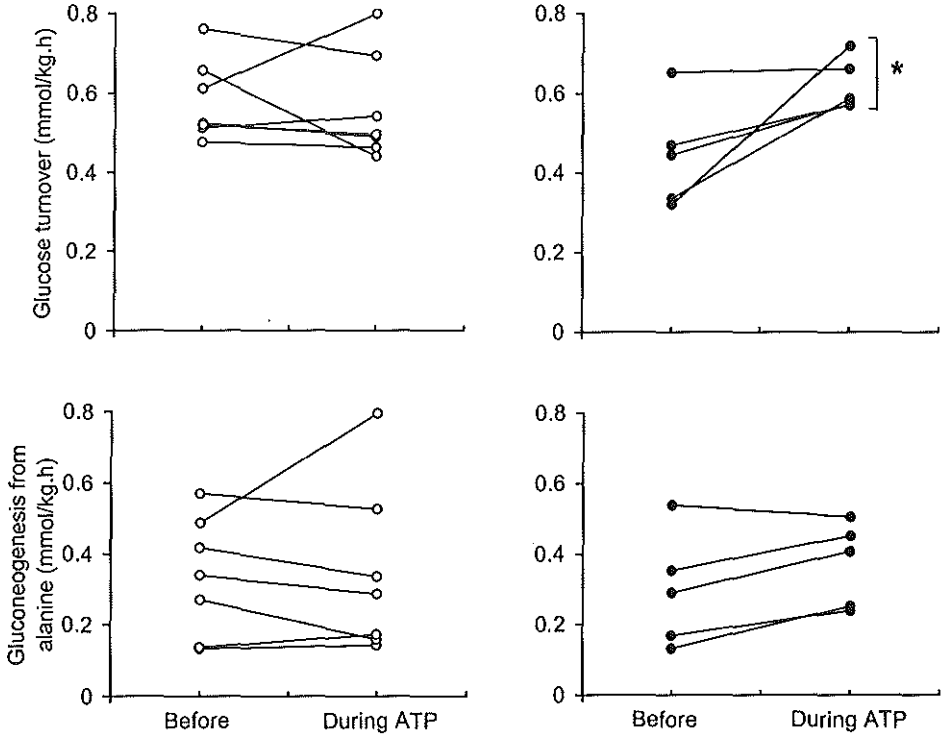

Figure 4.3.1. Whole body rate of turnover of glucose and gluconeogenesis from alanine before and during (22-24 hours) low-dose (37-50 μg/kg.min; O; n=7) and high-dose (75 μg/kg.min; ●; n=5) ATP infusion. Turnover rates were assessed using primed-constant infusions of [6,6-$^{2}H_2$]-glucose and [3-^{13}C]-alanine. *) Significantly different from baseline (P<0.05).

Mean glucose turnover rates in ATP-treated and control lung cancer patients, and healthy subjects are shown in **Figure 4.3.2.** Glucose turnover rates in lung cancer

patients during high-dose ATP infusion were similar to control (untreated) lung cancer patients, but significantly higher than in healthy control subjects (P=0.047). In contrast, baseline glucose turnover rates in high-dose ATP-treated patients were significantly lower when compared with both low-dose ATP and control cancer patients (P=0.04 and P=0.03, respectively), but were at a level similar to or even lower than in healthy subjects.

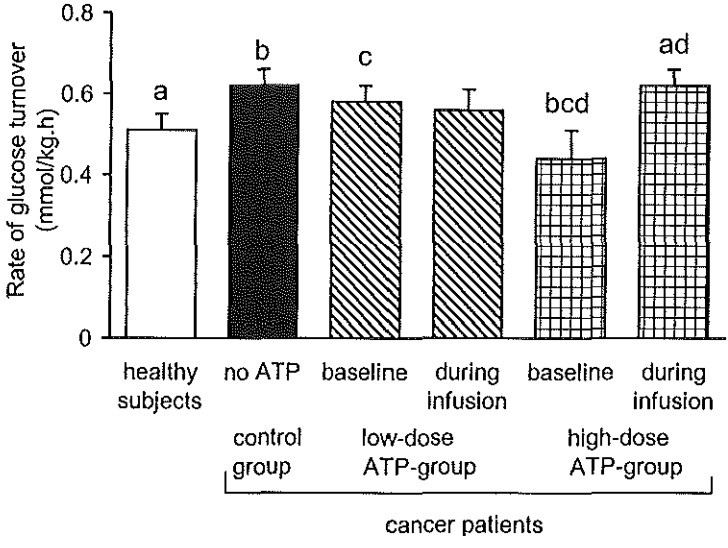

Figure 4.3.2. Whole body rate of glucose turnover in 11 healthy subjects (white bar), 13 control non-small-cell lung cancer (NSCLC) patients (black bar), seven low-dose ATP (37-50 μg/kg.min) treated NSCLC patients (hatched bars), and five high-dose ATP (75 μg/kg.min) treated NSCLC patients (cross hatched bars). Turnover rates were assessed using primed-constant infusions of [6,6-$^2H_2$]-glucose. Bars and error bars represent means ± SEM. Bars with the same superscript letter are significantly different (P<0.05).

DISCUSSION

The aim of the present pilot study was to explore effects of intravenous ATP infusion on whole-body glucose turnover, alanine turnover, and gluconeogenesis from alanine,

as possible pathways contributing to the reported beneficial effects of ATP on body weight and body composition in advanced lung cancer patients. The effect of ATP on gluconeogenesis from the amino acid alanine was studied because ATP was shown to inhibit loss of skeletal muscle mass[4] and muscle strength.[7] Turnover measurements were performed at 22 to 24 hours after starting ATP infusion, i.e., after ATP had reached plateau levels in erythrocytes.[5] All but one patients were studied during one of the subsequent ATP infusions.

During high-dose ATP infusion (75 μg/kg.min), whole-body glucose turnover increased by approximately 50%, whereas no change whatsoever was shown at low-dose ATP infusion (37-50 μg/kg.min). The ATP-induced increase of gluconeogenesis from alanine explained only part of the increase in total hepatic glucose production. This would suggest that ATP may also stimulate gluconeogenesis from other substrates and glycogenolysis. *In vitro* studies show that ATP administration stimulates gluconeogenesis from lactate,[133,366] pyruvate,[62,366] and glutamine.[133,229,366,406] Studies in isolated hepatocytes[64,97,219] and perfused rat liver[59,244] showed that ATP also stimulates glycogenolysis by activating glycogen phosphorylase. It is conceivable that high-dose ATP infusion evoked immediate stimulation of glycogenolysis in our patients since turnover measurements were performed after an overnight fast of 10 to 12 hours. Glycogen stores in healthy subjects were reported to be depleted only after 36 hours of fasting;[305] no data on glycogen stores in cancer patients are available.

The mechanisms responsible for increased glucose turnover and gluconeogenesis during 24 hours of high-dose ATP infusion remain to be elucidated. Mechanisms might include receptor-stimulating and catecholamine hormone-stimulating effects of ATP. Studies in isolated hepatocytes showed that extracellular ATP induced phosphatidylinositol hydrolysis, intracellular Ca^{2+}-mobilization, and extracellular Ca^{2+}-influx by stimulation of surface purinergic P2 receptors[64,311] which are involved in the control of gluconeogenesis[13] and glycogenolysis.[218] Furthermore, ATP was shown to act as a cotransmitter of the catecholamine noradrenaline in the nervous system, and was suggested to modulate the release of other neurotransmitters.[177] Noradrenaline is an activator of gluconeogenesis[105,366] and glycogenolysis.[105]

However, the above reasoning would only apply to the immediate effects of ATP infusion within NSCLC subjects. Notably, basèline glucose turnover rates in the present

study were significantly lower in patients who had previously received high-dose ATP infusions when compared to patients who had received low-dose ATP or no ATP at all (controls). Since patients in the high-dose group had already undergone an average of two ATP courses before the present turnover measurements, our data would suggest that the previous ATP infusions had induced a reduction in whole body glucose turnover on a longer term, which would be consistent with the observed inhibition of weight loss in our long-term clinical trial in NSCLC patients. This hypothesis is supported by the observation that baseline glucose turnover rates in patients who had already received one or more high-dose ATP infusions were similar to or even lower than in control subjects.

In contrast, baseline gluconeogenesis from alanine did not differ significantly between low- and high-dose ATP groups. This would be consistent with the finding in several experimental *in vivo* studies that the ATP degradation product adenosine inhibited gluconeogenesis from lactate,[241,242,264] pyruvate[242,264] and glutamine,[242,264] but not from alanine.[241, 242] In the present human study, direct effects of ATP on glucose turnover and gluconeogenesis cannot be separated from potential effects of adenosine.

In conclusion, the present study suggests that, despite a temporary dose-dependent increase in whole-body glucose turnover during high-dose ATP infusion in advanced lung cancer patients, ATP seems to inhibit glucose turnover in the long run. This hypothesis is supported by the observation of reduced baseline glucose turnover rates, to levels similar to turnover in healthy subjects, in patients who had previously received an average of two courses of high-dose ATP. Larger studies are indicated to confirm and elaborate these differential direct and long-term effects of ATP infusions on gluconeogenesis in NSCLC patients.

Acknowledgment

We are grateful to J.L.D. Wattimena for excellent technical assistance with the preparation and the actual performance of mass spectrometry analyses.

4.4

GROWTH INHIBITORY EFFECTS OF ADENOSINE TRIPHOSPHATE ON LUNG CANCER CELLS

Submitted for publication

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ABSTRACT

Background: Preliminary clinical data suggest that adenosine 5'-triphosphate (ATP) may inhibit lung tumor growth. Because studies to ATP on lung cancer cells are lacking, the aim of the present study was to explore effects of extracellular ATP on the growth and morphology of human lung tumor cells.

Methods: Five human lung tumor cell lines derived from tumors with different cellular characteristics i.e., a small cell carcinoma (GLC4), a large cell carcinoma (H460), a squamous cell carcinoma (H520), a mesothelioma (MERO82), and a papillary adenocarcinoma (H441) were exposed to 0, 0.5, 1, 2, and 3 mM ATP. Total cell numbers and dead or damaged cells were measured on days 1, 2, and 3.

Results: ATP induced a significant, dose-dependent growth inhibition in GLC4, H460, H520, and MERO82 cells. In contrast, H441 cells showed already maximal inhibition at 0.5 mM. Compared to untreated control cell lines, a significant growth inhibition (mean ± SEM) of 65±5% (GLC4), 59±5% (H460), 45±5% (H520), 38±2% (MERO82), and 55±8% (H441) was shown after 3 days incubation with 3 mM ATP. ATP also induced changes in morphology and attachment to the substratum. Although not demonstrated by the Trypan Blue exclusion test, on photographs it appears that ATP induces death of GLC4 and H460 cells at higher concentrations.

Conclusions: In four out of five explored lung tumor cell lines ATP induces a dose-dependent growth inhibition. Lung adenocarcinoma cells show already maximal inhibition at the lowest tested ATP dose. There is a relationship between growth inhibition and morphology changes.

INTRODUCTION

Adenosine 5'-triphosphate (ATP) is a naturally occurring nucleotide which is present in every cell of the human body. In addition to its well-established intracellular energy-transferring role, ATP extracellularly is involved in biological processes including neurotransmission, muscle contraction, cardiac function, platelet function, vasodilatation, and liver glycogen metabolism.[57] The physiological concentration of

ATP in plasma is low, i.e., 0.02 to 10 μM.[151,185] Extracellular ATP exerts its effects through cell surface P2 receptors, classified in G protein-coupled P2Y, and ionotropic P2X receptors.[155]

In vitro studies have shown that extracellular ATP can modulate the growth of neoplastic cells. In transformed cells, ATP induced mitogenesis at a concentration below 50 μM,[201,449] whereas higher ATP concentrations inhibited growth.[28,63,135,190,343,346,378,405,443] These effects were not observed in untransformed control cells.[189,190,224,299,343] In human tumor cell lines, ATP inhibited the growth of pancreatic adenocarcinoma cells,[343] colon adenocarcinoma cells,[343] melanoma cells,[224,343] androgen-independent prostate carcinoma cells,[135] breast cancer cells,[3,405,443] myeloid and monocytic leukemia cells,[190] and multidrug resistant colon carcinoma cells.[92] In rats and mice ATP was found to inhibit the growth of lymphomas,[301] colon carcinomas,[348] fibrosarcomas,[161] Ehrlich ascites tumors,[239] and breast tumors.[3]

Recently, 15 patients with advanced non-small-cell lung cancer were treated with courses of intravenous ATP. Stable disease was found in 10 of these patients suggesting an inhibitory effect of ATP on tumor growth.[187] So far, studies on growth inhibitory effects of ATP on lung cancer cell lines are lacking. Therefore, the aim of this study was to investigate the effects of ATP on the growth and morphology of different human lung cancer cell lines.

Materials and Methods

Chemicals

Adenosine 5'-triphosphate ($ATP\text{-}Na_2\cdot 3H_2O$; MW=605) of >98% purity was obtained from Merck (Darmstadt, Germany). Two stock solutions of ATP were prepared to a final concentration of 10.5 mM (stock A) and 21 mM (stock B). ATP was dissolved in RPMI-1640 medium, adjusted to pH 7.4 with NaOH and stored at -20 °C until use. ATP stock solutions were sterilized by filtration through 0.2 μm syringe filters. High-performance liquid chromatograph (HPLC) testing showed that under these conditions ATP remained stable in time (data not shown).

Cell lines, media, and culture conditions

Five cell lines were studied. Human papillary lung adenocarcinoma (H441), human large cell lung carcinoma (H460), and human squamous cell lung carcinoma (H520) cell lines were obtained from the American Type Culture Collection (Manassas, USA). Human small cell lung carcinoma (GLC4), and human mesothelioma cell lines (MERO82) were kindly given by Dr. T. Boersma (Department of Oncology, Erasmus University Medical Center Rotterdam, The Netherlands), and Dr. M. Versnel (Department of Immunology, Erasmus University Medical Center Rotterdam, The Netherlands), respectively.

Cells were cultured on RPMI-1640 culture medium (BioWhittaker Europe, Vervier, Belgium) containing L-Glutamine (2 mM), supplemented with 2% $NaHCO_3$ (BioWhittaker Europe), 100 units/ml penicillin (BioWhittaker Europe), 100 µg/ml streptomycin (BioWhittaker Europe), and supplemented with 10% heat inactivated fetal calf serum (GIBCO BRL, Life Technologies, Breda, The Netherlands). Cells were detached from their substratum by 0.5 g Trypsin / 0.2 g EDTA (GIBCO BRL, Life Technologies).

The different cell lines were cultured in 75 cm^2 flasks (Costar, USA) on RPMI-1640 culture medium at 37 °C in a 5% CO_2 humidified incubator. Heat-inactivated serum was prepared by heating FCS in a water bath at 60 °C for 30 min. The cells were routinely passaged at approximately 90% confluency with medium changes once per week.

Experimental protocol

On day –1 of the experiment, the different cell lines were seeded in a number of $4.75x10^4$ cells per well on 6-well culture plates (Costar, USA) and suspended in 5 ml RPMI-1640 medium per well. On day 0, cells were incubated in triplicate with ATP to final concentrations of 0, 0.5, 1, 2, and 3 mM. On days 1, 2, and 3, cell numbers and viability were determined. Cells were detached from their substrate by incubation with 1.2 ml Trypsin/EDTA per well for 10 min at 37 °C. The wells were rinsed once with 1 ml phosphate buffered saline (PBS). The solution (cells, Trypsin/EDTA and PBS) was centrifuged at 1000 RPM for 5 minutes at 22 °C and the supernatant was removed. Subsequently, the cells were resuspended in PBS and counted.

Determination of morphological changes due to ATP

Cells were inoculated and incubated as described. Photographs were taken at 1, 2, and 3 days after incubation with ATP, using a Sony RBG CCD video camera attached to a Zeiss Axiovert 100 microscope and stored on a videotape by use of a Panasonic time-lapse video recorder.

Cell growth determination

Cell numbers and viability were determined by Trypan Blue dye exclusion, and each sample was scored in triplicate microscopically

Statistical analysis

Growth data are expressed as cell numbers per well, and as percentage growth inhibition compared to control cell cultures. Cell death or damage is expressed as percentage of the total cells counted. Each value is the mean of 27 measurements: i.e., all experiments were performed three times, every incubation was performed in triplicate, and cell numbers were determined in triplicate. Values are expressed as mean ± SEM. Statistical significance of differences was appraised using Student's *t*-test. *P*-values below 0.05 were considered statistically significant.

RESULTS

Growth inhibition

Figure 4.4.1 A-E shows the growth curves of five human lung tumor cells exposed to ATP at concentrations of 0 (=control cell line), 0.5, 1, 2, and 3 mM ATP for a period of 3 days. As shown in this figure and in **Table 4.4.1**, ATP induced a significant, dose-dependent growth inhibition in GLC4, H460, H520, and MERO82 cells. Three days after exposure to ATP the growth inhibition of GLC4 cells was maximal at 3 mM ATP, less at 2 mM, minimal at 1 mM ATP, and absent at 0.5 mM ATP. At the same time point, the growth of H460, H520, and MERO82 cells was maximally inhibited at 3 mM, less at 2 mM, and not at either 1 and 0.5 mM ATP. In contrast, H441 cells showed already maximal growth inhibition at 0.5 mM ATP, with no additional growth inhibition at ATP concentrations of 1, 2, and 3 mM.

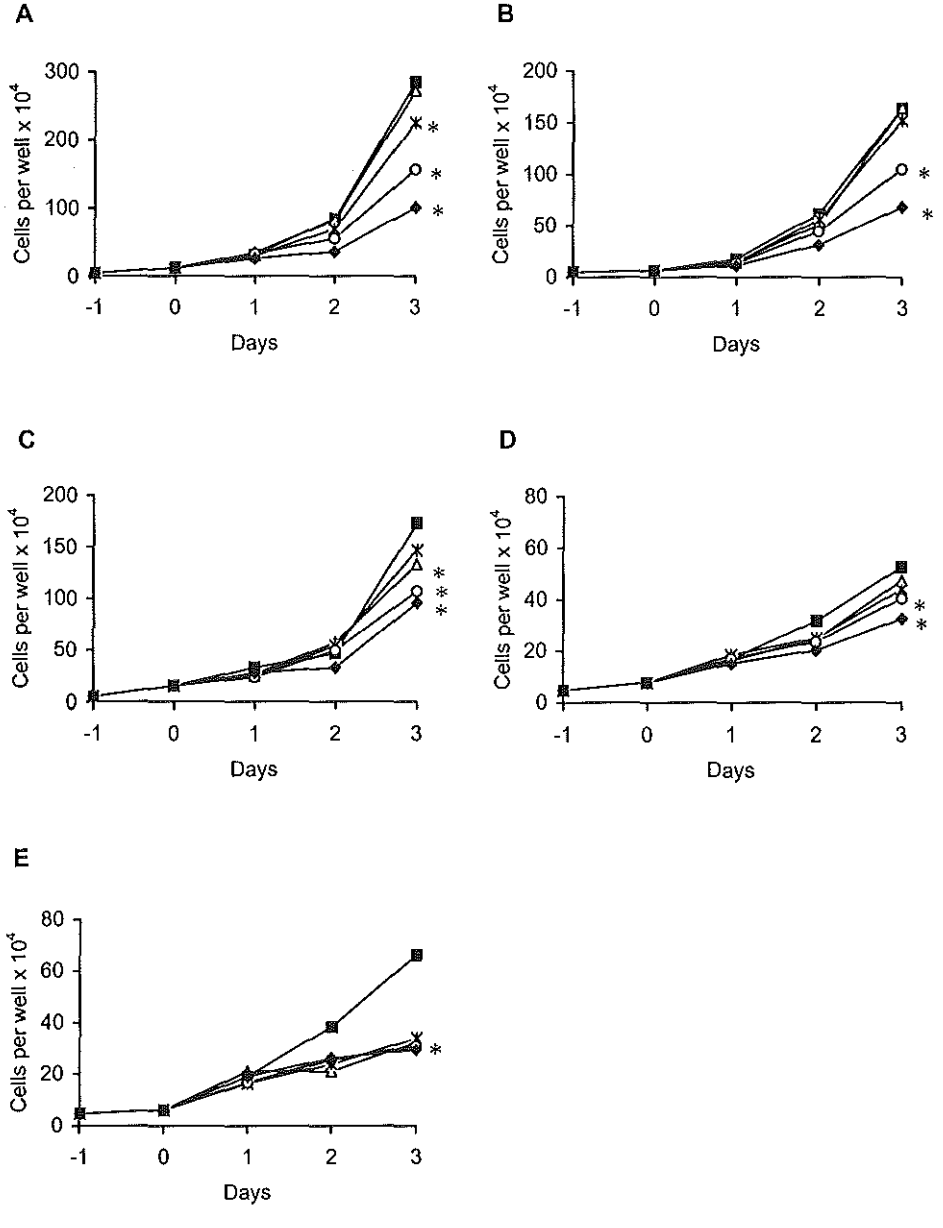

Figure 4.4.1. Growth of A) small cell carcinoma (GLC4), B) large cell carcinoma (H460), C) squamous cell carcinoma (H520), D) mesothelioma (MERO82), and E) papillary adenocarcinoma (H441) cell lines after administration of 0 (black square), 0.5 (open triangle), 1 (cross), 2 (open circle), and 3 (black diamont) mM adenosine 5'-triphosphate (ATP). *) Significantly different growth inhibition compared to the control cell line (0 mM ATP) at 3 days.

Table 4.4.1. Percentage growth inhibition of lung cancer cell after 3 days of exposure to different concentrations of adenosine 5'-triphosphate (ATP) as compared to control cell lines

	mM ATP			
	0.5	1	2	3
GLC4	5 ± 0	21 ± 1*	45 ± 4*	65 ± 5*
H460	0 ± 0	7 ± 0	36 ± 2*	59 ± 5*
H520	23 ± 2	15 ± 1	38 ± 4*	45 ± 5*
MERO82	10 ± 1	16 ± 1	23 ± 1*	38 ± 2*
H441	51 ± 7*	49 ± 8*	53 ± 7*	55 ± 8*

Values are mean of 27 measurements. Results are expressed as mean ± SEM.
*) indicates significant difference compared to control

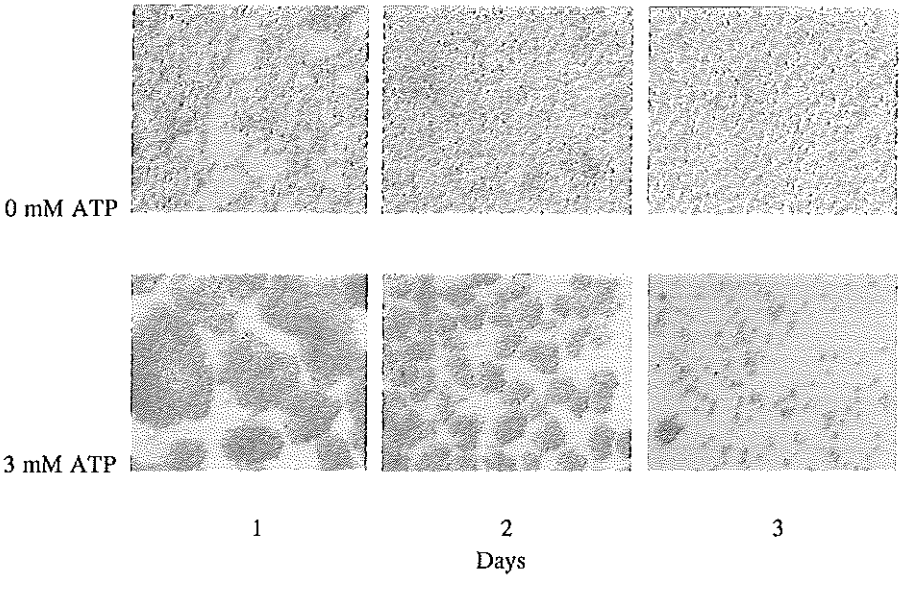

Figure 4.4.2. Growth and morfology changes of small cell lung carcinoma (GLC4) cells at 1, 2, and 3 days after administration of 0 and 3 mM adenosine 5'-triphosphate (ATP).

Morphological changes

Besides growth inhibitory effects, ATP had effect on the attachment of the lung tumor cells to the substratum and on their morphology. At higher ATP concentrations the cells became spheroidal, and detached from the substratum (**Figure 4.4.2**). As shown in **Figure 4.4.3** higher concentrations of ATP induced more pronounced changes in morphology of GLC4, H460, H520, and MERO82 cells than lower ATP concentrations. In contrast, H441 cells showed similar changes in morphology at both low and high ATP concentrations. Comparison of **Table 4.4.1** and **Figure 4.4.3** shows a relationship between growth inhibition and changes in morphology of lung cancer cells.

Table 4.4.2. Percentage dead or damaged cells on the total cell count of the different lung cancer cell lines 3 days after adenosine 5'-triphosphate (ATP) administration.

	mM ATP				
	0	0.5	1	2	3
GLC4	11 ± 2	16 ± 2	13 ± 1	11 ± 2	9 ± 1
H460	16 ± 1	13 ± 1	15 ± 1	11 ± 1	14 ± 1
H520	18 ± 1	13 ± 1	15 ± 2	18 ± 1	18 ± 3
MERO82	11 ± 1	9 ± 1	9 ± 1	13 ± 1	25 ± 3
H441	20 ± 3	9 ± 2	13 ± 2	12 ± 2	18 ± 3

Values are mean of 27 measurements. Results are expressed as mean ± SEM

Cell death

As tested by the Trypan Blue exclusion test, ATP at several concentrations did not have cytotoxic effects on the tested lung tumor cell lines (**Table 4.4.2**). In contrast, as seen in the photographs (**Figure 4.4.2**) ATP appears not only to exert cytostatic effects on lung tumor cell lines but also cytotoxic effects. Three days after 3 mM ATP administration, the number of the GLC4 cells decreased compared to days 1 and 2. This finding was also observed in H460 cells (data not shown).

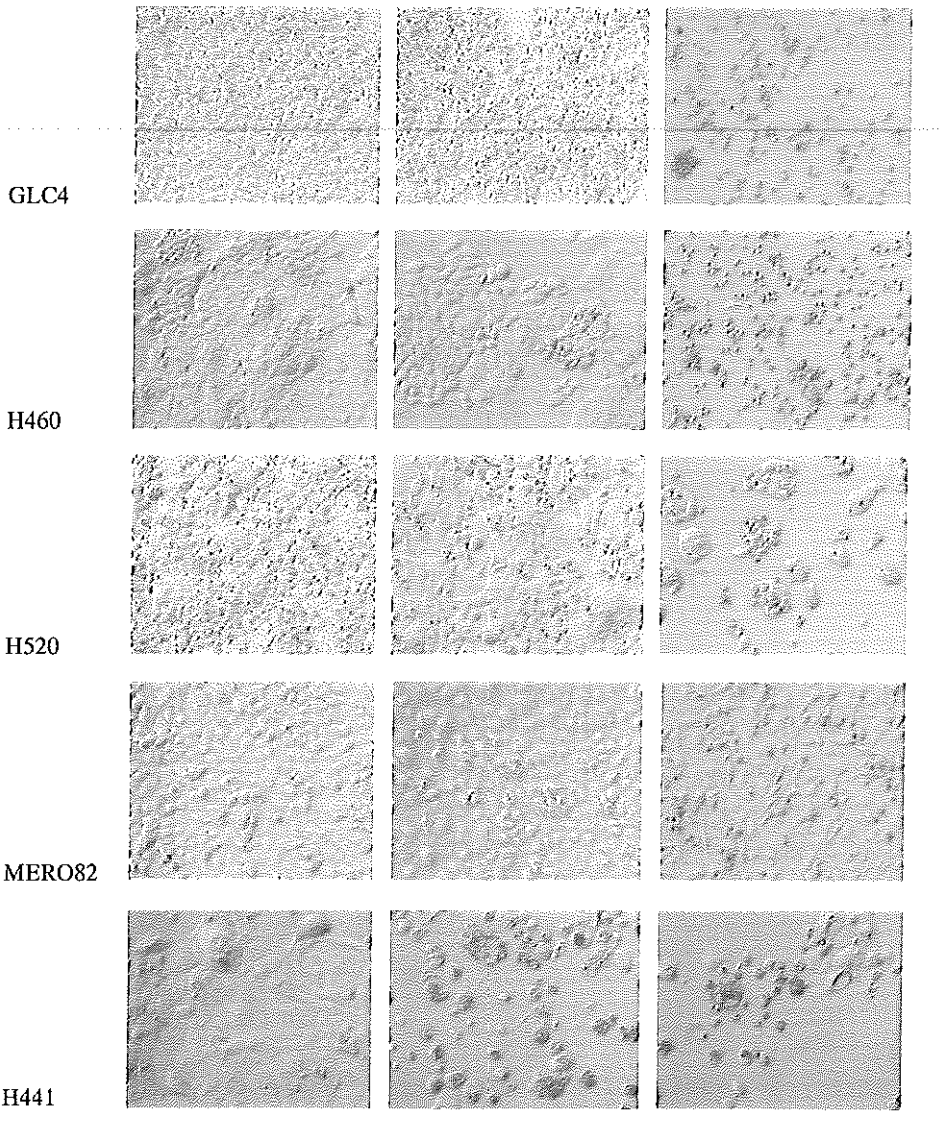

Figure 4.4.3. Effects of administered adenosine 5'-triphosphate (ATP) on the growth and the morphology of small cell carcinoma (GLC4), large cell carcinoma (H460), squamous cell carcinoma (H520), mesothelioma (MERO82), and papillary adenocarcinoma (H441) cell lines at concentrations of 0, 1, and 3 mM at 72 hours incubation period.

DISCUSSION

In the present study, we investigated the effects of extracellular ATP on the growth and morphology of five human lung cancer cell lines, i.e., a small cell lung carcinoma (GLC4), a large cell lung carcinoma (H460), a squamous cell lung carcinoma (H520), a mesothelioma (MERO82), and a papillary lung adenocarcinoma (H441).

Incubation with 0.5 to 3 mM ATP resulted in a concentration-dependent growth inhibition of GLC4, H460, H520, and MERO82 cell lines. In general, the most pronounced growth inhibitory effects (38 to 65% inhibition depending on cell type) were demonstrated at 3 mM ATP after an incubation time of 3 days. At this concentration GLC4 cells were most sensitive, whereas MERO82 cells were least sensitive. It may be noted that small cell lung carcinomas (GLC4) also have a high sensitivity to chemotherapy,[61] whereas mesotheliomas (MERO82) are almost completely resistant to this type of treatment.[47] In H441 cells, ATP induced significant growth inhibition at both high (3 mM) and low (0.5 mM) concentrations.

It is intriguing that ATP also influenced the morphology of the lung cancer cells in a concentration-dependent manner. Changes in morphology were related to the level of growth inhibition. Incubation with ATP induced changes in cell shape, membrane movement, cell agglutination, and attachment of cells to the substrate. It has been reported that extracellular ATP influences the cytoskeleton.[222,476] Possibly, ATP induces changes in cytoskeletal structures which may contribute to increased permeability of transformed cells.[476] In this connection it could be worthwhile to investigate effects of ATP on cell adhesion molecules including E-cadherin, integrins and vimentins, which may explain directly the spheroid nature of the cultured cells. It should be noted that loss of cell attachment by incubating the cells in 3 mM ATP during 3 days resulted not only in inhibition of growth, but also in cell death and cell loss as shown in **Figure 4.4.2.** The reason for not detecting ATP-induced cell death using the Trypan Blue test may be removal of dead cells which were detached from the substratum together with the supernatant. Possibly, as the photographs seem to suggest, there may have been more dead cells than were actually counted.

Several mechanisms have been proposed to explain the ATP-induced growth inhibition and death of tumor cells. Firstly, exposure of human adenocarcinoma cells to extracellular ATP has been reported to cause intracellular accumulation of ATP and

arrest of tumor cells in the S-phase of cell replication, followed by cell death.[343] A similar ATP-induced growth inhibition, due to prolonging of the S-phase, was found in human breast cancer cells.[405] Secondly, ATP-induced tumor growth inhibition is associated with a decrease in glutathione (GSH) content of the tumor, but not of normal tissues.[134,239] Thirdly, in various transformed cells ATP administration contributed to increased membrane permeability.[63,119,143,223,224,299,466,476] Increased cell permeability after exposure to extracellular ATP may be due to activation of $P2X_7$ receptors[154,413] which have been found on the cell surface of several tumor cells.[52,214] Activation of the $P2X_7$ receptors causes opening of intrinsic ion channels which leads to massive efflux of K^+, and influx of Ca^{2+} and Na^+, resulting in a decrease of the plasma membrane potential.[193,335] Activation of $P2X_7$ receptors further results in formation of non-selective pores, which induces an increase in nonselective membrane permeability for aqueous solutes that ordinarily do not cross the cell membrane.[73,130,271] These effects have been seen in many transformed cells[28,63,135,343,346,405,443] but not in untransformed cells.[117,190,224,361,460]

Preclinical studies have shown that ATP administration not only induces growth inhibition of tumor cells, but also potentiates the cytotoxic effects of several chemotherapeutic agents[222,281] and radiotherapy.[134] It would be interesting to explore effects of combinations of chemotherapeutic agents and ATP on lung carcinoma cell lines.

In summary, our results show that ATP induces a dose-dependent growth inhibition in four out of five lung cancer cell lines: GLC4, H460, H520 cells, and MERO82 cells. Lung adenocarcinoma cells (H441) show already maximal (55%) inhibition at the lowest ATP dose tested. There is a relationship between growth inhibition and morphology. Although not demonstrated by the Trypan Blue exlusion test, the photographs suggest that it seems that ATP may induce cell death at higher concentrations. Experiments to explore underlying processes contributing to ATP-induced cytostatic and cytotoxic effects, and morphology changes are warranted. Based on the marked growth inhibition of human lung adenocarcinoma cells by ATP at low dosage, further study with this tumor cell type would appear especially relevant.

Acknowledgement

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4.5

CHANGES IN BODY COMPOSITION IN ADVANCED LUNG CANCER MEASURED BY SKINFOLD ANTHROPOMETRY COMPARED WITH DEUTERIUM OXIDE DILUTION

Submitted for publication

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ABSTRACT

The accuracy of skinfold measurements to assess changes in fat and fat-free mass was estimated in 25 non-small-cell cancer patients (stage IIIB or IV), using the deuterium oxide dilution technique as a reference. Weight, skinfold thickness and total body water were measured at baseline and after 2 months. Fat mass (FM) and fat-free mass (FFM) were calculated from the sum of four skinfolds, and from the determination of total body water by deuterium oxide dilution. After 2 months, a mean weight loss (± SD) of 1.90±2.51 kg was observed ($P<0.001$). Body composition measurements by the two methods were highly correlated at t=0 (for FM: r=0.84; for FFM: r=0.92, both $P<0.001$) and t=2 months (r=0.82 and r=0.93, respectively, $P<0.001$). Significant positive correlations between the two techniques were also found when assessing changes in FM (r=0.63, $P<0.001$) and FFM (r=0.55, $P<0.01$) over a 2 months' period, with a mean overestimation of FM loss and underestimation of FFM loss of only 0.09±0.31 kg (mean ± SEM) by skinfold anthropometry. It is concluded that skinfold anthropometry is an useful and accurate technique for measuring changes in body composition in patient populations with advanced lung cancer, though not in individual patients.

INTRODUCTION

Patients with cancer cachexia suffer from extensive weight loss due to depletion of both adipose tissue and muscle mass.[194] Measurement of components of weight loss is important in order to assess the degree of depletion and to evaluate effects of nutritional and pharmacological interventions. A simple and non-invasive method to assess body composition is skinfold anthropometry. Although in several studies fat mass (FM) and fat-free mass (FFM) have been estimated by skinfold anthropometry in cancer patients,[8,115,194,373] there are only few longitudinal studies evaluating the validity of this simple technique in healthy subjects.[208,439,464]

In the present study, we estimated the accuracy of skinfold anthropometry to assess changes in FM and FFM in lung cancer patients over a period of two months, using deuterium oxide dilution (D_2O) technique as a reference.

PATIENTS AND METHODS

Patients

Thirty-six patients with stage IIIB/IV non-small-cell lung cancer (NSCLC) were included in the study. All patients had a Karnofsky performance status score of ≥60%. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. Written informed consent was obtained from all patients.

The D_2O dilution evaluation at 2 months could not be completed in 11 patients for reasons of physical deterioration or hospitalization (n=7), or because they found the return visit too burdensome (n=4). As a result, complete data for the present follow-up study were available from 25 patients (19 men, 6 women). Baseline characteristics of the patients participating in the present study are given in **Table 4.5.1.**

Anthropometry (test method)

At baseline and after 2 months, body weight and thickness of the skinfolds were measured. All measurements were performed as described by Durnin et al.,[131] by the same observer (HJA). Adipose patients with skinfold ticknessess >40 mm and pitting edema in the ankles or ascites were excluded for skinfold measurements.

Body height was determined to the nearest cm with patients standing barefoot. Body weight was measured in the overnight-fasted state, with patients standing without shoes in light clothing, and read to the nearest 0.1 kg on a electronic scale (SECA Ltd, Birmingham, UK).

Skinfold thicknesses were measured in triplicate to the nearest 0.2 mm using a Holtain® skinfold caliper (CMS weighing equipment LTD, London, UK). Total body fat mass (FM) was estimated from the sum of median skinfold thicknesses at four sites (triceps, biceps, subscapula, and supra iliac) using the age- and gender-specific tables from Durnin and Womersley.[132] Fat-free mass (FFM) was calculated by subtracting FM from body weight.

Table 4.5.1. Baseline characteristics of patients

	n = 25*	range
Sex (m/f)	19 / 6	
Age (y)	64 ± 12	30 - 85
Height (cm)	173 ± 5	160 - 185
Weight (kg)	70.0 ± 17.1	55.8 - 94.2
Body mass index (kg/m^2)	23.4 ± 4.9	17.0 - 30.8
Current weight (%)†	93.3 ± 6.2	79.6 - 110.4
Total body water (l)‡	35.5 ± 5.3	26.2 - 44.5
Fat mass (kg)‡	21.4 ± 6.8	10.9 - 39.0
Fat-free mass (kg)‡	48.6 ± 7.3	35.9 - 61.0

* Scores expressed as mean ± standard deviation
† As percentage of pre-illness weight
‡ Assessed by deuterium oxide (D_2O) dilution

Deuterium oxide dilution (reference method)

After an overnight fast of approximately 12 hours, patients received a oral dose of 15 or 20 grams deuterium-labeled water (99.9%; Isotec inc.,Miamisburg, USA) at about 9.30 hours a.m. Following deuterium oxide (D_2O) administration, patients had to abstain from eating and drinking until blood sampling completed. Venous blood samples were drawn into heparin tubes from the forearm at baseline and after 2½, 3, and 3½ hours. Blood samples were immediately placed on ice and then centrifuged for 10 min and 1300 x g at 4°C. Deuterium oxide in plasma was measured by infrared spectrophotometry (Miran 1FF, Foxboro, South Norwalk, USA). Total body water (TBW) volume was calculated by dividing the D_2O dose by D_2O concentration in plasma.[263,441] FFM was calculated using a constant water percentage of 73.[322] FM was calculated by subtracting FFM from total body weight.

Statistical analysis

Changes in time were tested for significance by Student's paired *t*-test. The test and reference methods were compared using Spearman's rank correlation coefficient and by graphic presentation according to Bland and Altman in which the difference

between measurements with both methods is plotted against their average.[37] *P*-values of less than 0.05 indicated significance.

RESULTS

During the follow-up period of two months, the patients lost 1.90±2.51 kg of weight (mean ± SD; $P<0.001$). Mean loss of FM obtained by skinfold anthropometry was 1.05±1.90 kg compared to 0.96±2.07 kg as assessed by deuterium oxide dilution. Mean loss of FFM as assessed by skinfold anthropometry was 0.85±1.52 kg, and 0.94±1.60 kg by deuterium oxide dilution. Comparison of the two methods showed an overestimation of loss in FM, and an underestimation of loss in FFM, of mean 0.09±0.31 kg (±SEM) when measured by skinfold anthropometry, relative to the deuterium dilution technique.

Table 4.5.2. Measurement of fat mass (FM) and fat-free mass (FFM):correlation coefficients between deuterium oxide (D_2O) dilution and skinfold anthropometry

	Baseline	At 2 months	Change 0-2 months
Fat mass	0.85†	0.82†	0.63†
Fat-free mass	0.92†	0.93†	0.55*

*) $P<0.01$, †) $P<0.001$

Table 4.5.2 shows that body composition measurements as based on the two techniques were highly correlated both on t=0 (for FM r=0.85; for FFM r=0.92, both $P<0.001$) and t=2 months (for FM: r=0.82; for FFM r=0.93, both $P<0.001$). Lower, but still highly significant positive correlations between the two techniques were observed for changes in FM (r=0.63, $P<0.001$) and FFM (r=0.55, $P<0.01$) over the 2 months' study period.

As shown in **Figure 4.5.1**, the Bland & Altman analysis revealed a lower limit of agreement of -2.83 kg and an upper limit of 3.02 kg for individual estimations of changes in FM by the two techniques. The 95% limits of agreement of estimation of changes in FFM were -3.02 and 2.83 kg.

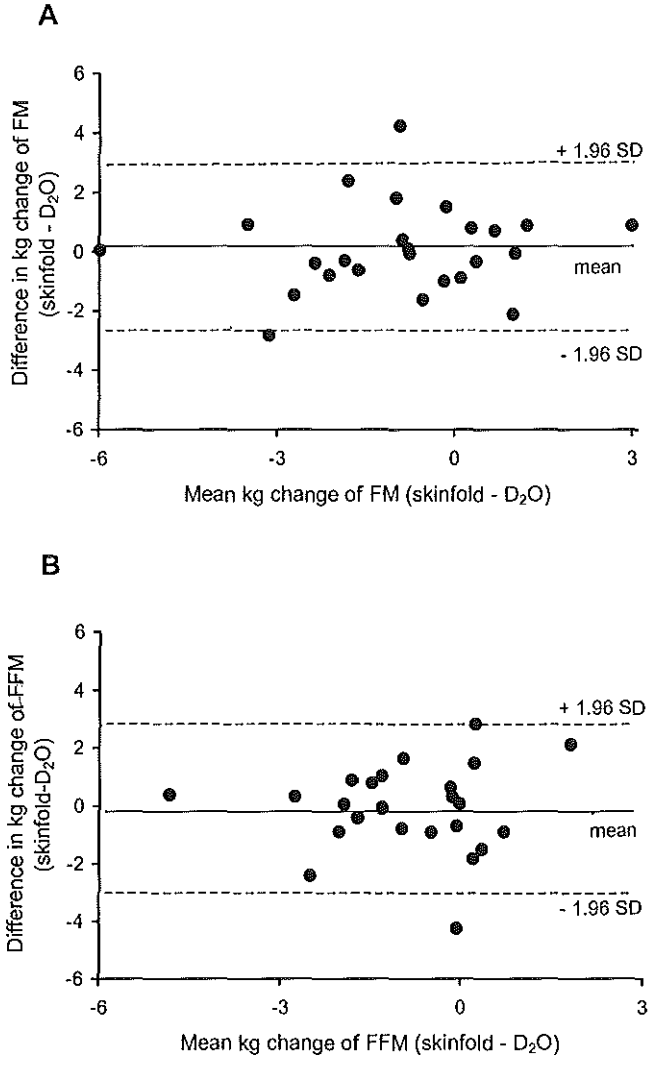

Figure 4.5.1. Individual differences of changes in fat mass (FM) and fat-free mass (FFM) over a 2 months' period as calculated from skinfold anthropometry and deuterium oxide (D_2O) dilution, plotted against the mean change measured by the two methods according to Bland & Altman. **A)** change of FM, **B)** change of FFM. The solid line represents the mean, the dotted lines represent upper and lower limits of agreement.

DISCUSSION

For use in epidemiological field studies and clinical practice a method to measure body composition must be both simple and reliable. Skinfold anthropometry is such a rapid and simple-to-operate method. Previous studies have shown that skinfold anthropometry is significantly correlated with computerized tomography[194] and underwater weighing.[132,205,243,256,447] Although skinfold anthropometry has been widely applied in clinical practice, information on the validity of this method for measuring changes of body composition over time is extremely scarce. In the present study, in NSCLC patients, the accuracy of skinfold anthropometry was compared with D_2O dilution as the reference method. Although no 'gold standard', D_2O dilution is commonly used as a reference method to validate other techniques.[285,339] Measurement of total body water by deuterium oxide dilution has been reported as an accurate method to predict body composition,[85,262,263,439] with a coefficient of variation of $<2.5\%$.[263,441] However, this method of measuring body composition puts a considerable strain on patients with advanced disease, as demonstrated by the present study in which 11 out of 36 patients dropped out for the D_2O dilution follow-up measurement at two months.

One reported disadvantage of skinfold anthropometry is that total body fat is assumed to have a constant proportion of subcutaneous fat which may lead to underestimation in elderly persons because of internal deposition of body fat reserves.[384,462] In spite of this, our study demonstrates that FM and FFM values obtained by skinfold anthropometry were highly correlated with those as assessed by D_2O dilution, both at baseline and at two months. During the 2 months' follow up period, patients lost approximately 2 kg of body weight. Over this period, mean loss in FM and FFM amounted to 1.0 and 0.9 kg, respectively, with only a small mean difference of 0.09 kg between skinfold anthropometry and D_2O dilution. This equal loss of FM and FFM is in line with the findings of Heymsfield et al.[194] who demonstrated that weight loss in cancer patients is due to both loss of FM and muscle mass.

We conclude that, although not in individuals, in patient populations with advanced cancer skinfold anthropometry is a useful and accurate technique for measuring changes in FM and FFM.

5

GENERAL DISCUSSION

This thesis describes results of the first randomized clinical trial on the effects of adenosine 5'-triphosphate (ATP) treatment in cancer patients. Fifty-eight patients with locally advanced (stage IIIB) and metastatic (stage IV) non-small-cell lung cancer were randomly assigned to receive either supportive care and 10 intravenous 30-hours ATP infusions every 2 to 4 weeks, or supportive care alone. Outcome parameters were assessed at 4-week intervals, until 28 weeks after randomization.

In this chapter, after a short description and interpretation of the clinical results in 5.1, the validity of this randomized clinical trial is discussed in 5.2. In 5.3, possible modes of action of ATP are briefly reviewed. Finally, the clinical implications of ATP infusions in oncology are discussed in 5.4.

5.1 CLINICAL RESULTS

Body weight

During the half-year study period, a significant difference in mean weight loss emerged between the ATP and control group (**Chapter 3.1**). Patients in the control group lost mean 6.5 kg over 6 months, whereas patients in the ATP group had an average stable weight. The effect of ATP on weight loss was especially marked in patients who were already cachectic at the start of the study, whereas no statistical significance was reached in non-cachectic patients.

Body composition

Longitudinal studies evaluating the validity of the simple and non-invasive skinfold anthrometry for measuring changes in body composition in weight-losing patients are scarse. Using deuterium oxide dilution technique as a reference, we showed that skinfold anthropometry is useful for measuring changes in fat and fat-free mass in advanced lung cancer patients (**Chapter 4.5**). Using skinfold anthropometry we found that the progressive weight loss in the control group was comprised of a mean loss of approximately 5 kg fat mass and 2 kg fat-free mass over the half-year study period (**Chapter 3.2**). In contrast, in the ATP group no significant reduction in fat mass and fat-free mass was observed. Furthermore, anthropometric measurements showed that ATP significantly inhibited the loss in upper arm muscle area (**Chapter**

3.2), as an indication of muscle mass.[262] The control group lost approximately 15% of muscle mass over 6 months, whereas the ATP group showed no change. Total and extracellular body water measurements suggest that ATP treatment counteracts body cell mass wasting in advanced lung cancer patients. This is an important finding since body cell mass is the vital compartment which contains the metabolically active cells. Whereas the control group demonstrated a significant decline in body cell mass, body cell mass in the ATP group remained stable over 4 months. In contrast with earlier pharmacological attempts to reverse cancer cachexia, ATP appears to be the first agent with beneficial effects on metabolically active tissue (skeletal muscles and body cell mass) in lung cancer patients.

Body function & quality of life

The loss of muscle tissue is thought to contribute to a number of symptoms which are part of the cancer cachexia syndrome, such as fatigue, weakness, compromised respiratory function, and gastrointestinal symptoms including nausea and constipation.[296] The described ATP-induced effects of counteracting loss of arm muscle mass and body cell mass (**Chapter 3.2**) may contribute to preventing these cachectic symptoms. Whereas elbow flexor muscle strength declined by 36% per 6 months in the control group, no such decline was shown in the ATP group. A similar difference was observed in knee extensor muscles. Like the effect of ATP on body weight, the effect of ATP on muscle strength was especially marked in patients who were already cachectic at study onset, whereas the effect of ATP was not statistically significant in non-cachectic patients.

Measures of several domains of quality of life, as assessed by the Rotterdam Symptom Checklist (**Chapter 3.1**) showed significant differences between the ATP and control group. In the control group, the deterioration expected in patients with progressive cancer was seen, whereas this was not observed in the ATP group. Over 6 months, the quality of life of control patients deteriorated significantly in the physical, functional, and overall domains. In contrast, practically no change in these quality of life domains was seen in the ATP group. The effect of ATP was statistically significant, except for the psychological domain where no significant difference was seen between the ATP and control groups. Items contributing to the better physical scores in the ATP group included lung cancer-related (shortness of

breath) as well as general symptoms (e.g. tiredness, lack of energy, lack of appetite, and constipation). It is noteworthy that these items include two of the most common cancer-related symptoms: tiredness and lack of energy.[11] The items contributing to the better functional scores in the ATP group include general activities including self-care, doing housework, climbing stairs, and walking outdoors.

Tumor response and survival

In contrast to the marked beneficial effects on both quality of life as reported by the patients and objective outcome parameters such as body weight and body composition, we did not find obvious effects of ATP on tumor response and survival which are the traditional outcome variables in clinical oncology (**Chapter 3.3**). No significant effect of ATP treatment on overall survival was demonstrated. Median survival was 5.6 months for the ATP group, and 4.7 months for the control group. In weight-losing patients with stage IIIB lung cancer, however, ATP treatment did contribute to a marked and statistically significant survival benefit (9.0 versus 3.4 months). It is possible that the observed survival benefit in these patients with non-metastatic disease is related with the inhibition of weight loss by ATP.

Pain reduction

Adenosine has been shown to reduce pain in healthy volunteers[380,381] and patient groups.[24,379,380,382,400] Because no data were available on the effects of adenosine in cancer a pilot study was performed in five advanced cancer patients with nociceptive pain (**Chapter 3.4**). During and after two 4-hour during intravenous adenosine infusions a substantial reduction in pain scores was seen. Further study of analgesic effects of adenosine as a new modality in cancer pain treatment seems warranted.

5.2 VALIDITY OF THE STUDY

Clearly, the ideal method for assessing the effect of treatment on quality of life would have been a double-blind, placebo-controlled study. However, blinding was not feasible because of the complexity of the ATP administration. Some side effects are relatively specific for ATP so that both patients and the investigator would have

easily recognized that ATP was being given. In addition, there were ethical reasons for not giving up to 10 placebo infusions to patients with advanced lung cancer with a short life expectation of between 3 to 6 months for the large majority of patients.

Despite the fact that maximal supportive care was given in both groups, a potential placebo effect of ATP infusions on measurements of muscle strength and quality of life cannot completely be excluded. In addition, there was some imbalance in baseline characteristics such as body weight, age and histology which could have affected the comparison of ATP and control groups.

Notwithstanding these limitations in our study design, several aspects seem to underline the validity of the results. Firstly, when we adjusted for possible confounders, in no case did this lead to significant alteration of the results. Secondly, there was a remarkable consistency of positive effects of ATP on quality of life measurements and objective parameters such as body weight and serum albumin levels (see 5.3). The same consistency was also found in self-reported appetite and objective food records, as well as between muscle strength and physical activities including housework, climbing stairs and walking outdoors, which contributed to the significant difference in functional score between ATP and control patients. Changes in individual scores of arm muscle strength and functional quality of life score over the 28-week study period showed a highly significant correlation of $r=0.68$ ($P<0.0001$). And thirdly, the psychological quality of life score did not differ significantly between the two groups, in contrast with physical and functional scores which did show significant positive effects of ATP.

5.3 MODES OF ACTION

Cellular uptake, transport, and receptor stimulation

Our pharmacokinetic results show that during continuous intravenous ATP infusions in the dose range of 25 to 75 μg/kg.min, ATP levels within erythrocytes increase to dose-dependent plateau levels 1.5 to 1.7 fold above baseline values at approximately 24 hours. After discontinuation of the ATP infusion, half-life of ATP disappearance from erythrocytes was approximately 6 hours (**Chapter 4.1**). This is in line with the suggestion that ATP from erythrocytes is slowly released into plasma.[345] The clinical effects of ATP on nutritional status and quality of life described in 5.1 suggest a

prolonged duration of efficacy of ATP after stopping the infusion. Administered ATP may interact with specific purinergic receptors on the surface of many cells. These receptors play a fundamental role in cell physiology, and are divided in two major classes: P1 and P2 receptors. In general, it is thought that the effects of adenosine are mediated through P1 receptors, whereas ATP binds to P2 receptors. P2 receptors are subclassified in G-protein-coupled receptors termed P2Y receptors, and intrinsic ion channels termed P2X receptors.[130] Stimulation of these purinergic receptors evokes diverse biological responses depending on the cell type. P2 receptors are expressed in many tissues including heart, brain, spleen, lung, liver, kidney, and skeletal muscles.[428]

Appetite and energy intake

Despite these proposed P1 and P2 receptor-evoked effects, the precise mechanisms underlying the observed effects of ATP on quality of life and nutritional status remain to be elucidated. The scores on the item 'lack of appetite' as part of the Quality of Life questionnaire revealed a significant difference in appetite between the ATP and control group. This finding was consistent with food records kept by the patients which showed a significant decline in energy intake in the control group but not in the ATP group (**Chapter 3.2**). It is therefore possible that ATP has appetite-regulatory effects contributing to increased food intake, and further study of the underlying mechanisms of these effects seems warranted.

However, model calculations of differences between energy intake and estimated energy needed for restoration of fat and fat-free mass suggest that differences in food intake cannot entirely account for the effects of ATP on weight and body composition between the two groups. Moreover, it is unlikely that the inhibitory effects of ATP on loss of body cell mass and skeletal muscles would be simply caused by appetite stimulating effects since appetite stimulators like corticosteroids[56] and cyproheptadine[215] did not influence body weight, and megestrol acetate was shown only to increase fat mass.[396] Our results therefore suggest that ATP may also influence specific metabolic pathways involved in weight loss.

Liver metabolism

In experimental cancer models, ATP levels in liver[12,101,374,434] and skeletal muscle[374] have been reported to be significantly reduced. Recently, reduced liver ATP levels were also demonstrated in lung cancer patients, particularly weight-losing patients.[249] It is interesting that, in the latter group only, ATP infusion was shown to lead to a 40% rise in liver ATP pools indicating restoration of depleted hepatic energy stores (**Chapter 4.2**). It could be hypothesized that the increase in hepatic ATP levels by ATP infusion restores the energy stores in the liver which were depleted by the elevated gluconeogenesis in weight-losing lung cancer patients. However, this relation does not seem straightforward since changes in liver ATP content during ATP infusion were not correlated with changes in glucose turnover or gluconeogenesis from alanine. Other mechanisms by which ATP infusion could stimulate glucose production by the liver may be through binding on purinergic P2 membrane receptors[311] which are involved in the control of gluconeogenesis[13] and glycogenolysis,[218] or by acting as cotransmitter or noradrenaline in the nervous system thereby indirectly stimulating glucose production.[64] ATP was also reported to inhibit binding of insulin to this receptor, which may stimulate glucose production as well.[433] Although ATP infusion appears to stimulate glucose production at short term (**Chapter 4.3**), to rates of glucose turnover similar to control lung cancer patients, our data (low glucose turnover between ATP infusions similar to that in healthy subjects) suggest that ATP infusion may in fact induce a reduction in glucose turnover at longer term after the infusion has been stopped, which would be consistent with the observed attenuation of weight loss.

Inflammatory response

Biochemical support for the beneficial effects of ATP on nutritional and functional status of advanced lung cancer patients is given by the significant difference in serum albumin concentrations between the ATP and control group. It is noteworthy that the effect of ATP on serum albumin levels was especially marked in patients who were already cachectic at the start of the study, whereas no statistical significance was reached in non-cachectic patients (**Chapter 3.1**). Since albumin levels are affected by both nutritional status and acute phase response, the stabilization of serum albumin levels by ATP could in fact be caused by inhibition of the acute phase response. In animals ATP has been shown to downregulate the production of the proinflammatory

cytokines interleukin (IL)-1 and IL-6, and tumor necrosis factor α (TNFα).[453] This is noteworthy since increased serum levels of these cytokines,[19,291] and also C-reactive protein (CRP),[19] have been found in patients with advanced cancer, and were suggested to play a prominent role in progressive weight loss in cancer.[316] As seen in **Figure 5.1**, and tested by repeated-measures analysis, the control patients demonstrated a significant increase in CRP levels of 12.1 mg/l (95% CI= 3.0 to 21.2) per 4 weeks (P=0.01). The absence of this increase in the ATP group (0.3 mg/l per 4 weeks; 95% CI= -6.3 to +6.9; P=0.93) indicates that ATP may inhibit the inflammatory response in cancer patients (between-group difference: P=0.04). As serum levels of CRP, IL-6, and TNF receptors in cancer patients are positively correlated,[19] ATP infusion may contribute to downregulation of these proinflammatory cytokines. Furthermore, the degradation product of ATP, adenosine, is known to inhibit neutrophil degranulation,[43] neutrophil superoxide production,[43] activation of anti-oxidants,[273,340] adhesion of neutrophils,[98] and expression of adhesion molecules,[45] eicosanoids,[232] and complement.[238] These effects suggest that ATP and/or adenosine inhibit inflammatory mechanisms in cancer patients which are related to the phenomenon cancer cachexia.

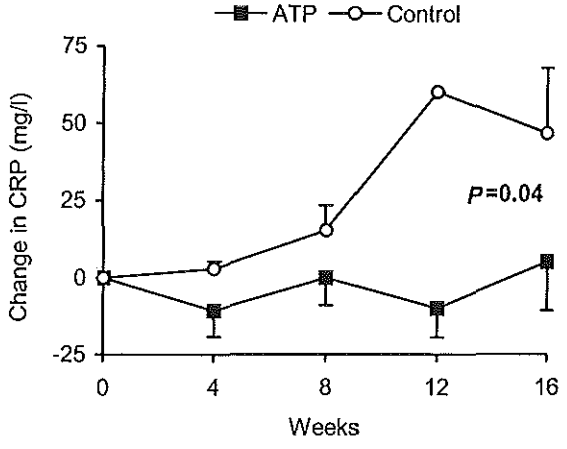

Figure 5.1. Changes in serum C-reactive protein (CRP) concentration (mean ± SEM). Two-sided P-values for difference between patients in the adenosine 5'-triphosphate (ATP) group and patients in the control group were determined with the use of repeated-measures analysis.

Table 5.1. Effects of drugs on cancer-associated cachexia as tested by randomized clinical trials

Category	Specific drug	Reference	n	Dose	Appetite	Cal.int.	Weight	FM	FFM	Perf	QoL	Albumin	Tumor	Survival
Progestagens	MA	55	31	480 mg/d	+	+	+	+	o	.	o	.	.	.
	MA	137	150	240 mg/d	o	.	o	.	.	o	.	.	.	.
	MA	257	133	800 mg/d	+	+	+	.	.	.	.	.	.	.
	MA	260	342	160-1200 mg/d	+	.	o	.	.	.	.	o	.	.
	MA *	261	12	800 mg/d	.	.	+	+	o	.	.	.	.	.
	MA	360	243	800mg/d	.	.	+	.	.	.	o	.	o	o
	MPA	395	206	500 mg bid	+	.	+	.	.	.	o	.	.	o
	MA	27	240	160-480 mg/d	+	.	o	o	o	.	+	.	.	.
	MPA *	396	54	500 mg bid	.	+	.	+	o	.	.	.	.	.
	MA	108	33	320 mg/d	+	o	o	.	.	.	o	.	.	.
	MA	437	150	160-480 mg/d	.	.	+	+	.	o	o	.	.	.
	MA	54	53	160 mg tid	+	o	o	.	.	+	o	.	.	.
	MA	472	130	160 mg tid	.	.	.	.	.	.	o	.	o	o
	MA	426	67	1,600 mg/d	+	+	o	.	.	.	o	.	.	.
	MA	141	61	160 mg/d	.	.	+	+	.	.	o	o	.	.
NSAID	Indomethacin	268	68	50 mg tid	.	.	o	.	o	+	.	o	.	o
Prog.+ NSAID	MA + Ibuprofen	284	41	160+400 mg tid	o	.	+	o	o	.	+	o	.	.
Corticosteroids	Dexamethasone	290	116	0.75-1.5 mg qid	+	.	o	.	.	o	.	.	.	o
	Methylprednisolone	56	40	32 mg/d	o	.	o	.	.	o	o	.	.	.
	Methylprednisolone	114	403	125 mg/d (iv)	+	.	.	.	.	.	+	.	.	o
	Methylprednisolone	336	173	125 mg/d (iv)	+	.	o	.	.	.	+	.	.	o
	Prednisolone	469	41	5 mg tid	+	o	o	.	.	.	+	.	.	.
	Prednisolone	268	68	10 mg bid	.	.	+	.	+	+	.	o	.	o
Serotonin antagonist/ inhibitor	Cyproheptadine	215	295	8 mg tid	+	.	o	.	.	.	.	.	.	.
	Melatonin	253	86	20 mg/d	.	o	+	.	.	.	.	.	.	.
Inhibitor of gluconeogenesis	Hydrazine sulfate	74	65	60 mg tid	.	+	o	.	.	.	.	+	.	o
	Hydrazine sulfate	259	243	60 mg qid	o	.	o	.	.	.	o	.	o	o
	Hydrazine sulfate	231a	291	60 mg tid	o	.	o			o	o	o	o	o
Methylxanthine derivate	Pentoxifylline	174	70	400 mg tid	o	o	o	.	.	.	o	.	.	.
Nucleotide	ATP	This thesis	58	25-75 mg/kg.min (iv)	+	+	+	+	+	+	+	+	o	o

*satellite study; MA = megestrol acetate; MPA = medroxyprogesterone acetate

5.4 CLINICAL IMPACT

ATP – a new approach in oncology

In **Table 5.1**, we have listed all known randomized clinical trials investigating the effects of drugs in cachectic cancer patients. It should be noted that drugs which are used in routine clinical practice including progestagens (Megace^{R}) and corticosteroids (Prednison^{R}, Dexamethason^{R}) influence only part of characteristics of cancer cachexia. In contrast, as shown in this table, ATP induces beneficial effects on a broad spectrum of cancer cachexia-related symptoms. Moreover, ATP would appear the first agent with demonstrated beneficial effects on skeletal muscle mass and strength of cachectic cancer patients. The ATP-induced counteracting of the loss of skeletal muscles may contribute to the maintenance of well-being and performance status in patients suffering from progressive lung cancer.

ATP was administered without side effects in the majority of courses, and if side effects occurred, they disappeared rapidly on lowering the infusion rate. In the large majority of cases, reported side effects were of type 1 (=mild). As in addition no side effects were observed between the ATP infusions, ATP treatment may be considered as an effective non-toxic palliative therapy in patients with advanced lung cancer.

We conclude that the natural compound adenosine 5'-triphosphate (ATP) has potential in the palliative management of lung cancer. ATP may increase the comfort of terminally ill cancer patients. Further clinical trials using an appropriate placebo control are warranted.

6

SUMMARY / SAMENVATTING

SUMMARY

Despite the continuing exploration of new cytotoxic drugs the prognosis of lung cancer is poor. The high morbidity and mortality in this disease are not only due to progressive tumor growth but also to the high frequency of cachexia. Cachexia is characterized by involuntary weight loss, fatigue, impaired physical performance and general malaise.

This thesis presents the results of a randomized clinical trial of intravenous adenosine 5'-triphosphate (ATP) infusions on quality of life, body weight, muscle strength, nutritional status, tumor response, and survival in 58 advanced non-small-cell lung cancer (NSCLC) patients (stage IIIB or IV). In addition, supportive studies in order to explore underlying mechanisms are described.

In **Chapter 1** an introduction is given about the development of ATP. Extracellular ATP has been known to exert its effects through P2 receptors on the surface of many cells. Preclinical studies showed that extracellular ATP induced growth inhibition of various human tumor cell lines. In animals ATP administration resulted in growth inhibition of implanted tumors, inhibition of weight loss, and prolonged survival. Previous uncontrolled phase I and II studies suggest that ATP modulate loss of weight, performance status and quality of life in lung cancer patients.

Chapter 2 gives an overview of the potential and established clinical applications of ATP. It is suggested that intravenous administered ATP may be useful in, amongst others, the management of pain, cardiovascular and pulmonary diseases, and cancer.

In **Chapter 3** results of a randomized clinical trial on the effects of ATP in patients with NSCLC, stage IIIB or IV, and effects of adenosine in patients suffering from cancer pain are presented.

Chapter 3.1 discusses the beneficial effects of ATP on quality of life, muscle strength, weight loss, and serum albumin concentration in the patients. The quality of life of control patients deteriorated significantly over a half year at both physical

(16%), functional (36%) and overall level (23%), whereas ATP-treated patients practically showed no change. No significant effect of ATP on psychological scores was seen. Arm muscle strength declined by 35% per 6 months in the control group, but remained stable in the ATP group. A similar pattern was observed in leg muscles. Mean weight loss was 6.5 kg over 6 months in the control group, and none in the ATP group. In the control group serum albumin level declined by approximately 6 g/l (=15%) per half year, but remained stable in the ATP group. The effect of ATP on muscle strength, weight loss, and serum albumin levels was especially marked in patients who were already cachectic at the start of the study, whereas no statistical significance was reached in non-cachectic patients.

In **Chapter 3.2** it is shown that inhibitory effects of ATP on weight loss are due to counteracting loss of both metabolically inactive and active tissues. The control group lost fat mass, fat-free mass, muscle mass and body cell mass, whereas in the ATP group no change in these parameters was found. Food records as kept by the patients indicate that these inhibitory effects of ATP are partly, but not solely, due to maintenance of energy intake.

In **Chapter 3.3**, it is reported that ATP as a single therapy does not lead to tumor regression or increased survival in patients with advanced lung cancer. As shown by the median time to progression of 3.9 months in the ATP group, compared to 3.0 months in the control group, ATP does not inhibit tumor growth. In weight-losing stage IIIB lung cancer patients the anti-cachectic effects of ATP appear to lead to increased survival.

In **Chapter 3.4**, a pilot study on the effect of intravenous adenosine infusions in five patients with cancer pain is presented. Results showed that during two 4-hour infusions of adenosine, a degradation product of ATP, pain scores decreased substantially together with a trend for reduced need for rescue opioids. Significant pain reduction was still present at 24 hours after adenosine administration.

In **Chapter 4**, four satellite studies on the underlying mechanisms and a validation study on skinfold anthropometry are reported.

Chapter 4.1 discusses the pharmacokinetics of intravenous ATP in cancer patients. During continuous ATP infusions in the dose range of 25 to 75

μg/kg.min, ATP was taken up by the erythrocytes and reached dose-dependent plateau levels 50 to 70% above basal concentrations at approximately 24 hours. After discontinuation of the ATP infusion, half-time of ATP disappearance from erythrocytes was approximately 6 hours. Constant intravenous infusion of ATP did not induce side effects in the majority of courses, and if side effects occur they were mild and disappeared rapidly when the dose was lowered.

The study described in **Chapter 4.2** was aimed at investigating whether ATP infusion restores liver ATP levels in advanced lung cancer patients. Patients with advanced NSCLC were studied one week before and at 22 to 24 hours of continuous ATP infusion. During ATP infusion ^{31}P magnetic resonance spectroscopy-detected hepatic ATP levels in lung cancer patients increased to a level similar to that in healthy subjects. ATP levels tended to increase more in patients with ≥5% weight loss than in patients without weight loss.

Chapter 4.3 describes the effects of intravenous ATP infusion on whole-body glucose turnover and gluconeogenesis from alanine in 12 patients with NSCLC, compared to 13 control NSCLC patients and 11 healthy subjects. Results of this study indicate a dose-dependent increase in glucose turnover. During high-dose ATP infusion (75 μg/kg.min) glucose turnover was higher than at baseline, and was similar to that during low-dose ATP infusion (37-50 μg/kg.min) and to control NSCLC patients. However, between ATP infusions, glucose turnover in the high-dose ATP-treated patients was significantly lower than in both low-dose and control NSCLC patients, and similar to that in healthy subjects. This would suggest that high-dose ATP infusion may inhibit glucose turnover in the long run.

Chapter 4.4 shows that ATP induces growth inhibition in various lung cancer cell lines. This growth inhibition is dependent of the ATP dose (0.5 to 3 mM) in small cell lung carcinoma cells, large cell lung carcinoma cells, squamous cell lung carcinoma cells, and mesothelioma cells. Papillary lung adenocarcinoma cells show already maximal inhibition at the lowest ATP dose tested (0.5 mM). Growth inhibition is related to cell morphology.

Chapter 4.5 describes the validity of skinfold measurements to assess changes in body composition in cancer patients, using the deuterium oxide dilution technique as a reference. In 25 patients with advanced lung cancer, positive correlations between the two techniques were found when assessing changes in fat mass

and fat-free mass over a 2 months' period. Skinfold anthropometry gives with 0.1 kg a marginal overestimation of fat mass loss and underestimation of fat-free mass loss.

Finally, in **Chapter 5**, the role of ATP in patients with advanced NSCLC is discussed. For ethical reasons in the randomized trial presented above, no placebo-treatment was given to these advanced cancer patients with a short life expectation between three and six months. Notwithstanding this limitation in the study design, the validity of the results is underlined by the consistency of findings regarding quality of life, food intake, muscle mass, muscle strength, and serum albumin and C-reactive protein concentrations.

We conclude that:

1. In patients with non-small-cell lung cancer (stage IIIB or IV) ATP-treatment inhibits loss of:
   a. body weight;
   b. fat mass, fat-free mass, muscle mass, and body cell mass;
   c. muscle strength;
   d. quality of life.
2. In patients with advanced lung cancer ATP-treatment does not influence:
   a. tumor response;
   b. survival.
3. ATP inhibits growth of several human lung tumor cell lines.
4. Adenosine infusion may lead to pain reduction in cancer patients.

Effects above described may be caused by:

1. Increase in intracellular ATP stores as shown for erythrocytes and the liver.
2. Stimulation of appetite.
3. Inhibition of inflammatory responses.

SAMENVATTING

De prognose van patiënten met longkanker is nog steeds slecht te noemen, ondanks de voordurende ontwikkeling van nieuwe therapieën. De hoge ziekte- en sterftecijfers zijn niet alleen het gevolg van de snelle groei van de tumor, maar ook van de vaak bij deze aandoening bestaande cachexie. Het woord 'cachexie' is afkomstig van twee Griekse woorden: *kakos* (=slecht) en *hexis* (=conditie). Cachexie is een syndroom dat gekarakteriseerd wordt door gewichtsverlies, vermoeidheid, verminderde activiteit en algehele malaise.

In dit proefschrift worden de resultaten gepresenteerd van een gerandomiseerd (=loting bepaalt de groep) klinisch onderzoek naar het effect van intraveneuze (= in de bloedbaan toegediende) infusen met een natuurlijke stof, adenosine 5'-trifosfaat (ATP). Bij 58 patiënten met een niet-kleincellige vorm van longkanker, bij wie uitzaaiingen naar de lymfeklieren (stadium IIIB) of naar andere organen (stadium IV) waren aangetoond, is het effect van ATP onderzocht op de kwaliteit van leven, het gewicht, de spierkracht, de voedingstoestand, de tumorgroei en de overlevingsduur. Verder wordt een aantal studies beschreven waarin gepoogd wordt enkele van de onderliggende processen te ontrafelen.

Hoofdstuk 1 bevat een korte inleiding tot dit proefschrift. ATP dat zich buiten de cel bevindt, bindt zich aan P2-receptoren die aanwezig zijn op de celmembraan van de cellen. Vervolgens wordt aan de binnenkant van deze cellen een signaal afgegeven, leidend tot een bij de celsoort passend effect. Laboratoriumstudies laten zien dat ATP groeiremming van diverse soorten tumorcellen bewerkstelligt. Bij dieren met een geïmplanteerde tumor en gewichtsverlies leidt ATP-toediening tot remming van de tumorgroei en van verder gewichtsverlies, en tot een hogere overleving. Een tweetal ongecontroleerde studies bij patiënten met longkanker doen vermoeden dat ATP invloed heeft op het gewicht, het welbevinden en de kwaliteit van leven.

In **hoofdstuk 2** wordt een literatuuroverzicht gegeven over de werkingsmechanismen en de toepassingsmogelijkheden van ATP. Beschreven wordt dat intraveneus ATP bruikbaar is -of zou kunnen zijn- bij de bestrijding van pijn, hart- en vaatziekten, taai-slijmziekte, kanker en als combinatie-therapie met chemotherapie of bestraling.

In **hoofdstuk 3** worden de resultaten van het gerandomiseerd klinisch onderzoek naar de effecten van ATP bij patiënten met niet-kleincellige longkanker (stadium IIIB of IV) gepresenteerd.

Hoofdstuk 3.1 beschrijft het gunstige effect van ATP op de kwaliteit van leven, de spierkracht, het gewichtsverlies en de concentratie van het eiwit albumine in het bloed. Metingen gedurende een half jaar lieten in de controlegroep een verslechtering zien van de kwaliteit van leven op een drietal gebieden: lichamelijk (16%), activiteit (36%) en algeheel (23%). Daarentegen toonden de met ATP behandelde patiënten praktisch geen achteruitgang op bovengenoemde gebieden. De psychologische scores lieten geen verschil zien tussen de controle- en de ATP-groep. De armspierkracht verslechterde in 6 maanden met 35% in de controlegroep, maar bleef stabiel in de ATP-groep. De beenspierkracht vertoonde eenzelfde patroon. De controlegroep verloor in een half jaar gemiddeld 6,5 kg gewicht, terwijl de ATP-groep geen gewicht verloor. Ook in het bloed was een verschil tussen beide groepen te meten. In 6 maanden daalde de concentratie van albumine bij de controlepatiënten ongeveer 6 gram per liter (=15%), maar bleef stabiel bij de met ATP behandelde patiënten.

Het effect van ATP op de spierkracht, het gewichtsverlies en albumine-concentraties werd met name gezien bij patiënten die aan het begin van het onderzoek reeds meer dan 5% gewichtsverlies hadden. Bij patiënten die geen gewichtsverlies hadden werd dit effect van ATP niet aangetoond. Het effect van ATP op de kwaliteit van leven trad echter op bij alle patiënten.

In **hoofdstuk 3.2** wordt het gunstige effect van ATP op gewichtsverlies verder uitgewerkt. Het blijkt dat dit het gevolg is van het remmen van zowel afbraak van vet- als spierweefsel. De controlegroep verloor vetmassa, vetvrije massa, spiermassa en celmassa, terwijl dit in de ATP-groep niet werd aangetoond. Aan de hand van de door patiënten bijgehouden voedingsboekjes werd de hoeveelheid genuttigde voeding berekend. Patiënten in de controlegroep gingen steeds minder eten, terwijl patiënten in de ATP-groep evenveel of zelfs meer aten. Dit doet vermoeden dat een deel van het gunstige effect van ATP kan worden verklaard door stimulering van de eetlust.

In **hoofdstuk 3.3** wordt aangetoond dat toediening van ATP niet leidt tot verkleining van de tumor of tot een langere overleving van patiënten met progressieve longkanker. Bij gewichtsverliezende patiënten in een relatief vroeger stadium van uitgezaaide longkanker (stadium IIIB) lijken de anti-cachexie-effecten van ATP wel te leiden tot een verlengde

levensduur.

In **hoofdstuk 3.4** wordt het effect van adenosine -een afbraakproduct van ATP- bij vijf patiënten met kankerpijn beschreven. Een adenosine-infuus van vier uur, gedurende twee opeenvolgende dagen, leidde tot verminderde pijnbeleving en tot verminderd gebruik van morfine. Vierentwintig uur na het laatste adenosine-infuus werd nog steeds minder pijn ervaren. Deze resultaten moeten met voorzichtigheid worden bekeken omdat in dit kleine deelonderzoek geen controlegroep was opgenomen.

In **hoofdstuk 4** wordt een viertal deelstudies naar de onderliggende mechanismen van de werking van ATP beschreven. Ook wordt de waarde van de huidplooi-meetmethode aangetoond.

In **hoofdstuk 4.1** wordt de farmacokinetiek (=het gedrag van het lichaam op een medicijn) gepresenteerd van intraveneus toegediend ATP bij patiënten met longkanker. ATP, gedoseerd in concentraties van 25 tot 75 µg/kg per minuut, werd opgenomen door de rode bloedcellen. Na ongeveer 24 uur was de ATP-concentratie in de rode bloedcellen met 50 tot 70% toegenomen. Ongeveer 6 uur na het stoppen van het ATP-infuus bleek de helft van het ATP uit de rode bloedcellen te zijn verdwenen.

De ATP-infusies leidden niet tot ernstige bijwerkingen. Als bijwerkingen optraden waren deze mild van aard en verdwenen deze snel na het verlagen van de pompsnelheid.

In het onderzoek beschreven in **hoofdstuk 4.2** is nagegaan of een infuus met ATP het ATP-gehalte in de lever bij longkankerpatiënten kan verbeteren. Dit werd onderzocht met behulp van een magnetische resonantie scan. Deze methode is gebaseerd op het meten van verschillen in magnetische eigenschappen tussen verschillende natuurlijke moleculen die een fosfor atoom bevatten. Negen patiënten met longkanker werden tweemaal onderzocht, en wel vóór en tijdens 22 tot 24 uur continu ATP-infuus. Het ATP-gehalte in de lever van longkankerpatiënten nam toe van 9% van de totale hoeveelheid fosfor in de lever in de uitgangssituatie tot 12% tijdens het ATP-infuus. Deze laatste waarden waren gelijk aan die bij gezonde controlepersonen. Vermeldenswaard is dat het ATP-gehalte meer toenam bij patiënten mét gewichtsverlies dan bij patiënten zónder gewichtsverlies.

Door glucose en alanine, een aminozuur afkomstig uit de afbraak van spierweefsel, in het bloed te 'merken' met deuterium (^2H) en koolstof-13 (^{13}C), kan de omzettingssnelheid van glucose en alanine gemeten worden. In **hoofdstuk 4.3** wordt het effect van het ATP-infuus op de glucose-omzettingssnelheid en op de glucoseproductie uit alanine

(=gluconeogenese) in twaalf patiënten met niet-kleincellige longkanker beschreven. Dertien patiënten met dezelfde ziekte en elf gezonde vrijwilligers dienden als controlegroepen. ATP-infuus leidde tot een dosis-afhankelijke stijging van de glucose-omzettingssnelheid. Een hoge ATP-dosis (75 μg/kg per minuut) bleek bij longkankerpatiënten een stijging van de glucose-omzettingssnelheid van 50% en een tendens tot stijging van de gluconeogenese uit alanine van 40% te veroorzaken. Lagere doses ATP (37 tot 50 μg/kg per minuut) bereikten dit effect niet. Hoewel tijdens hoog gedoseerde ATP-infusies de glucose-omzettingssnelheid gestimuleerd werd, zou deze op langere termijn juist verminderd kunnen zijn. Tussen de ATP-infusies werd namelijk een lage waarde gemeten, vergelijkbaar met die bij gezonde vrijwilligers.

Hoofdstuk 4.4 laat zien dat ATP de groei van longkankercellen remt. In kleincellige, grootcellige, plaveiselcelcarcinoom en mesothelioom cellijnen is de mate van groeiremming gerelateerd aan de ATP-dosis (0,5 tot 3 mmol/liter). Hoe hoger de ATP-dosis, des te meer groeiremming er optreedt. Adenocarcinoomcellen laten daarentegen reeds een maximale groeiremming zien bij de laagste ATP-dosis (0,5 mmol/liter).

In **hoofdstuk 4.5** wordt de waarde aangetoond van een methode om de lichaamssamenstelling (=onderverdeling in vetmassa en vetvrije massa) bij een groep kankerpatiënten op een eenvoudige wijze te bepalen: namelijk door het meten van de dikte van enkele huidplooien. Als referentiemethode werd de deuterium-verdunningsmethode gebruikt. Metingen van de verandering van vetmassa en vetvrije massa in 2 maanden bij 25 longkankerpatiënten laten vergelijkbare uitkomsten zien tussen beide methoden. De huidplooi-meetmethode geeft met 0,1 kg verschil slechts een minimale overschatting van het verlies van vetmassa en onderschatting van het verlies van vetvrije massa.

In **hoofdstuk 5** wordt de rol van ATP in patiënten met niet-kleincellige longkanker bediscussieerd. In het hierboven beschreven gerandomiseerde klinische onderzoek naar het effect van ATP bij patiënten met progressieve longkanker die slechts een korte levensverwachting van drie tot zes maanden hebben, is om ethische redenen een placebobehandeling in de controlegroep achterwege gelaten. Desondanks kan geconcludeerd worden dat de resultaten van deze studie voldoende betrouwbaar zijn.

Op grond van de resultaten in dit proefschrift zijn de volgende conclusies te trekken:

1. ATP-behandeling bij patiënten met niet-kleincellige longkanker (stadium IIIB of IV) leidt tot remming van verlies van:
   a. lichaamsgewicht;
   b. vetmassa, vetvrije massa, spiermassa en celmassa;
   c. spierkracht;
   d. kwaliteit van leven.
2. ATP-behandeling bij patiënten met dit eindstadium van niet-kleincellige longkanker (stadium IIIB of IV) heeft geen invloed op de:
   a. tumorrespons;
   b. overlevingsduur.
3. Adenosine-behandeling bij kankerpatiënten kan leiden tot vermindering van pijnbeleving.
4. ATP remt de groei van longkankercellijnen.

Bovengenoemde effecten worden onder andere bereikt via:

1. Aanvulling van aanwezige ATP-tekorten in de lever bij gewichtsverliezende patiënten;
2. Stimulering van de eetlust;
3. Onderdrukking van ontstekingsprocessen.

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Ellen van Rooijen heeft tijdens een onderzoekstage in het kader van haar biologiestudie een enorme inzet bewezen met de uitvoering van de celstudie, daarbij

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Diverse internisten, longartsen, radiotherapeuten en arts-assistenten in het Academisch Ziekenhuis Rotterdam en perifere ziekenhuizen waren bereid om patiënten voor deelname aan de ATP-studie te vragen.

Het belangrijkste deel van het onderzoek vond plaats op de Clinical Research Unit in het Dijkzigt Ziekenhuis. Hier kwamen de patiënten voor een polibezoek of opname. De verpleegkundigen Ana, Christie, Hannie, Hans, Jacqueline, Jeanne, Juul, Karin, Leo, Marijke en Titia hielpen bij de patiëntenverzorging, ATP-toediening en bloedmonsterverzameling. Co, je bureaustoel en weegschaaltje kwamen goed van pas.

Op de verpleegafdelingen B-O en de Palliatieve Unit in de Daniël den Hoedkliniek werden ook patiënten opgenomen en behandeld. De neuro-oncologen Vecht en Sillevis Smitt in de Daniël den Hoed Kliniek hielpen bij het opzetten en uitvoeren van de pijnstudie.

Op de zaterdagochtenden vonden metingen plaats m.b.v. de magnetische resonantie scan in de Daniël den Hoed Kliniek. Kwart over zeven beginnen was best wel vroeg. Peter, Christien en Paul waren hierbij bereid tot hulp.

De beide supervisoren van afdeling 4-noord, Gerard de Jong en Roel Swart hebben tijdens bijeenkomsten van de sectie 'voeding en metabolisme' meegedacht over de interpretatie van de resultaten van het ATP-onderzoek. Ik waardeer verder de vrijheid die jullie gaven om tussen de klinische bezigheden door m'n onderzoek te mogen afronden.

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Vaak wordt in de laatste zin de wens uitgesproken dat er meer tijd zal zijn voor nieuwe dingen. Laat ik het maar niet doen.

CURRICULUM VITAE

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LIST OF ABBREVIATIONS

^{13}C	carbon-13
^{2}H	deuterium
^{3}H	tritium
^{31}P	phosphorus-31
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
BCM	body cell mass
BW	body weight
Ca^{2+}	calcium
CL_R	renal clearance
CRP	C-reactive protein
ECW	extracellular water
FFM	fat-free mass
FM	fat mass
HPLC	high-performance liquid chromatograph
IL	interleukin
IP_3	inositol 1,4,5-triphosphate
MRS	magnetic resonance spectroscopy
MTD	maximally tolerated dose
NRS	numerical rating scale
NSCLC	non-small-cell lung cancer
PME	phosphomonoester
PDE	phosphodiester
P_i	inorganic phosphate
QOL	quality of life
TNF	tumor necrosis factor
r	correlation coefficient
Ra	rate of appearance
REE	resting energy expenditure
RSCL	Rotterdam Symptom Checklist
SD	standard deviation
SEM	standard error of the mean
$t½$	half-time
TBW	total body water
TR	repetition time
VRS	verbal rating scale
UTP	uridine 5'-triphosphate