Research Article

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Protective effect of dexpanthenol (vitamin B5) in a rat model of LPS-induced endotoxic shock

Dekspantenol (Vitamin B5)'ün LPS ile indüklenen endotoksik şok modelinde koruyucu etkisi

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Abstract

Objectives: This study investigated the protective effect of dexpanthenol (DEX) in the septic shock model of rats with biochemical parameters.
Methods: 12–15 weeks old male 32 Wistar rats has been used for this study. Sepsis was induced by a single intraperitoneal injection of lipopolysaccharide (LPS) (5 mg/kg) and treatment groups received single intraperitoneal injection of DEX (500 mg/kg) just 30-min before. The blood and tissue samples were obtained 16 h later of LPS intervention under the ketamine and xylasine (50 and 5 mg/kg, respectively) anesthesia.
Results: Giving alone DEX did not alter any physiologic levels of biochemical markers. Induction of sepsis resulted in a marked increase in ALT, AST, urea, creatinine, lactate, procalcitonin, TNF-α, IL-1β levels to show the tissue damage. In all serum parameters, liver's GSH, CAT levels and kidney's CAT, GSH, MDA and NO levels have ameliorated by DEX treatment in sepsis group.
Conclusion: Along with the standard therapy of sepsis, DEX can be used as a safe way of restoring (anti)oxidant status of kidney and liver tissues. It can be effective to control cytokine pathway, to decrease procalcitonin and regulate the metabolic process of sepsis, such as lactate.

Keywords: Dexpanthenol; Endotoxemia; Lactate; Lipopolysaccharide; Procalcitonin; Rat; Sepsis.

Özet

Amaç: Bu çalışma, dekspantenol (DEX)'ün sıçan septik şok modelinde koruyucu etkisini biyokimyasal verilerle araştırdı.
Metod: 12–15 haftalık 32 erkek Wistar sıçanı bu çalışma için kullanıldı. Tedavi gruplarına 30 dakika öncesinden periton içine tek doz DEX (500 mg/kg) verildi ve sepsis periton içine uygulanan tek doz Lipopolisakkarid (5 mg/kg) enjeksiyonu ile başlatıldı. Lipopolisakkarid uygulamasından 16 saat sonra kan ve doku örnekleri ketamin ve ksilazin (50 and 5 mg/kg) anestezisi altında alındı.
Bulgular: Tek başına DEX uygulaması, biyokimyasal parametrelerin fizyolojik düzeyinde herhangi bir değişiklik yapmadı. Sepsisin başlatılması, organ hasarını gösteren ALT, AST, üre, kreatinin, lactate, prokalsitonin, TNF-α, IL-1β düzeylerinde belirgin artış ile sonuçlandı. Sepsis grubunda, tüm serum değerlerinde, karaciğerin GSH, CAT düzeyleri ile böbreğin CAT, GSH, MDA ve NO düzeylerinde iyileşme DEX tedavisi ile sağlandı.
Sonuç: Sepsisin standart tedavisinin yanı sıra, DEX karaciğer ve böbrek dokularında (anti)oksidan dengeyi onarmak için güvenli bir yol olarak kullanılabilir. Sitokin yolağını kontrol etmek için, prokalsitonini azaltmak için, sepsisin laktat üretimi gibi bir metabolik sürecini düzenlemek için etkin olabilir.

Anahtar Kelimeler: Dekspantenol; Endotoksemi; Laktat; Lipopolisakkarid; Prokalsitonin; Sıçan; Sepsis.

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Introduction

Sepsis causes a life-threatening organ dysfunction and underlying mechanisms are included tissue ischemia, direct cell injury by proinflammatory mediators and altered rate of apoptosis [1]. Many treatment options have been used for in sepsis clinics so far to aim to restrict the tissue injuries of endothelial, liver, kidney etc. Some of the therapeutic approaches are antibiotics, strategy of fluid resuscitation, the modulation of inflammatory mediators, counteracting of endocrine, metabolic and bioenergetics modulations have been found partially effective, but still remained for further investigations [2]. As the patients highly vulnerable to iatrogenic complications [2]; the safest effective medication should be chosen in the right doses.

Dextrorotary isomer of panthothenic asid (vitamin B5) is called as dexpanthenol (DEX) and it possesses biological activity [3]. Many clinical and experimental researches has revealed its beneficial effects on wound healing [3], testicular ischemia and reperfusion injury [4], diabetes [5], APAP hepatotoxicity [6] and so on. In these literatures, prominent mechanism of its effect is mainly attributed on preventing of lipid peroxidation. Wojtczak and Slyshenkov [7] also proved that DEX modulates bioenergetics of cells and increases glutation biosynthesis. Recently, similar study has been done on LPS induced acute lung injury model and DEX has been found controlling effect on cytokine pathway [8].

The aim of the study was to determine the safety and efficacy of DEX on endotoxemia. Therefore the hepatic and kidney tissues' injury has been biochemically evaluated in an accepted animal model.

Materials and methods

Chemicals and equipments

Lipopolysaccharide (LPS from *Escherichia coli* serotype 055:B5, L-2880) was purchased from Sigma Chemicals (Interlab, Izmir, Turkey). Dexpanthenol (DEX, Bepanthene amp., Istanbul, Turkey) has obtained from the local pharmacy store.

Animals

12–15 weeks old male 32 Wistar rats were obtained from Experimental Animal Center of University and all experiments were performed according to the principles and guidelines of Universities' Animal Ethical Committee's approval (HADYEK 2016/30).

Experimental design

The rats randomly assigned in to four groups of eight animals each. For the development of animal model, single dose of LPS (5 mg kg^{-1} in 1 mL of saline) has been given intraperitoneally to the rats as in a previous study [9]. Etensel et al. [4] have been determined 500 mg/kg single dose of DEX is effective against testicular ischemia and reperfusion injury, Demirci et al. [5] have been shown that administration of 500 mg/kg/day DEX during 6-week is safe and effective for diabetes treatment. Additionally, Uysal et al. [6] have been found that 500 mg/kg single dose of DEX alleviates hepatocelluler damage in paracetamol poisining of rats. Therefore 500 mg/kg single administration of DEX has been chosen in this study too, and similar to Can et al. paper, treatment agent (DEX), was given 30 min before LPS challenge [4, 6].

1. Control group: The rats in this group were administered a single i.p. injection of saline solution.
2. LPS group: The rats in this group were administered a single i.p. injection of LPS.
3. DEX group: 500 mg/kg were given i.p. to see the safety of dexpanthenol in this dose.
4. LPS + DEX group: 500 mg/kg dexpanthenol was given by a single i.p. injection to the right side of the abdomen, 30 min before LPS intervention of the left side.

Sixteen hours later of the interventions, under the anesthesia with ketamine and xylasine (50 and 5 mg/kg, respectively), blood sample was obtained by cardiac puncture meanwhile, liver and kidney tissues were harvested. Blood was centrifuged ($1000 \times g$ for 10 min) and their sera separated and stored at −80°C.

Blood analyses

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea and lactate were immediately determined by spectrophotometric autoanalyzer (Architect C 8000, Abbott, IL, USA). Malondialdehyde (MDA) production and hence lipid peroxidation were assessed in the serum by the method of Ohkawa et al. [10]. Nitric oxide (nitrite + nitrate; NO) was assayed by a modification of the cadmium-reduction method of Navarro-Gonzalvez et al. [11]. For determination of cellular total glutathione (GSH), the colorimetric Beutler method [12] was used. Catalase (CAT) activity was measured by the

method of Aebi [13]. The reduction rate of peroxide (H_2O_2) was measured at a wavelength of 240 nm for 30 s at room temperature.

In serum samples, procalcitonin (PCT) levels were determined via commercial Fine Test Rat PCT ELISA kit (catalog number: ER1235, Wuhan Fine Biotech Co. Ltd., East Like High-tech Development District, Wuhan, China); tumor necrosis factor-α (TNF-α) levels were determined via commercial Fine Test Rat TNF-α ELISA kit (catalog number: ER1094, Wuhan Fine Biotech Co. Ltd., East Like High-tech Development District, Wuhan, China); interleukin 1-β (IL-1β) levels were determined via commercial Fine Test Rat IL-1β ELISA kit (catalog number: ER1094, Wuhan Fine Biotech Co. Ltd., East Like High-tech Development District, Wuhan, China). The test results were calculated by Bioelisa reader (ELX800, BioTek Instruments, Inc., Winooski, VT, USA) using standard curve via 450 nm. The limit of detection for both TNF-α and PCT were given as 9.37 pg/mL, and IL-1β was given as 18.75 pg/mL. The calculated overall intra-assay coefficient of variation was 8%. The calculated overall inter-assay coefficient of variation was 10%. Assay-range was given both TNF-α and PCT as 15.6–1000 pg/mL and for IL-1β as 31.25–2000 pg/mL. Procedures were performed according to manufacturer's instruction.

Tissue analyses

Preparation of tissue homogenates

Specimens from the liver and kidney were weighted and homogenized separately with tissue homogenizer (PRO 250 Scientology Inc., Monreo, CT, USA). Tissues were homogenized for detect of tissue GSH, MDA, NO (nitrite + nitrate) levels and the activities of CAT in phosphate buffer saline 50 mM pH 7.4. The crude tissue homogenate was centrifuged at 20,000 *g*, for 15 min in ice-cold centrifuge, and the resultant supernatant was collected and stored at −80°C.

The MDA production and hence lipid peroxidation were assessed in the tissues by the method of Ohkawa et al. [10]. MDA forms a colored complex in the presence of thiobarbituric acid, which is detectable by measurement of absorbance at 532 nm. Absorbance was measured with Shimadzu UV-160 spectrophotometer. 1,1′,3,3′ Tetraethoxy propane used as a standard and the results were expressed as μmol/mg protein.

Nitric oxide (nitrite + nitrate) was assayed by a modification of the cadmium-reduction method of Navarro-Gonzalvez et al. [11]. The nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylethylenediamine. The samples were analyzed spectrophotometrically using a microplate reader (ELX800, BioTek Instruments, Inc., Winooski, VT, USA) and quantified automatically against KNO_3 standard curve and the results were expressed as μmol/mg protein.

CAT activity in tissue was measured by the method of Aebi [13]. The reduction rate of H_2O_2 was followed at 240 nm for 30 s at room temperature. Catalase activity was expressed as nmol/min/mg protein.

GSH content in tissue supernatants was measured according to the method of Beutler et al. [12]. The absorbance was measured at 412 nm using a Shimadzu UV-160 spectrophotometer. The GSH concentration was determined using standard aqueous solutions of GSH. Results were expressed as μM/mg protein.

Data presentation and statistics

All biochemical parameters were assessed by using non-parametric Mann-Whitney U-test. Data were presented as mean ± SEM, p values below 0.05 were considered significant.

Results

Blood results

When given alone DEX treatment did not make any differences from control animals on studied blood parameters (Table 1). LPS intervention has increased all the serum biochemical markers 3–4-fold, except CAT which has 64% declined.

LPS + DEX treatment has lowered the liver (ALP, AST) and kidney (urea, creatinine) function tests, lactate, PCT and nitrite/nitrate increment (all $p < 0.001$). While oxidant marker MDA level has been decreased, antioxidant CAT level restored by DEX (Table 1).

Both TNF-α and IL-1β have been folded nearly three-times with LPS challenge. DEX + LPS treatment group's TNF-α level was increased only 40% from 36.53 ± 1.45 to 50.57 ± 3.55, IL-1β increment was completely blocked by DEX treatment.

Liver tissue (anti)oxidant levels

LPS treatment increased MDA and NO levels of the liver tissue, GSH and CAT levels diminished. While MDA level

Table 1: Blood parameters of all groups, mean ± SEM.

Group	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)	Lactate (mg/dL)	Nitrite+ nitrate (µmol/L)	MDA (µmol/L)	Catalase (nmol/min/mg)	Procalcitonin (pg/mL)	TNF-α (pg/mL)	IL-1β (pg/mL)
Control	63.38 ± 2.95	77.13 ± 2.92	38.50 ± 0.80	0.42 ± 0.01	22.65 ± 1.09	21.63 ± 0.78	11.81 ± 0.53	147.75 ± 10.46	35.25 ± 2.794	36.53 ± 1.45	49.59 ± 3.10
LPS	183.50 ± 23.25[b]	241.25 ± 19.27[b]	105.50 ± 7.83[b]	1.51 ± 0.02[b]	90.39 ± 2.83[b]	94.84 ± 2.52[b]	47.28 ± 1.65[b]	53.25 ± 2.93[b]	107.63 ± 4.34[b]	116.86 ± 7.20[b]	140.19 ± 11.15[b]
Dexpanthenol	60.75 ± 3.26	77.38 ± 2.78	39.50 ± 1.46	0.41 ± 0.01	25.08 ± 1.22	17.67 ± 2.05	12.11 ± 0.58	155.88 ± 13.35	37.88 ± 3.39	35.28 ± 1.32	35.29 ± 1.32
Dexpanthenol + LPS	86.13 ± 5.41[a,d]	91.63 ± 3.69[a,d]	58.50 ± 5.16[a,d]	0.77 ± 0.04[b,d]	30.80 ± 2.63[b,c]	18.08 ± 2.10[d]	25.04 ± 1.32[b,d]	90.57 ± 5.14[b,d]	44.29 ± 4.86[d]	50.57 ± 3.55[b,d]	64.60 ± 2.57[b,c]

[a]$p < 0.01$, [b]$p < 0.001$ according to control; [c]$p < 0.01$, [d]$p < 0.001$ according to LPS group.

was significantly ameliorated, GSH and CAT levels were completely reversed by DEX treatment. NO level was diminished only from 85.51 to 77.12 which was insignificant (Table 2).

Kidney tissue (anti)oxidant levels

LPS treatment increased MDA and NO levels of the kidney tissue, GSH and CAT levels diminished. GSH, CAT, MDA and NO tissue levels were insignificant from the control kidney tissue levels by DEX treatment (Table 3).

Discussion

In this study the possible benefits of DEX was investigated in LPS-induced endotoxemia by determining with some of biochemical parameters. With regard to this hypothesis, we tried to reveal some of underlying mechanisms in animal model of septic shock such as (anti)oxidant and cytokine pathways, additional to clinical liver and kidney functions findings.

In the present study, 18 h later of LPS administration, ALT and ASP activity in plasma were markedly elevated, compared with saline control rats, indicative of hepatocellular damage. Plasma creatinine and urea levels were also elevated by LPS treatment, indicating renal damage. Treatment with DEX group did not show any tissue damage findings against LPS challenge. While DEX significantly prevented elevation of lipid peroxidase marker MDAs' increment in serum, liver and kidney; antioxidant catalase and GSH levels were restored in the tissues. These experimental evidences about the anti-oxidative features of DEX are consistent with previous studies. DEX significantly decreased lipid peroxidation in many studies, LPS induced acute lung injury [7], ischemia-reperfusion-induced testicular oxidative damage [4], cardiovascular injury of diabetes [5] and APAP toxicity of liver [6].

After triggering of signaling pathways on septic shock, released pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and NO should be balanced with anti-inflammatory mediators for the less tissue destruction [1, 14]. Otherwise, they lead to serious inflammatory cascade injury and organ failure [8]. This mediator systems detection can be helpful to understand the degree of pathological process, diagnosis and management of sepsis [1]. It has been reported that DEX is able to decrease TNF-α and IL-1 levels in LPS induced acute lung injury [8]. NO overproduction and mitochondrial dysfunction is associated with sepsis severity [14–16]. In APAP toxicity study, DEX has been

Table 2: Liver tissue levels of (anti)oxidants, mean ± SEM.

Group	MDA (μmol/mg protein)	NO (μM/mg protein)	GSH (μM/mg protein)	Catalase (nmol/min/mg protein)
Control	0.72 ± 0.03	25.04 ± 2.81	27.05 ± 1.16	28.45 ± 1.56
LPS	2.28 ± 0.14[b]	85.51 ± 4.08[b]	10.84 ± 0.73[b]	12.69 ± 0.81[b]
Dexpanthenol	0.80 ± 0.05	29.71 ± 1.73	24.23 ± 1.80	28.95 ± 1.23
Dexpanthenol + LPS	1.07 ± 0.09[a,c]	77.12 ± 5.41[b]	26.44 ± 2.10[c]	27.52 ± 2.08[c]

[a]$p < 0.05$, [b]$p < 0.001$ according to control; [c]$p < 0.001$ according to LPS group.

Table 3: Kidney tissue levels of (anti)oxidants, mean ± SEM.

Group	MDA (μmol/mg protein)	NO (μM/mg protein)	GSH (μM/mg protein)	Catalase (nmol/min/mg protein)
Control	1.24 ± 0.15	26.63 ± 2.04	15.58 ± 1.40	20.32 ± 1.29
LPS	2.84 ± 0.11[a]	69.93 ± 3.74[a]	4.86 ± 0.63[a]	7.78 ± 0.70[a]
Dexpanthenol	1.07 ± 0.09	24.58 ± 1.16	15.93 ± 1.00	18.70 ± 0.86
Dexpanthenol + LPS	1.46 ± 0.18[b]	26.54 ± 2.44[b]	13.52 ± 1.66[b]	16.45 ± 1.78[b]

[a]$p < 0.001$ according to control; [b]$p < 0.001$ according to LPS group.

determined that it has significant capability to decrease NO level both in liver and kidney tissues [6]. Similarly, in our study we have also shown that DEX significantly prevents TNF-α and IL-1β release and NO levels' elevation in sepsis model.

Mitochondrial functions play an important role in both inflammatory responses and metabolic dysfunction. Metabolic disturbances associated with sepsis include insulin resistance, hyperglycemia, hyperlactatemia are linked to inflammatory response [17]. Cellular ATP depletion resulting from mitochondrial dysfunction can cause organ dysfunction and death [17]. LPS infusion has produced mitochondrial swelling, respiratory dysfunction and partially uncoupling of oxidative phosphorylation [18]. Wojtczak and Slyshenkov [7] incubated the Jurkat cells with pantothenic acid and concluded that DEX increases the level of mitochondrial CoA, stimulates ATP synthesis and glutathione synthesis in the cells. This mechanism has been suggested as an additional protecting mechanism of DEX against cell injury. Unfortunately, we were unable to assess mitochondrial functions such as ATP amount and degree of complex I inhibition by NO, but as a marker of metabolic inflammation, we could determine the lactate level of serum in this study. Lactate level's follow-up is recommended to improve the diagnosis of sepsis [1]. Decreased ATP synthesis in sepsis can be compensated for by increased glycolytic ATP synthesis which is associated with increased lactate production [17]. In our study, lactate levels of septic animals were tripled. DEX treatment was highly diminished lactate production. Lactate decreasing effect of DEX has been published in a report [3]. Indeed, TNF-α induces lactate production [17] and at some point controlling of TNF-α increment might be responsible of diminishing of lactate level. Additionally, DEX treatment has decreased glucose level 10%–15% in diabetic rats [5] and reported reduced the insulin resistance in hypothalamic obesity model [19]. If we consider that sepsis is a metabolic inflammation [17], it seems that DEX is effective on pathologic metabolic pathways of sepsis as well.

Besides the classical pro-inflammatory mediators (TNF-α, IL-1 and IL-6), many new biomarkers such as PCT, lactate level's follow-up are recommended to improve the diagnosis of sepsis [1, 20]. LPS induce sepsis is an accepted and widely used animal model in studies [9], here we have also shown that this is a suitable model in researches for many new biochemical parameters follow-up including lactate and PCT.

Conclusion

In conclusion, the results of this study have demonstrated that DEX ameliorates liver and kidney functions on sepsis-induced organ damage. DEX treatment shows antioxidant power by regulating MDA, GSH, CAT levels. It has anti-inflammatory action which has been assessed by TNF-α, IL-1, NO and PCT levels. It is effective for hyperlactatemia which is also an important marker of cell metabolic stress in sepsis. Any side effect of DEX cannot be determined, therefore it should be considered as an effective, safe and economical way of adjuvant therapy in sepsis clinic.

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