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Original Article

Determination of the stability of plasma ATP in vitro

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Abstract: Background: Adenosine 5'-triphosphate (ATP) is the most direct source of energy in organisms. Recently, it is evident that ATP plays an essential role in the immune and inflammatory systems. However, ATP is unstable when it exposed to room temperature in vitro. Therefore, our article is aim to explore the stability of ATP. Methods and Results: 28 samples of ATP were detected. Student's t test or one-way ANOVA was used to compare multiple groups. It shows that during the storage process from day 1 to day 70, the overall levels tend to decrease. Conclusion: The level of ATP does not reduce at least in the first month when stored at -80°C. On the 70th day, there was a star drop, and the levels were lower than before.

Keywords: ATP, concentration, stability

Introduction

ATP is an unstable high-energy compound composed of 1 molecule of adenine, 1 molecule of ribose and 3 molecules of phosphate groups. Under the action of ATP hydrolase, it hydrolyzes the high-energy phosphate bond, generating ADP and Pi, to release energy. As the most direct source of energy in organisms, it can transform with ADP to realize energy storage and release, thus ensuring the energy supply for activities in the cell [2-5].

In addition to being an energy substance in the body, ATP also plays an important role in signal transduction as an active substance [6-12]. Purinergic signaling was thought to be an essential signaling pathway related to physiology exclusively in the nervous system [1, 13-15]. In cardiovascular system, Burnstock G reported that ATP can regulate the systole and diastole of blood vessels through different mechanisms [16]. Furthermore, purinergic signaling is also involved in the physiology of erythrocytes, platelets, and leukocytes which mediate platelet aggregation and deformation [17]. Recently, it is increasingly evident that ATP also plays an essential, even more important, role in the immune and inflammatory systems [1, 18, 19].

However, although we all know that ATP is unstable when it exposed to room temperature in vitro, how is its stability and how long it can maintain at a stable concentration in a low temperature environment remains to be resolved. We searched previous literatures and found there are few studies on that. Chen Y reported that ATP solution is not stable at room temperature and the validity period is only 69 days [20]. Wang G found that the longer ATP solution is placed at room temperature, the lower concentration is [21]. Regrettably, the above findings were reported in 20th century, and the source of ATP was standard solution, so our study is aim to: 1) Prove the previous view that the level of ATP concentration reduce along with the storage time gradually; 2) Explore whether the validity period of ATP in human plasma is also in that range.

Methods

Sample processing

The research samples come from the patients admitted to the Fourth Affiliated Hospital of Zhejiang University School. All the participants signed a written informed consent form and the ethics committee of the hospital approved the use of clinical data. Patients with histories of

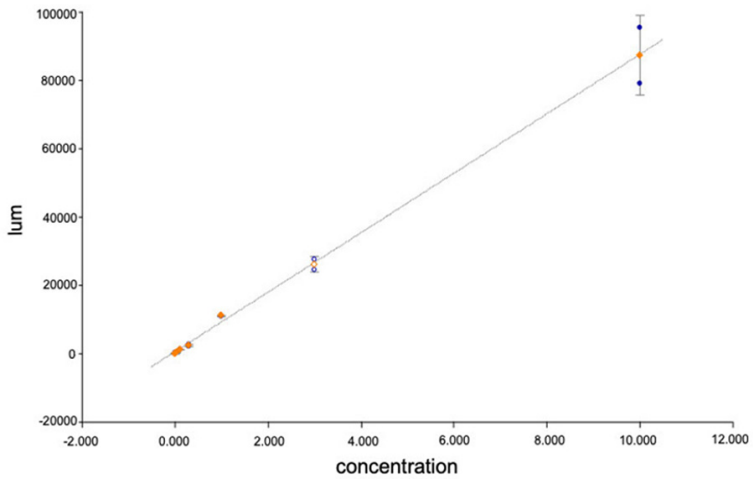

Figure 1. The standard curve.

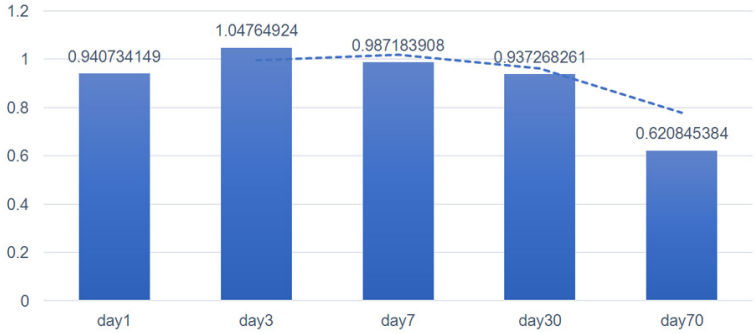

Figure 2. The concentration of ATP solution from day 1 to day 70.

Table 1. Sample ATP concentration under different storage time

Time	Number of cases	Concentration (μM)	Concentration (μM)	
Day 70	28	0.687		
Day 30	28		1.038	
Day 7	28		1.093	
Day 3	28		1.160	
Day 0	28		1.042	
P		1.000	0.693	0.000

autoimmune diseases, lung diseases, asthma, stroke, AIDS, cancer, transplantation and those having symptoms or history of infection in the last month were excluded. Finally, 31 patients were elected in our study.

Plasma ATP analysis

The fasting blood (4 ml) of 31 selected subjects were collected into heparin-treated anticoagulation tubes. Plasma was isolated by centrifuging the whole blood at 1000 g for 15 min. Hemolyzed samples were not used. All blood samples used were not subjected to a freeze-thaw cycle at any step in the process. The separated plasma is aliquoted in 50 ul and stored at a low temperature of -80°C. Plasma ATP levels were assessed with an ATP assay bioluminescence detection kit (Beyotime) [22]. They were tested on days 0, 3, 7, 30, and 70 since centrifugation.

Statistical analysis

Categorical variables were presented as numbers and continuous variables were presented as mean ± SD. Samples were categorized according to using aspirin or not, and each group was further divided into hypertension group (HTN) and normotensive (NTN). Student's t test or one-way ANOVA was used to compare groups, then the least squares method (LSD) was used to perform multiple comparisons of data in each group. Statistical analyses were performed using SPSS software (version 25.0, SPSS Inc.).

Results

Unqualified samples will be eliminated during the ATP detection process, eventually, a total of 28 samples were included. The standard curve is showed in **Figure 1** and the results are basically distributed on a straight line. The **Figure 2** shows that during the storage process of ATP solution from day1 to day 70, the overall levels tend to decrease. The **Table 1** shows further that there is a statistical difference between 5 results, among which there is no statistical difference between the first 4, that is, the levels of ATP do not show a star drop in at least the first month when stored at -80°C. On the 70th day, there

Table 2. Difference in ATP concentration between different groups

*	Conc (μM)		Conc (μM)			Conc day 0-70 (μM)	
NO (n=13)	1.184±0.744	HTN (n=9)	1.377±0.106	P<0.000	P=0.051	0.408±0.100	P=0.169
		NTN (n=4)	0.751±0.375				
YES (n=15)	0.918±0.519	HTN (n=11)	0.909±0.232	P=0.883		0.307±0.113	
		NTN (n=4)	0.941±0.628				

*samples were categorized according to using aspirin or not; HTN: hypertension; NTN: normotensive.

was a statistical difference in the value, and the ATP concentration was lower than before.

Also, we combined the basic information of these samples to obtain that ATP levels were significantly higher in the HTN than NTN group [median ATP, 1.377 μM versus 0.751 μM; P<0.000] who had no use of aspirin on admission, otherwise, there was no difference (aspirin is considered to have anti-inflammatory effects and may will affect the level of ATP). In addition, there also have no statistically significance in the degree of ATP reduction between two groups (aspirin was used or not) (**Table 2**).

Discussion

In the healthy organism, ATP almost present inside the cells, where it reaches a several millimolar concentration. In the extra-cellular environment, the ATP concentration is negligible, i.e. in the low nanomolar range [23]. When the body causing inflammation, ATP release from the cells into the extracellular (plasma or interstitial) to participate in the reaction. Therefore, the detection of plasma ATP concentration may be helpful to the pathophysiological process of some clinical diseases.

ATP is unstable when exposed to room temperature in vitro. Our experiment concluded that under the environment of -80°C, the levels of ATP will not decease for at least the first month and the validity period of ATP in human plasma is also between 30 days and 70 days, which is consistent with the previous conclusions. Based on it, I think that ATP injections used in clinical can be made into powders, including laboratory ATP-related reagents that need to be prepared on the process of usage.

Furthermore, it suggests that individuals with hypertension are accompanied by systemic chronic low-grade inflammation. Serving as a DAMP, ATP plays an important factor in the pathological process of hypertension [24]. Our small sample study also found that the level of ATP was higher in HTN, which is consistent with previous studies. However, when drug which may affect inflammation, such as aspirin, was used, this difference disappeared, which further proves the inflammatory effect of ATP.

However, aspirin does not show its effect in the decay speed of ATP level, which we guess that drugs like aspirin may only affect its generation, and not degradation.

Our study has several limitations: 1) although the process of detecting ATP was performed on an ice bath, it cannot be guaranteed completely that there is no consume of it, which may affect the experimental results; 2) how long will the concentration of ATP be stable also needs to be further explored; 3) a large sample study is needed to further prove the relationship between ATP and inflammation, which we are proceeding and have gotten some preliminary gratifying results.

Conclusions

ATP is unstable when exposed to room temperature in vitro, but the levels of ATP in vitro under the environment of -80°C will not change for at least the first month and the validity period of ATP in human plasma is also between 30 days and 70 days. Besides, ATP was higher in HTN and drugs like aspirin may reduce its concentration.

Disclosure of conflict of interest

None.

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