



ATP in red blood cells as biomarker for sepsis in humans

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ABSTRACT

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to an infection. Due to the lack of causative immune treatment, mortality of sepsis remains at a high level and represents one of the main disease burdens globally. Adenosine 5' triphosphate (ATP) levels in red blood cells (RBC) are modulated by various factors during sepsis, including a decrease in ATP production, an increase in ATP catabolism and alterations in ATP release. Therefore, we hypothesize that intracellular ATP levels in RBC can serve as potential biomarker for sepsis and support sepsis diagnosis. This will facilitate early treatment and could improve the outcome of this serious condition.

Introduction

Despite significant advances in critical care medicine, sepsis - defined as organ dysfunction caused by dysregulated systemic immune response to infection [1] - is still the leading cause of death in surgical intensive care units worldwide [2]. Every year, 20–30 million patients are diagnosed with sepsis and daily about 24,000 people die from sepsis. In developing countries, more than 6 million neonates and children suffer from sepsis every year [3]. Thus, it is of great importance to identify effective sepsis biomarkers to improve the diagnosis and treatment of sepsis.

Adenosine 5' triphosphate (ATP) provides energy for cells and tissues and plays important roles as signaling molecule [4]. In inflammation, extracellular ATP (eATP), released from various cells, activates purinergic P2 receptors, thereby facilitating diverse effects on immune cells and vascular tone. Thus, increases in eATP levels have also been observed in systemic inflammation, i.e. sepsis [5]. Cell populations responsible for ATP release include red blood cells (RBC): they function not only as oxygen provider but act also as sensor of local oxygen gradients and modify oxygen delivery by ATP release and subsequent vasodilation [6]. Sepsis induces decreased RBC deformability [7,8] which results in RBC entrapment in the microcirculation of specific regions [9]. The exposure to reactive oxygen species (ROS) stimulates intracellular RBC proteolysis, membrane lipid peroxidation

and nitric oxide (NO) generation. Alterations of the RBC cell membrane have been reported including changes of membrane pumps, decreased ATP reserve and modified 2,3-diphosphoglycerate (2,3-DPG) concentrations [10]. In sepsis, organ and tissues are frequently exposed to hypoxia. Therefore, ATP release by RBC is expected. However, it is not fully elucidated, whether or not this results in a decrease of intracellular ATP (iATP) levels in RBC.

Hypothesis. We hypothesize that sepsis is accompanied by decreased ATP levels in RBC and measuring RBC ATP levels can be used as a novel biomarker for sepsis.

Discussion

Experimental and clinical evidence

Only a few studies evaluated iATP levels in RBC under experimental conditions similar to clinical sepsis. Our group studied RBC ATP levels in experimental endotoxemia induced by lipopolysaccharide (LPS), an experimental model of sepsis. Endotoxemia caused a significant decrease of ATP levels in RBC [11]. We also found that tigecycline, an antibiotic widely used in sepsis treatment, increased RBC ATP levels in experimental sepsis. This shows that preserving RBC ATP levels is a tangible pharmacological target in sepsis treatment [12].

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A common observation in septic patients are significantly elevated blood lactate levels [13,14]. Rozier et al. incubated RBC obtained from experimental animals with lactate or lactate-free solution, with and without hypoxia [15]. The iATP levels were significantly lower in the groups incubated with lactate compared to control groups with and without hypoxia. Therefore, in sepsis a decrease of RBC ATP levels can be expected.

Todd et al. reported decreased RBC ATP levels in experimental sepsis, causing energy deficiency for the Ca^{2+} membrane pump, thereby increasing intracellular Ca^{2+} . Pretreatment or posttreatment with ATP increased iATP content and reversed the increase of RBC intracellular Ca^{2+} [16,17]. Kalan et al. measured ATP in RBC of sixteen neonates with sepsis and found that RBC ATP levels in septic neonates were significantly lower than that in controls [18].

In summary, several studies demonstrated direct or indirect evidence supporting our hypothesis that the concentration of ATP in RBC decreases in sepsis which could be used as potential biomarker for sepsis.

Potential mechanisms

The following are several potential mechanisms for the decreased ATP levels in RBC found in sepsis. There might be more than one mechanism responsible for the changes in iATP concentration and the final change in iATP levels may be the product of a combination of different mechanisms.

ATP production

Since mature mammalian RBC lack mitochondria, which is the main cell organelle producing ATP, the way RBCs generate ATP is different from the cells containing mitochondria. The synthesis of ATP in RBC is mainly through anaerobic glycolysis which requires no mitochondria and no oxygen. This process can be divided into two phases. In the first phase, glucose is converted into two three-carbon molecules of glyceraldehyde 3-phosphate (GAP). The first phase consumes two molecules of ATP instead of producing it. Then GAP is turned into pyruvate in the second phase which produces four molecules of ATP. Because of the lack of mitochondria in RBC, pyruvate is converted into lactate instead of entering the TCA cycle which happens in the mitochondrial matrix. So, the final balance of this process is that one molecule of glucose taken up by RBC through glucose transporters (GLUT) is metabolized into two molecules of ATP and two molecules of lactate [19]. In addition, production of ATP can also occur using adenosine, AMP or ADP as precursors via the salvage pathways particularly when there is an increase in demand of energy [20]. In sepsis, with a compromised microcirculation, high blood lactate levels are a consistent finding [14]. Exposure of RBC to high levels of lactate, a product of glycolysis, which can inhibit glycolysis through negative feedback control, will affect the production of ATP in RBC and lead to significant decreases in ATP levels in RBC [15]. Furthermore, during sepsis, decreased ATP production has been observed in a state of 'cytopathic dysoxia' [21,22].

2,3-DPG is an allosteric regulator of hemoglobin. When it binds to hemoglobin, the affinity of hemoglobin for oxygen decreases. 2,3-DPG concentrations in RBC were shown to increase in the early phase of experimental endotoxemia I [23]. In mammalian RBC, 2,3-DPG is synthesized and dephosphorylated by the unique Rapoport-Luebering glycolytic bypass. 1,3-diphosphoglycerate (1,3-DPG) can be converted to 3-phosphoglycerate by phosphoglycerate kinase which generates ATP directly or through the Rapoport-Luebering glycolytic bypass which synthesizes 2,3-DPG without ATP generation [24]. Therefore, increased 2,3-DPG levels may reduce ATP production in sepsis.

ATP metabolism

In RBC and other cells, iATP is first catabolized to adenosine 5'-

diphosphate (ADP) and then to adenosine 5'-monophosphate (AMP) and subsequently to adenosine (ADO) by 5'-nucleotidase [25]. Yeung et al. found that RBC concentration of iATP decreased and the concentrations of ADP and AMP in RBC increased in a rat model of cardiovascular toxicity induced by isoproterenol, suggesting an increase of iATP breakdown in RBC occurred with cardiovascular toxicity [26]. Considered that cardiovascular dysfunction often happens in sepsis and ATP breakdown increases in cardiovascular dysfunction, it can be speculated that the increase of ATP breakdown in RBC is related to the decrease of ATP concentration in RBC in sepsis. In addition, a massive increase of ROS is seen in sepsis [27]. Increased ROS levels, which creates an adverse milieu of oxidative stress for cells, induce a significant increase of ATP catabolism [28]. Therefore, the increased catabolism of ATP in RBC affected by sepsis might be another reason for the decreased ATP levels in RBC.

ATP release

ATP acts not only as an essential energy source but also as a signaling molecule. It has been demonstrated that ATP release from RBC is triggered by hypoxia [29] and the release of ATP from RBC is linearly proportional to hemoglobin saturation with oxygen; the greater deoxygenation the hemoglobin undergoes, the more ATP is released [30]. The ATP released in response to hypoxia stimulates the synthesis of vasodilators, such as NO via activating purinergic P2 receptors present on the vascular endothelium [31]. Hypoxia often appears in sepsis and systemic concentration of ATP increases in sepsis due to the release of ATP from the intracellular into the extracellular space [32]. Sumi et al. found that compared to sham control mice, the plasma ATP level was higher in septic mice [33]. The experimental results from Sumi et al. in mice are contrary to the clinical findings of Chida et al. [34] in critically ill patients, where the median eATP levels in severely ill patients at ICU admission were significantly lower than the levels in moderately ill patients. This might be related to the observation, that the RBC response to hypoxia is disturbed in sepsis, resulting in decreased RBC O_2 -dependent ATP efflux [6]. Taken together, it is possible that increased ATP release from RBC contributes to the decrease of iATP levels in RBC in sepsis. However, how ATP release from RBC is changed by sepsis still needs further studies.

RBC ATP as sepsis biomarker

The ideal biomarker is defined as a characteristic biological parameter that is objectively measured and evaluated as reproducible indicator of physiological processes, pathological changes, and therapeutic responses to an intervention with high specificity and sensitivity and rapid measurement time [35].

At present, there is no such biomarker available for sepsis. Firstly, many potential biomarkers for sepsis cost considerable time, money and effort to measure, which limits their application [36]. In contrast, RBC ATP levels can be measured via rapid, sensitive and reproducible HPLC assays at moderate costs [37].

Secondly, inflammation is an important part of the complex pathophysiology of sepsis and many suggested biomarkers are based on inflammatory changes. Actually, procalcitonin (PCT), an inflammatory biomarker widely used in recent years for sepsis diagnosis, has low specificity because increased PCT levels can also be found in other inflammatory conditions [38,39]. The same is true for C-reactive Protein or White Blood Cell count. Changes of iATP levels reflect sepsis pathology from a different perspective. It has been reported that ATP levels in RBC are useful biomarkers for cardiovascular dysfunction [26] which often occurs in sepsis independent of the immune status (hyper-versus hypo-inflammation) [40,41]. Potentially, the combination of ATP levels in RBC and inflammatory biomarkers can overcome the failure of single (un-)specific inflammatory biomarkers and improve sepsis diagnosis. Early sepsis diagnosis facilitates early treatment and

therefore, improves outcome.

Conclusion

There is a growing body of experimental evidence showing that iATP levels in RBC are decreased in sepsis due to a potential combination of decreased ATP production, increased ATP breakdown (catabolism) and release. Therefore, iATP levels in RBCs may be useful as a biomarker for sepsis. However, due to the complex pathophysiology of sepsis, more research is required in order to elucidate the mechanism responsible for the pathological changes of iATP levels in RBC.

Conflict of interest

The authors declared that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.02.014>.

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