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Original Research Article

Evaluation of changes in rat fatigability and biochemical parameters after oral and intra-peritoneal administration of adenosine tri phosphate: an experimental study

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ABSTRACT

Background: Adenosine tri phosphate (ATP) is an important intracellular energy source and has many extracellular functions meadiacating through purine receptors. Currently ATP is available in the market as oral dietary supplement. However there are inconclusive studies regarding its efficacy through oral route. Hence this study was carried out to evaluate efficacy of oral and intraperitoneal administration of ATP in experimental rats by comparing swim exhaust time and associated biochemical changes.
Methods: 18 Swiss albino rats of either gender were grouped randomly into three groups, consisting of group -1 control group which receive distilled water (5ml/kg body weight) whereas group 2 and 3 received oral and intraperitoneal ATP (60mg/kg body weight) for 8 days. On 8th day all rats all rats were evaluated for extent of physical fatigue by using exhaustive swimming test time required to attain immobility status is noted. Under aseptic precautions blood samples were drawn from rat tail vein and biochemical parameters like uric acid, triglycerides, total cholesterol, random blood sugar and c-reactive protein levels were measured.
Results: There was a significant ($p<0.05$) increase in serum uric acid, blood sugar and urea in Group 2and3 compared control group 1. There was no statistically significant increase in physical strength in group 2 and 3 as compared to group 1.
Conclusions: Oral and intraperitoneal administration of ATP may lead to hyperglycaemia, hyperuricemia and dyslipidaemia without significant increase in muscle strength.

Keywords: Adenosine tri phosphate, Hyperuricemia, Hyperglycemia

INTRODUCTION

It is well known fact that adenosine tri phosphate (ATP) an adenine nucleotide bound to three phosphates is involved in various cellular functions and is primarily an intracellular energy packet. Whereas extracellular ATP is involved in various biological processes including neurotransmission, cardiac function, muscle contraction and platelet aggregation via purinergic membrane receptors.[1-4] In addition it has been implicated in changes in arterial smooth muscle tone.[5]

Various dietary food products like soya, meat and breast milk contain nucleotides.[6,7] Currently Oral ATP is available in the market as dietary supplement. Studies have been conducted evaluating its efficacy in low back pain, post total knee arthroplasty.[8,9] There are studies demonstrating oral ATP efficacy as antihyperlipidemic supplement.[10] However majority of studies have also reported that there is increase in uric acid level with oral ATP administration and variable bioavailability.[11,12] ATP undergoes dephosphorylation by the enzyme ecto-nucleoside triphosphate diphosphohydrolase present on

the luminal side of enterocytes via ADP (Adenosine diphosphate) to AMP (Adenosine monophoshate). AMP in turn, is further metabolized by ecto-5'-nucleotidase and alkaline phosphatase to adenosine.[13] Adenosine can be taken up into the enterocytes of the intestinal wall, through concentrative or equilibrative nucleoside transporters present on the basolateral side of enterocytes.[14] Adenosine is taken up by erythrocytes, provided adenosine is released intact into the vascular bed and will come in contact with erythrocytes.[15-17] Then it will quickly enters into liver. In liver, adenosine will be broken down to uric acid by the enzymes adenosine deaminase and xanthine oxidase.[18] Hyperuricemia is an independent risk factor for development of metabolic syndrome, gout and insulin resistance.[19-21]

Hence this study was undertaken with an objective to evaluate efficacy of ATP by oral and intraperitoneal route using rat fatigability model and associated changes in biochemical parameters.

METHODS

Study was conducted in department of Pharmacology, S Nijalingappa medical college Bagalkot, Karnataka, India. Approval for study protocol was obtained from Institutional Animal Ethics committee and by the animal regulatory body (Registration No: 829/AC/04/CPCSEA). After obtaining clearance, eighteen Swiss albino rats of either gender were selected from the central animal house of S Nijalingappa Medical College for the study. No protocol related procedures were undertaken before taking animal ethics committee approval. All procedures carried out in the study were as per CPCSEA guidelines. Selected animals were examined and screened for general health condition including vital parameters. All the animals were acclimatized to laboratory for one week before starting the study. The animals were housed under standard laboratory conditions maintained at 25 ± 5^0C and exposed to 12 hr dark and 12 hr light cycle and fed with standard pellet diet and water ad libitum.

Then animals were randomly tagged with serial numbers and then with computer generated list were grouped in to three groups, six animals in each group. For Group I (6) - distilled water was administered orally at the dose of 5ml/kg body weight, which acts as control group. Group II (6): ATP was given orally and the dose is 60mg/kg body wt. Group III (6): received ATP Intraperitoneal 60mg/kg body wt. Sample size of 18 was chosen as purposive sampling.

On the 8th day of experiment, all animals were evaluated for extent of physical fatigue by using exhaustive swimming test, using a cylindrical glass container, containing water at 25°C. Immobility time i.e. time taken by the animal to reach a stage where it makes only those movements required to keep its head above water is measured in minutes using stop watch. Then blood samples were collected from tail vein under aseptic precautions. Serum uric acid, urea, RBS, TGL, TC, hsCRP were measured. All the biochemical parameters were estimated using STATFAX 3300, semi-auto analyser and kits were supplied by the BIOSYSTEMS Pvt Ltd.

All values were expressed in mean± SD. The data was statistically analysed using unpaired 't' test , one-way ANOVA followed by Dunnett multiple comparison test with equal sample size. $p<0.05$ was considered as statistically significant.

RESULTS

Table 1: Body weight, swim time and biochemical parameters control and oral group.

Parameter	Gp-I (Control) Mean +SD	Gp II (oral ATP) Mean +SD	T	P
Body wt (Gm)	163.0±31.7	162.5±30.7	0.02	0.978
Swim time (Min)	2.5±0.7	2.1±0.0	1.35	0.204
Uric acid (mg/dl)	2.2±0.9	4.5±0.1	19.22	0
Urea(mg/dl)	30.3±2.1	37.3±3.9	3.74	0.004
TGL(mg/dl)	69.8±7.3	71.0±1.1	0.14	0.68
TC (mg/dl)	45.7±1.5	46.0±5.4	0.13	0.89
RBS(mg/dl)	73.1±6.0	192.1±19.5	14.24	0
hsCRP(µgm/L	1.3±0.8	1.4±0.9	0.26	0.798

Table 2: Body wt, swim time and biochemical parameters Control and IP group.

Parameter	Gp-I (Control) Mean +SD	Gp II (oral ATP) Mean +SD	T	P
Body wt (Gm)	163.0±31.7	149.1±19.8	0.9	0.387
Swim time (Min)	2.5±0.7	3.1±0.1	1.98	0.075
Uric acid (mg/dl)	2.2±0.9	9.4±2.9	9.51	0
Urea(mg/dl)	30.3±2.1	37.3±3.9	10.33	0
TGL(mg/dl)	69.8±7.3	43.4±2.1	0.82	0.42
TC (mg/dl)	45.7±1.5	47.91±3.0	1.57	0.14
RBS(mg/dl)	73.1±6.0	168.0±10.8	18.7	0
hsCRP(µgm/L	1.3±0.8	2.2±0.3	2.4	0.037

Body weights of rats were comparable among three groups, no statistical significant difference was observed as depicted in tables 1to3. Swim time among the three groups was also statistically significant even though swim duration was on higher side with intraperitoneal route .i.e. group III but it was not statistically significant as shown in figure 1. There was significant increase in uric acid, blood sugar level and hsCRP in groups II and III as shown in tables 1 to3 and figure 2. During study period general health condition of all animals was well maintained and there was no mortality.

Table 3: Body wt, swim time and biochemical parameters oral and IP group.

Parameter	Gp-I (Control) Mean +SD	Gp II (oral ATP) Mean +SD	T	P
Body wt (Gm)	162.5±30.7	149.1±19.8	0.9	0.387
Swim time (Min)	2.1±0.0	3.1±0.1	1.98	0.075
Uric acid (mg/dl)	4.5±0.1	9.4±2.9	9.51	0
Urea(mg/dl)	37.3±3.9	37.3±3.9	10.33	0
TGL(mg/dl)	71.0±1.1	43.4±2.1	0.82	0.42
TC (mg/dl)	46.0±5.4	47.91±3.0	1.57	0.14
RBS(mg/dl)	192.1±19.5	168.0±10.8	18.7	0
hsCRP(µgm/L	1.4±0.9	2.2±0.3	2.4	0.037

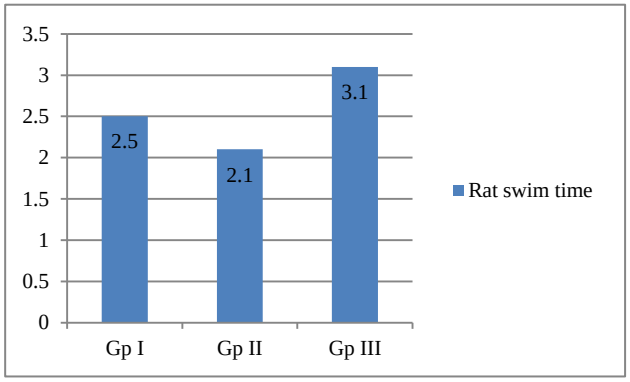

Figure 1: Rat immobility time (swim time) expressed in minutes.

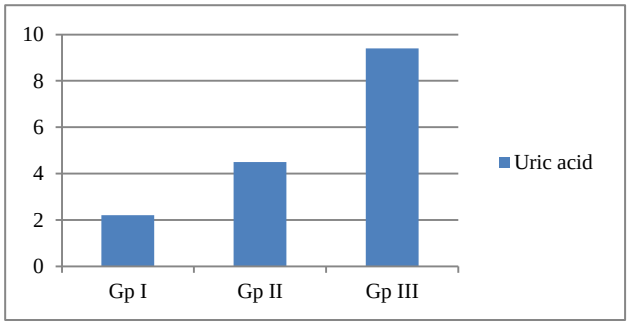

Figure 2: Serum uric acid levels on 8th day.

DISCUSSION

This was a basic study conducted to evaluate efficacy of ATP administered by different routes i.e. oral and intraperitoneal. 18 Swiss albino rats of either gender were randomly grouped into three groups containing six in each. On 8th day of experiment they were evaluated for the efficacy of ATP and biochemical parameters. Rat swim exhaust time was taken as surrogate marker for efficacy assuming ATP will increase the strength of muscles.

However in this study we observed there was no statistically significant difference among the groups even though swim time was comparably on higher side in Group III, depicting there was no improvement in muscle strength or stamina of the rats in all three groups.

Animal and human study conducted by Jäger R et al. concluded that oral ATP administration can increase post-exercise blood flow, hence can be effective during exercise recovery.[11] Long term oral administration of ATP has been shown to increase both the uptake and synthesis of ATP in the erythrocytes of rodents.[22]

Animal studies reporting alterations in cardiac, vascular and pulmonary function after 30 days of oral ATP supplementation, also found no increase in systemic concentrations of plasma or erythrocyte ATP.[22,23] However, the concentration of ATP in plasma taken from the portal vein of rats increased rapidly up to a 1000-fold after instillation of ATP in the small intestine.[22]

Study conducted by Rathmacher JA.et al, a placebo controlled double blind study have shown that Supplementation with 400 mg ATP daily for 2 weeks tended to reduce muscle fatigue and improved a participant's ability to maintain a higher force output at the end of an exhaustive exercise bout.[24] Similarly Wilson JM et al, conducted a 12 week long term phase III randomized, double-blind, placebo- controlled study with oral ATP supplementation have shown no decrement in performance following overreaching and without significant biochemical changes.[25]

Recently study by Ju J et al, in patient with Alternating hemiplegia of childhood demonstrated supplementation of oral ATP for 2 years at dose of 24mg/kg/day has shown significant improvement and patient tolerated intervention well with occasional rise in serum uric acid levels.[26]

However in other studies, Arts ICW et al, concluded that ATP administered orally is not absorbed and there is associated significant increase in uric acid levels.[11] Findings of our study are consistent with them as there is no significant increase in swim time and presence of significant hyperuricemia. Hyperuricemia is an independent risk factor for various conditions like gout, metabolic syndrome, insulin resistance, renal dysfunction and hypertension.[27,28]

Exact mechanisms of insulin resistance and metabolic syndrome in relation to hyperuricemia have not been clearly established and are under investigation. Krishna E et al. Evaluated the association between serum uric acid level and incidence both of type 2 diabetes and pre-diabetes, and their study concluded that hyperuricemia can be a useful predictor of diabetes mellitus and urate concentration can be considered as an inexpensive marker for assessing the risk of future incident type 2 diabetes and diabetes-related outcomes in non-obese individuals.[29] In vitro study consisting of Human aortic endothelial cells demonstrated that uric acid induced endothelial

dysfunction is associated with mitochondrial alterations and decreased intracellular ATP.[30]

A meta-analysis conducted by Quin Lv and group have concluded the strong evidence exists between high level of serum uric acid and development metabolic syndrome component- type 2 diabetes in middle-aged and older people.[31] Our study results are consistent with that there was a significant rise in blood sugar levels as depicted in Tables 2 and 3.

There are reported studies suggesting increased uric acid levels are associated with alteration in lipid levels and positively correlated to hypertriglyceridemia, hypercholesterolemia and increased arterial tone, whereas HDL levels were inversely correlated with serum uric acid levels.[32,33] In our study there was slight elevation triglycerides and total cholesterol but values were not statistically significant as compared to control group. In addition there is correlation between serum uric acid levels and elevated hs-CRP, which in turn associated with stroke, cardiovascular events and immunological diseases like psoriasis.[34-36] However in our study mild elevation of hs-CRP was not statistically significant.

On the other side elevated uric acid levels have positive outcomes like reduced incidence of Parkinsonism and reduced uric acid levels were found in patients with multiple sclerosis.[37,38]

In this study there was significant elevation of serum uric acid level when given parenteral route as well as oral route($P<0.005$), however there was no improvement in swim time suggesting poor bioavailability. However rats swim time was higher with intra peritoneal route compared to oral route but statistically not significant, suggesting relatively poor efficacy through oral administration. Limitations of this study were small sample size and short duration of study. There is scope for study with larger sample size, long term administration of ATP and emphasising on measurement of ATP concentrations along with clinical correlation.

In conclusion, oral and parenteral ATP supplementation is associated with hyperuricemia, hyperglycaemia without much beneficial effects.

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