Indian Journal of Forensic Medicine and Toxicology
Volume 16 No. 3, July-September 2022
DOI: http://dx.doi.org/xxxxx/ijfmt.xxxx.16322.10

Changing of ATP and Its Metabolites in Blood Samples for Post Mortem Interval: In Vitro Study

Warangkool Chanpan[1,4], Churdsak Jaikang[2], Chaturong Kanchai[3]

[1]Resident Training in Forensic Medicine, [2]Assistant Professor, [3]Associated Professor, Department of Forensic Medicine, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand, [4]Somdejphajaotaksinmaharaj Hospital, 16/2 Rahang Sub-district, Meuang District, Tak Province Thailand.

How to cite this article: Warangkool Chanpan, Churdsak Jaikang, Chaturong Kanchai. Changing of ATP and Its Metabolites in Blood Samples for Post Mortem Interval: In Vitro Study. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):48-52.

Abstract

Background: Postmortem interval (PMI) is an important in forensic practice. Many studies in blood and tissue of animals have revealed correlation between adenosine triphosphate (ATP) level and PMI. In this study aimed to explore the correlation between human blood ATP and its metabolites level and PMI in vitro model.

Methods: Ten milliliter of venous blood samples were collected form four healthy men and contained in EDTA tube. One milliliter of the blood sample was divided at 0, 1, 2, 4, 8, 12 and 24 h, respectively. The blood samples were extracted and measured ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) level by High performance liquid chromatography- diode array. Energy charge value was calculated to predict charge of ATP metabolites.

Results: The blood ATP, ADP and AMP levels increased in the first hour. The blood ATP, ADP and AMP levels did not obviously change before 12 hour. The blood ATP level trended to decrease but the blood ADP and AMP levels trended to increase after 24 hour. Energy Charge was not change in first 12 hour but trended to decrease after 24 hour.

Conclusion: The blood ATP level and its metabolites changed after 12 hour and apply for the PMI investigation.

Keywords: Post-mortem interval; ATP degradation; ATP metabolites; Forensic Science.

Introduction

The estimation of the time since death or postmortem interval (PMI) is an important step in criminal investigation and is the highest frequency question in forensic practice.[1-2] Early stage of PMI investigation is based on algor, livor and rigor mortis for assessments.[3] Ambient temperature, body structure, cause of death, climate and diseases are associated factors and need evidences to describe the PMI processes.[3] Many methods including molecular biology, spectroscopic technology, entomological and Thanatos chemistry, biochemical reaction and fluid concentration have been improved to help the PMI investigation.[2, 4]

Corresponding Author: Chaturong Kanchai, Associated Professor, Department of Forensic Medicine, Faculty of Medicine Chiang Mai University, Chiang Mai, 50200 Thailand.

E-mail: Chaturong.k@cmu.ac.th

Adenosine Triphosphate (ATP) is an essential substance in cellular metabolism and widely uses for the time elapsed since death. The degradation of ATP in post-mortem tissue is an important biochemical reactions. The oxygen supply to the tissue is stopped after death and the normal physiological metabolic functions are damaged. Normally source of ATP comes from erythrocytes. Imbalance of ATP production and consumption appear after death. The ATP synthesis was stopped and rapidly degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP) which can be summarized as: ATP→ADP→AMP.[5-6]

An extensive metabolomics and biochemical change in all body tissue due to lack of circulating oxygen, altered enzymatic reactions, cellular degradation and cessation of anabolic production of metabolites after death. The biochemical changes provide information about the effect of death on cellular function especially ATP blood level. Blood sample has an advantage including easy sampling and sufficient specimens for the PMI estimation. There are many studies have revealed the relationship between ATP blood levels with PMI by using animal blood and tissues. In this study aimed to explore the correlation between human blood ATP levels and PMI in vitro model to provide a preliminary research basis for PMI investigation in forensic science.

Materials and Methods

Chemicals and reagents

Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were purchased from Med Chem Express (New Jersey, USA) Ammonium hydrogen phosphate and methanol were purchase from BDH (Fontenay-Sous Boi, France). Chloroform was purchased from Labscan Limited, Thailand.

Subject and study design

Four healthy men Thai aged range 20-40 years were included in this study. The subjects who were the anemia and red blood cell diseases were excluded. Written inform consent was obtained. The Study protocol was approved by the Research Ethic committee Faculty of Medicine, Chiang Mai University (FOR 2564-07900).

Collection and specimen preparation

Venous blood approximately 10 mL was collected and contained in EDTA tube. The blood samples were stood at 25 °C through experiment. One milliliter of the blood sample were divided every 0, 1, 2, 4, 8, 12 and 24 hr. Each blood samples were extracted with methanol and chloroform in ration 1:2:2 according the method of Nagana *et.al*.[7] Then, the solution was centrifuged at 4,000 g at 4 °C for 10 min. The organic solvent layers was collected and evaporated under nitrogen gas. The residue was re-dissolved with ammonium phosphate buffer pH 6.0 before HPLC analysis.

ATP and it metabolites measurement

ATP, ADP and AMP were measured by high performance liquid chromatography- diode array (Agilent 1260 Infinity Binary LC, Santa Clara, CA, USA) by apply the method of Menegollo *et. al*. [8] The HPLC condition comprised of Purospher® Star PR-8 endcapped column (150 × 4.60, 5 µm). The mobile phase consisted of 100% 0.1 M ammonium hydrogen phosphate pH 6.0. Ten microliter of the sample was injected and the spectra were determined at 250 nm. The identification of the chromatographic peak was achieved by comparing the retention times and spectral characteristics (200-400 nm) of the eluted peaks with the standards.

For, energy charge value was calculated by as follows: EC = [ATP] + 0.5 [ADP]/ {[ATP] + [ADP] + [AMP]}[9]

Statistical Analysis

Quantitative analysis of ATP and its metabolites were done by external standard curve under the same liquid chromatography condition. The data of ATP, ADP and AMP were expressed as mean ± standard deviation (mean± S.D). The data were evaluated using the SPSS version 22 (New York, USA) The One- Way ANOVA was evaluated for identifying significant level between ATP and its metabolites and time. The threshold for statistical significance was $p<0.05$.

Results and Discussion

In this study, under HPLC-DAD condition, the ATP, ADP, AMP were appeared at 5.32, 5.96 and 8.59 min, respectively. Changing of ATP, ADP and AMP levels is demonstrated in Table 1 and Fig.1.

Table 1: Concentration of ATP, ADP and AMP levels in the blood at different post-mortem interval.

Time (hr)	ATP (μM x 10^2)	ADP (μM x 10)	AMP (Mean ± SD)	Energy charge
0	21.14 ± 3.56[a]	18.45 ± 3.92[a]	46.18 ± 22.69[a]	0.94
1	48.32 ± 5.27[b]	37.03 ± 2.07 [a]	48.72±7.77[a]	0.95
2	8.03 ± 0.19[c]	15.23 ± 1.74[b]	1.78 ± 0.15[b]	0.91
4	15.94 ± 0.17[d]	25.51± 1.93[b]	18.80 ± 0.33[b]	0.92
8	13.51 ± 0.22 [d]	24.06 ± 1.72[b]	11.27 ±8.23[b]	0.91
12	16.25 ± 0.21[d]	20.71 ± 11.09[b]	17.43 ±0.21[b]	0.93
24	10.31 ± 0.24[e]	49.49 ± 1.30[c]	112.61 ± 9.44[c]	0.78

The values are presented in mean ±S.D. (n=4). Different small letters indicate significant difference between time in column at $p<0.05$ which analyzed by One-way ANOVA followed by Bonferroni.

In the first hour, the ATP level significantly increased ($p<0.05$) and the level did not changed duting 4-12 h. After 12 h incubation, the ATP level significantly decreased comparing with the other times ($p<0.05$). Highly activity ATPase enzyme and more glucose and nutrition contained in the blood can generate ATP molecule during the first period.[10] Butt and his colleages found that blood ATP level decreased during 0-72 h after death.[4] The ATP level with in the first 8 h increased following not significant change within 8-16 h in skeletal rat muscle model.[6] However, these results from in vitro model presented that the ATP decreased after 12 h and need to confirm in the crops study.

ADP is a by- product from ATP degradation in both the cells and in vitro models. In the first hour, the ADP level increased due to the ATP molecules were used in the cell resulting the ADP level rapidly decreased. We hypothesized that the ADP was used for the ATP synthesis by ATPase enzyme or it was degraded to AMP for energy production. Zhu et al. presented that ADP level did not significant change within the first 8 h but within 16-24 h the ADP increased.[6] At the 2-12 h, the AMP level significant decreased after that the AMP level significant increased ($p<0.05$). Zhu et.al showed that over all of the AMP level trended to increase in the first 144 h after death.[6]

Energy is an important biochemical molecule for the adequate turnover of the biomolecular structures and the functional metabolic viability in unicellular organisms. ATP, ADP and AMP level and energy charge (EC) reflect to the energetic status of the cell.12 The EC value in physiological system presents in range 0.7-0.9 and reflects to a balance with the major ATP-consuming reaction. The value closely to 0.9 in living cell but it is dropped off provoking cell to die.[13,14] Our results showed that the EC value in the first 12 h did not change but after 12 h the value was dropped indicating an imbalance of energy status. The ATP and its metabolites changed in the in human blood in vitro model. The ATP and its metabolites experienced "rise-decline-constant and increase within 24 h after starting experimentation. The possible reason is that residual enzymes in the blood continue to catalyze the decomposition of glycolysis and phosphocreatine and ADP continues to produce AMP and ATP ($2ADP \leftrightarrow ATP+AMP$).[15] The degradation of ATP, ADP and AMP are not obvious and cannot be used to estimate PMI by itself during the first 12 h.[16] Imbalance in energy homeostasis will be occurred after 12 h then determination of the blood ATP and its metabolites might be useful for PMI investigation. The correctly of PMI estimation has not significantly improved and less reliable methods.[9] After death, a biochemical changes will occur under the influence of various intrinsic and environmental factors.[6] These processes are non-reversible, progressive and time-sequence.

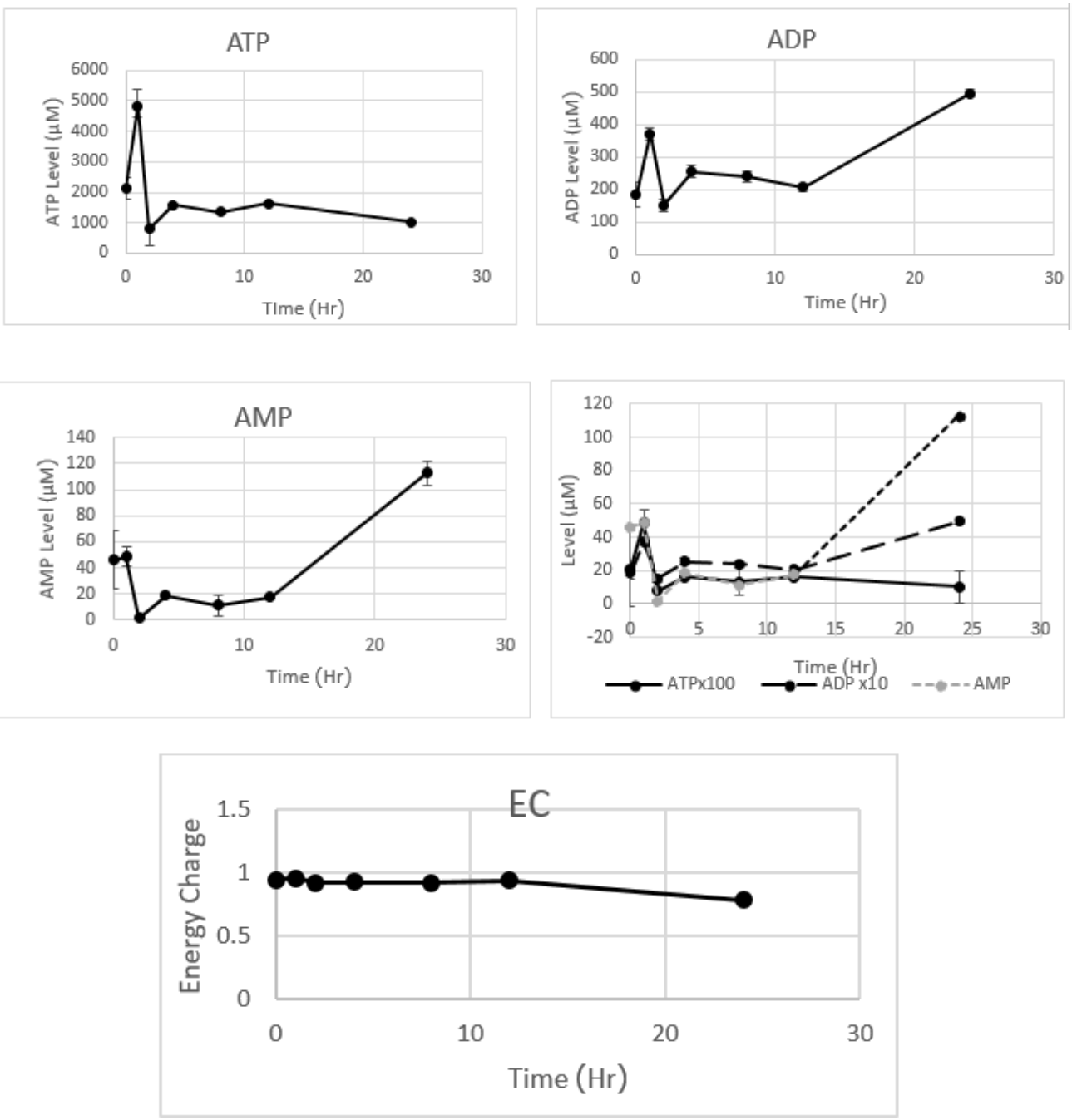

Figure 1: Change in the blood concentration of ATP, ADP AMP and energy charge in vitro model

Conclusion

For in vitro study, changing of ATP and its metabolites after death within 12 h might be applied for postmortem interval investigation. However, study on human body should be studied further to give more information and the factors involved in postmortem interval interpretation.

Disclosure

The authors declare that there is no conflict of interest in this work.

Ethical Clearance

Taken from Faculty of Medicine, Chiang Mai University committee.

Source of Funding: Self

References

1. Alibegovic, A., Cartilage: a new parameter for the determination of the postmortem interval? *J Forensic Leg Med* 2014;*27*:39-45.
2. Li, C.; Wang, Q.; Zhang, Y.; Lin, H.; Zhang, J.; Huang, P.; Wang, Z., Research progress in the estimation of the postmortem interval by Chinese forensic scholars. *Forensic Sci Res* 2016;1(1):3-13.
3. Sun, T.; Yang, T.; Zhang, H.; Zhuo, L.; Liu, L., Interpolation function estimates post mortem interval under ambient temperature correlating with blood ATP level. *Forensic Sci Int* 2014;*238*:47-52.
4. Butt, U. S.; Iqbal, A.; Zaheer, S.; Nazir, S.; Rakha, A., ATP levels and its degradation process as a post-mortem interval indicator. *Pakistan Journal of Physiology* 2021;17(1):23-25.
5. Aliani, M.; Farmer, L. J.; Kennedy, J. T.; Moss, B. W.; Gordon, A., Post-slaughter changes in ATP metabolites, reducing and phosphorylated sugars in chicken meat. *Meat Sci* 2013;94(1):55-62.
6. Zhu, W.; Zhai, X.; Zheng, Z.; Sun, K.; Yang, M.; Mo, Y., New contributions to the relationship between sequential changes of ATP-related metabolites and post-mortem interval in rats. *Leg Med (Tokyo)* 2021;48: 101809.
7. Nagana Gowda, G.; Raftery, D., Whole blood metabolomics by 1H NMR spectroscopy provides a new opportunity to evaluate coenzymes and antioxidants. *Analytical chemistry* 2017;89(8):4620-27.
8. Menegollo, M.; Tessari, I.; Bubacco, L.; Szabadkai, G., Determination of ATP, ADP, and AMP levels by reversed-phase high-performance liquid chromatography in cultured cells. In *Calcium Signalling*, Springer: 2019;pp.223-32.
9. Guimaraes, P. M.; Londesborough, J., The adenylate energy charge and specific fermentation rate of brewer's yeasts fermenting high- and very high-gravity worts. *Yeast* 2008;25(1):47-58.
10. Bonora, M.; Patergnani, S.; Rimessi, A.; De Marchi, E.; Suski, J. M.; Bononi, A.; Giorgi, C.; Marchi, S.; Missiroli, S.; Poletti, F.; Wieckowski, M. R.; Pinton, P., ATP synthesis and storage. *Purinergic Signal* 2012; 8(3):343-57.
11. Silva-Vilches, C.; Ring, S.; Mahnke, K., ATP and Its Metabolite Adenosine as Regulators of Dendritic Cell Activity. *Front Immunol* 2018;9:2581.
12. De la Fuente, I. M.; Cortes, J. M.; Valero, E.; Desroches, M.; Rodrigues, S.; Malaina, I.; Martinez, L., On the dynamics of the adenylate energy system: homeorhesis vs homeostasis. *PLoS One* 2014;9(10):e108676.
13. Ball, W. J., Jr.; Atkinson, D. E., Adenylate energy charge in Saccharomyces cerevisiae during starvation. *J Bacteriol* 1975;121(3):975-82.
14. Swedes, J. S.; Sedo, R. J.; Atkinson, D. E., Relation of growth and protein synthesis to the adenylate energy charge in an adenine-requiring mutant of Escherichia coli. *J Biol Chem* 1975;250(17):6930-8.
15. Gastin, P. B., Energy system interaction and relative contribution during maximal exercise. *Sports Med* 2001;31(10):725-41.
16. Mao, S.; Fu, G.; Seese, R. R.; Wang, Z. Y., Estimation of PMI depends on the changes in ATP and its degradation products. *Leg Med (Tokyo)* 2013;15(5):235-8.