Original Article / Özgün Araştırma

Protective Efficacy of Thiamine (Vitamin B_1) Alone on LPS-induced Acute Kidney Injury

Tiamin (Vitamin B_1) Tedavisinin LPS ile Indüklenen Akut Böbrek Hasari Üzerine Koruyucu Etkileri

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Keywords
sAKI, thiamin, sepsis, kidney injury, rat, vitamin B_1

Anahtar Kelimeler
sABH, tiamin, sepsis, böbrek hasarı, rat, vitamin B_1

Received/Geliş Tarihi : 11.06.2022
Accepted/Kabul Tarihi : 29.07.2022

doi:10.4274/meandros.galenos.2022.70456

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Abstract

Objective: Sepsis-induced acute kidney injury (sAKI) is the leading cause of renal dysfunction and mortality in intensive care unit. We must target its multidimensional pathogenesis for new treatment strategies. Therefore, we decided to reveal the protective efficacy of thiamin on sAKI in terms of its antioxidant, anti-inflammatory and mitochondrial regulatory effects.

Materials and Methods: Four rat groups were formed as; healthy (HG), sepsis (SG), thiamine (TG), sepsis + thiamine (TSG) groups. In kidney tissue, oxidant [malondialdehyde (MDA), nitric oxide (NO)] and antioxidant (glutathione (GSH), catalase (CAT)] levels were measure. In serum, tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), procalcitonin (PCT) and urea, creatine (Cr), lactate were also surveyed.

Results: There were significant decreases in MDA and NO levels ($p<0.001$, $p<0.001$, respectively) and increases in GSH and CAT levels ($p<0.001$, $p<0.001$, respectively) when TSG and SG groups were compared. Proinflammatory cytokine levels were significantly elevated in SG ($p<0.001$). In the TSG group, PCT, IL-1β and TNF-α levels were decreased compared with SG ($p<0.001$ $p<0.001$, $p<0.001$, respectively). In the SG group versus HG group results, lactate levels were found to be 4-fold higher ($p<0.001$) and urea, Cr levels were 3-4 fold higher ($p<0.001$, $p<0.001$, respectively). In the TSG group, there was an obvious decrease in lactate and urea, Cr levels compared with SG ($p<0.001$, $p=0.002$, $p<0.001$, respectively).

Conclusion: We revealed the preventive efficacy of thiamin against sAKI by reducing the oxidant parameters and proinflammatory cytokine levels and increasing the antioxidant parameter levels with the attainment of near normal kidney functions, simultaneously. With its cheap, readily available, and good safety profile in adults, thiamin appears like a tempting adjunctive therapy for sAKI.

Öz

Amaç: Sepsinin indüklediği akut böbrek hasarı (sABH), yoğun bakımda renal disfonksiyon ve mortalitenin önde gelen sebebidir. Yeni tedavi stratejileri için sepsisin çok yönlü patogenezini hedeflemeliyiz. Bu nedenle, sABH'da tiaminin antioksidan, antiinflamatuvar ve mitokondrial düzenleyici etkilerini kullanarak koruyucu etkilerini ortaya koymayı amaçladık.

Gereç ve Yöntemler: Ratlar sağlıklı (HG), sepsis (SG), tiamin, sepsis + tiamin (TSG) olmak üzere dört gruba ayrıldı. Böbrek dokusunda oksidan [malondialdehit (MDA),

nitrik oksit (NO)] ve antioksidan [glutatyon (GSH), katalaz (CAT)] seviyeleri ölçüldü. Serumda TNF-α, IL-1β, prokalsitonin (PCT) ve üre, kreatinin (Cr), laktat değerlendirildi.
Bulgular: TSG grubunda, SG grubuyla karşılaştırıldığında, MDA ve NO seviyelerinde anlamlı bir azalma (p<0,001, p<0,001, sırasıyla) ve GSH ve CAT seviyelerinde artış (p<0,001, p<0,001, sırasıyla) saptandı. SG grubunda, proinflamatuvar sitokin seviyelerinde anlamlı bir artış mevcuttu (p<0,001). TSG ve SG karşılaştırıldığında, PCT, IL-1β ve TNF-α seviyerinde belirgin bir düşüş saptandı (p<0,001, p<0,001, p<0,001, sırasıyla). SG grubunda HG grubuna kıyasla laktat seviyelerinde 4 kat artış (p<0,001) ve üre ve Cr seviyelerinde 3-4 kat artış gözlendi (p<0,001, p<0,001, sırasıyla). TSG grubunda SG grubuna kıyasla laktat, üre ve Cr seviyelerinde aşikar bir düşüş vardı (p<0,001, p=0,002, p<0,001, sırasıyla).
Sonuç: Tiaminin sABH üzerinde, oksidan parametreleri ve proenflamatuvar sitokinleri azaltarak ve antioksidan seviyelerini yükselterek neredeyse normal böbrek fonksiyonuna ulaşmayı sağlayabildiğini ortaya koyduk. Ucuz, kolay ulaşılabilir ve iyi bir güvenlik profiline sahip tiaminin sABH tedavisinde cazip bir ek tedavi seçeneği olabileceği kanısındayız.

Introduction

Sepsis is a life-threatening organ dysfunction shaped by host response and pathogen factors (1). Sepsis-induced acute kidney injury (sAKI) is the leading cause of renal dysfunction which accounts nearly 50% of all cases and has 41% mortality in intensive care units (2). Currently used early goal-directed strategies are reactive but nonspecific (1). The way to develop effective treatments for sAKI is through a good understanding of its pathogenesis.

Although renal ischemia is trusted on to be the main reason of sAKI, many conflicting studies have been presented. Animal models of sAKI revealed that kidney injury may develop without renal ischemia. Renal medullar microcirculatory disturbances may lead to medullar oxygen delivery failures resulting hypoxia which may be the major trigger point of renal injury (3).

In sepsis induced organ injury, tissue and vascular injury and the inflammatory host response initiates a multidirectional cascade resulting to endothelial dysfunction. The over expression of inducible nitric oxide synthase (iNOS) elevates nitric oxide (NO) production, tissue hypoxia and additionally the generation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) (4).

Concurrently, neutrophils generate an oxidative stimulus initiating a cytokine storm including pro-inflammatory molecules especially like interferon-gamma, tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). This cascade triggers the downstream production of ROS and RNS and propagate sAKI (4).

As a result of all; oxidative stress, inflammation, mitochondrial dysfunction, renal medullar hypoxia and endothelial dysfunction seem to be interrelated mechanisms in the sAKI pathophysiology. By this way the term 'metabolic resuscitation' for sepsis treatment got in range of new studies (5,6). With its role as a constant co-factor of mitochondrial aerobic metabolism and redox cycle and with antioxidant/anti-inflammatory effects thiamin has caught the attention in sepsis-induced organ failure therapy studies (7).

Thiamine is a water-soluble vitamin. Some essential nutrients like thiamine may be deficient during sepsis (8,9). In septic patients, the prevalence of thiamine deficiency is between 20-71% (10,11). Thiamine deficiency may increase lactic acid generation and the existing metabolic stress in sepsis may increase consumption of antioxidant molecules like thiamine (7,9). Whether thiamin deficiency is the cause or consequent effect of sepsis is not clear. All these steps above, creates a vicious cycle between thiamine and sepsis pathophysiology.

Transketolase, α-ketoglutarate dehydrogenase, branched chain amino acid dehydrogenase and pyruvate dehydrogenase are all thiamine pyrophosphate (TP) -dependent enzymes having key roles in Krebs cycle (aerobic metabolism) and pentose phosphate pathway. Shortened TP levels lead to decreased enzymatic activity of these enzymes resulting decreased oxidative phosphorylation, lactic acidosis, decreased production of adenosine triphosphate (ATP) and causing dysfunctions in high metabolic demand organs (12). Additionally, thiamine is critical for; ATP energy production, metabolism of some oxidant molecules such as ROS and for aerobic metabolism (13). It is considered that with all these overlapping pathogeneses of both thiamine metabolism and sepsis; bioenergetic especially mitochondrial failure resulting to organ injuries in especially high metabolic demand organs like kidneys, brain and heart, may exist (14).

Considering all these alterations of sepsis, we decided to reveal the protective efficacy of thiamine on sAKI by the means of its antioxidant and anti-inflammatory and mitochondrial regulatory effects.

Materials and Methods

Animals

A total of 32 male, Wistar albino rats were provided from Experimental Animal Unite of Aydın Adnan Menderes University, Turkey. Animals were kept in colony room, 12 hours of light/dark cycle and let to reach freely to water and food. All experimental design was consistent with the guide for the care and use of laboratory animals. Study has been approved by Animal Experiments Local Ethical Commission of Aydın Adnan Menderes University, Medical Faculty (approval number: 64583101/2016/49, date: 25.02.2016).

Chemical Substances

Lipopolysaccharide (LPS), (obtained from serotype 055:B5 *Escherichia coli*, L-2880) and TP was both obtained from Sigma Chemicals.

Experimental Study Design

Rats were fasted night before experiment and separated into four different groups, randomly (8 in each) as; healthy (HG), sepsis (SG), thiamine (TG), LPS + TP group (TSG). TP alone (TG) was created to investigate if TP alone may cause any deleterious effect on study parameters. By intraperitoneal (ip) route, saline was applied to HG rats. We performed preliminary studies to determine the exact LPS and TP doses and appropriate time for gathering tissue and blood samples after drug administrations (15). We have been revealed that 100 mg/kg TP dose has a protective effect on the oxidative stress in rats, in our previous study (16). Consequently, LPS at a dose of 5 mg/kg was given by ip injection to the right side of abdomen of SG and TSG rats. Thirty minutes before the ip administration of LPS, TP was administered ip. to the left abdomen of TSG rats. Eighteen hours after these interventions, blood samples were taken by cardiac puncture and kidney tissues were gathered under ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) anesthesia.

Blood analyses

Collected serum samples were kept at -80 °C. Serum urea and creatine (Cr) and lactate levels were surveyed using an autoanalyzer (C8000, Abbott, USA).

In serum specimens, by some merchant rat ELISA kits (Biotech Co. Ltd, China); TNF-α, IL-1β and procalcitonin (PCT) levels were evaluated via an ELISA reader (ELX800). The limit of detection for TNF-α was 9.37 pg/mL, and for PCT was 18.75 pg/mL. Assay range was determined as 15.6-1000 pg/mL for PCT and TNF-α, 31.25-2000 pg/mL for IL-1β.

Preparation of Tissue Homogenates

All tissues were homogenized in 50 mM, pH 7.4 phosphate-buffered saline for the measurement of tissue malondialdehyde (MDA), NO and glutathione (GSH) levels and to set up catalase (CAT) activity.

MDA was determined by the standard Ohkawa method (17). If thiobarbituric acid exists, MDA creates a complex which may be detected via the measurement of 532 nm wavelength absorbance. Results were indicated as µmol/g protein.

NO; was analysed by Navarro-Gonzalves method (18). By diazotization of sulphanilamide the emerging nitrite was measured. Via a microplate reader, samples were quantified versus a KNO^3 curve and analysed spectrophotometrically. Results were indicated as µmol/g protein.

CAT; to measure the activity method of Aebi (19) was used. The H^20^2 reduction rate was administered for 30 seconds at 240 nm. Results were indicated as U/g protein.

Glutatione (GSH); Beutler method was used to measure the GSH content (20). Via Shimadzu UV-160 spectrophotometer at 412 nm wavelength, the absorbance was detected. Results were indicated as µM/g protein.

Statical Analysis

To evaluate the normal distribution of numeric variables Kolmogorov-Smirnov test was used. All data were analyzed by the PAWS Statistics version 18 software SPSS using Kruskal-Wallis test to determine the significant differences between groups of the independent variables. The results are defined as mean ± standard error of the mean. Further, one-way analysis of variance (ANOVA) tests with Tukey-Kramer HSD or Tamhane as post-ANOVA tests were operated to determine the group differences. Statistical significance was stated at $p \leq 0.05$.

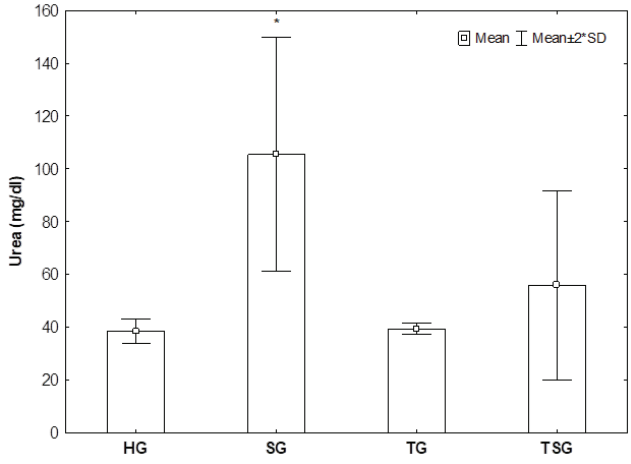
Results

The levels of oxidant/antioxidants in kidney tissues are shown in Table 1. When HG, TG and TSG were compared to SG, significant differences were revealed among all groups (p<0.001). A very prominent increase in all oxidants (MDA, NO) besides an evident reduction in antioxidants (GSH, CAT) was detected in SG. Statistically, no significant difference was defined among HG and TG (p>0.05) indicating TP alone had no harmful effect in renal tissue. There was an obvious decrease in MDA and NO levels (p<0.001, p<0.001, respectively) and a significant increase in GSH and CAT levels (p<0.001, p<0.001, respectively) in TSG when compared with SG. These results were indicating the antioxidant efficacy of thiamine alone on renal tissue with no harmless effect on healthy tissues.

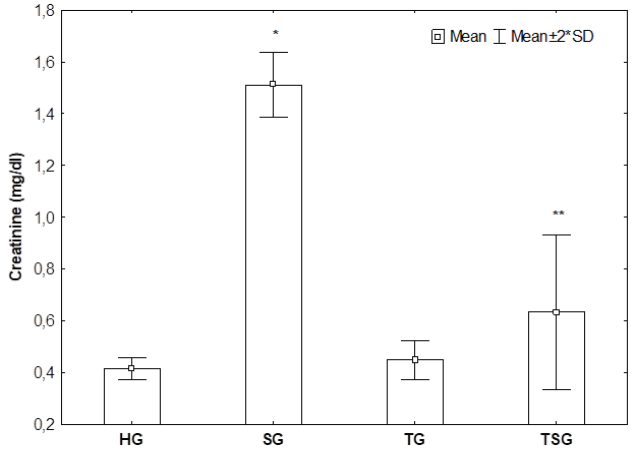
Table 2 shows the pro-inflammatory agents and lactate levels among groups. The markers of inflammation PCT, TNF-α and IL-1β levels were elevated significantly in SG versus HG (p<0.001). In TSG; PCT, IL-1β and TNF-α levels were all decreased when SG results were considered (p<0.001, p<0.001, p<0.001, respectively). There was no significant difference between HG and TSG in terms of proinflammatory agents (all p>0.05, respectively).

Lactate levels were nearly 4-fold higher in SG, when compared with HG (p<0.001). In TSG, lactate levels reduced nearly HG levels, as there was no significant difference between HG and TSG in terms of lactate levels (p>0.05).

Serum Cr levels were 0.41±0.00 mg/dL in HG, 1.51±0.02 mg/dL in SG, 0.44±0.01 mg/dL in TG and 0.63±0.05 mg/dL in TSG. There were nearly 4-fold increase in serum Cr levels in SG when compared with HG (p<0.001). With the comparison between SG and HG there were nearly 4-fold increase in serum urea levels in SG (p<0.001). These results were defining the establishment of severe sepsis induced AKI in our study model. When given alone TP had no adverse effect on kidney function tests as seen in TG results. A significant reduction was found in urea and creatinine levels of TSG when compared with SG (p=0.002, p<0.001, respectively). In TSG, Cr levels reduced nearly 2-fold with TP treatment when compared with sepsis group but there was still statistically difference between HG and TSG Cr levels (p=0.026). All these data may be seen in Graphic 1 and 2.



Graphic 1. Urea levels among groups
*All groups were compared with SG group. The bars were created using mean ± SEM values. Statistically significant value defined as p<0.05
SG: Sepsis group, SEM: Standard error of the measurement



Graphic 2. Creatine levels among groups
*All groups were compared with SG group. The bars were created using mean ± SEM values. Statistically significant value defined as p<0.05
**All groups were compared with TSG group. The bars were created using mean ± SEM values. Statistically significant value defined as p<0.05
SG: Sepsis group, TSG: Sepsis + thiamine pyrophosphate group, SEM: Standard error of the measurement

Discussion

In this study, our goal was to examine the protective efficacy of thiamine alone on sAKI in a model of LPS-induced sepsis. To the best of our knowledge, this is the first study examining thiamine efficiency alone

Table 1. The oxidant and antioxidant levels in kidney tissue among rat groups[1]

	MDA µmol/g protein	NO µmol/g protein	GSH µM/g protein	CAT U/g protein
HG n=8	1.23±0.14*	26.62±2.04*	15.64±1.39*	20.32±1.29*
SG n=8	2.83±0.10	69.92±3.73	4.85±0.62	7.78±0.69
TG n=8	1.10±0.10*	25.81±1.96*	16.80±1.24*	17.28±1.31*
TSG n=8	1.22±0.12*	31.51±2.65*	13.09±0.98*	15.38±0.91*

[1]The comparison of MDA, NO, CAT and GSH levels of each group with the SG group using one-way ANOVA and post-hoc Tamhane range test.

*Statistically significant value defined as p<0.001. All values are expressed as the mean ± standard error of the measurement.

MDA: Malondialdehyde, NO: Nitric oxide, GSH: Glutation, CAT: Catalase, HG: Healthy group, SG: Sepsis group, TG: Thiamine group, TSG: Sepsis + thiamine pyrophosphate group

Table 2. The pro-inflammatory agents and lactate levels among rat groups

	PCT ng/mL	TNF-α pg/mL	IL-1β pg/mL	Lactate U/L
HG n=8	35.25±2.94*	36.52±1.45*	49.58±3.10*	22.65±1.08*
SG n=8	107.62±4.33	116.86±7.20	140.18±11.14	90.38±2.83
TG n=8	33.62±3.76*	35.80±0.95*	46.42±2.50*	23.06±1.31*
TSG n=8	47.14±7.63*	47.81±3.18*	62.00±2.70*	26.35±1.26*

*Statistically significant value defined as p<0.001. All data are defined as the mean ± standard error of the measurement. All values were compared with SG group based on one-way ANOVA and post-hoc Tukey's range test.

PCT: Procalcitonin, TNF-α: Tumor necrosis factor alpha, IL-1β: Interleukin 1 beta, HG: Healthy group, SG: Sepsis group, TG: Thiamine pyrophosphate (TP) group, TSG: Sepsis + TP group

in rat sepsis model. We also determined to compare the efficacy of thiamine on oxidant/antioxidant parameters in kidney tissue. Furthermore, we tried to reveal the thiamine-related interactions on inflammatory parameters of sepsis and lactate level changes in LPS-induced septic rats.

In sAKI; there is an imbalance between oxidative host parameters like ROS/RNS and antioxidant defense mechanisms in the favor of oxidative parameters (4).

NO is a key molecule in the intersection point of oxidative stress, inflammation and vascular injury in sepsis (6). By the inflammatory mediators and proinflammatory cytokines, iNOS expression is increased. Consequently, iNOS leads the production of NO causing the oxidative stress in sAKI (21).

Besides, lipid peroxidation leads to a molecular cell damage. MDA is the last product of lipid production pathway. As an oxidative stress marker, MDA levels are elevated in LPS-induced sAKI patients (22).

As consistent, we have revealed a significant establishment of oxidative stress in our sepsis model. On the other hand, there was a significant decrease in MDA and NO levels in TSG group, demonstrating the renal antioxidant efficacy of thiamine.

GSH has a key role in the protection against septic oxidative stress. GSH may convert peroxides and ROS to innocuous compounds and mends oxidative tissue damage (12). In thiamine deficiency, pentose phosphate pathway may malfunction leading reduction in nicotinamide adenine dinucleotide phosphate (NADPH) production resulting in decreased GSH production (9).

CAT is an enzymic antioxidant protecting tissues from lipid peroxidation. Hence, the reduction of CAT levels in tissues may result in the accumulation of ROS (12). In a sAKI mice model, the prominent decrease of CAT levels has been revealed (23). In our study, we demonstrated the antioxidant efficacy of thiamine.

It is well known that one of the main pillars of sepsis is inflammatory host response (7). In renal tissues of septic mice, the marked elevation of TNF-α and IL-1β has been revealed (23). Increasing oxidative stimuli activates leucocytes more to generate a cytokine storm which may lead the overwhelming production of ROS. This inflammatory cascade plays a critical role in propagating the oxidative stress in sepsis causing negative effects on renal microcirculation (4).

An early marker of sepsis is PCT. PCT may also be used to predict the development of AKI in sepsis (24). As consistent, in our SG group serum PCT levels were significantly elevated, accompanied by a 3-4-fold

increase in urea and Cr levels, when compared with HG.

Mitochondrial dysfunction appears in early sepsis, determining survival (13,14). In sepsis cellular mitochondrial dysfunction prevents oxygen utilization and shifts the pathway to anaerobic metabolism. In thiamine deficiency accompanying sepsis, pyruvate is transformed to lactate leading to lactic acidosis and a deficit of ATP generation (25). This point of view opened a new era in septic organ injury treatment modalities called 'metabolic resuscitation'. Metabolic resuscitation focuses on augmenting oxygen utilization and properly maintenance of the redox balance (5). In this setting, thiamine has gained attention in sAKI with its pivotal role in mitochondrial respiration and cellular redox as a metabolic resuscitator.

High lactate levels are representative of tissue hypoperfusion and cellular hypoxia. Increased lactate levels are known to associate higher mortality in sepsis (25). Donnino et al. (10) revealed the high prevalence of thiamine deficiency in critically ill septic patients, and they also reported a negative correlation between lactic acidosis and thiamine levels. As consistent with literature, we found nearly 4-fold increase in lactate levels in SG.

In summary, despite early treatment goals and quick action plans mortality and sepsis induced organ failures still stands at high rates. Pathophysiologically, sepsis is a highly heterogenous clinical syndrome therefore, its treatment requires a multidimensional approach. Thiamine is a very important vitamin with powerful and beneficial effects on multiple metabolic steps. Thiamine acts like an antioxidant by preventing lipid peroxidation and propagates GSH production acting as a cofactor of NADPH production. Some thiamine dependent enzymatic pathways play a role in reducing intracellular ROS production and allowing the generation of antioxidants. Additionally, thiamine has key roles in the maintenance of aerobic respiration and cellular energy production. Owing to all the features of thiamine, we think that it has a pivotal role in sAKI.

Conclusion

We revealed the preventive efficacy of thiamine against sAKI by reducing the oxidant parameters and rising the antioxidant parameter levels with the attainment of near to normal kidney functions, simultaneously. Consequently, thiamine is a cheap, economic and readily available drug. It has a very good safety profile in adults with no significant side effects in long term. With this excellent profile thiamine looks like a tempting adjunctive therapy for sAKI.

Ethics

Ethics Committee Approval: Study has been approved by Animal Experiments Local Ethical Commission of Aydın Adnan Menderes University, Medical Faculty (approval number: 64583101/2016/49, date: 25.02.2016).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.B.U., B.D., Concept: H.B.U., B.D., Design: H.B.U., B.D., Data Collection or Processing: H.B.U., M.Y., İ.K.Ö., Analysis or Interpretation: H.B.U., M.Y., İ.K.Ö., Literature Search: H.B.U., B.D., Writing: H.B.U., B.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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