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Does dexmedetomidine prevent colistin nephrotoxicity?

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Abstract

Background: In this study, we aimed to investigate the effect of dexmedetomidine on colistin nephrotoxicity in rats.

Methods: Thirty-two Wistar albino rats were allocated into four groups. Intraperitoneal (ip) saline at $1\,mL.kg^{-1}$ was administered to the control group and $10\,mg.kg^{-1}$ ip colistin was given to the colistin group. In the DEX10 group $10\,mcg.kg^{-1}$ dexmedetomidine ip was given 20 min before the injection of $10\,mg.kg^{-1}$ ip colistin. In the DEX20 group ip $20\,mcg.kg^{-1}$ dexmedetomidine was injected 20 min before the administration of $10\,mg.kg^{-1}$ ip colistin. These treatments were continued twice a day for seven days. Samples were taken on the eighth day. BUN, Cr, KIM-1, TAS, and TOS were examined in blood samples and caspase-3 was examined in kidney tissue samples.

Results: The values for BUN, Cr and TOS were significantly higher in the colistin group than in the control group. BUN, Cr and TOS changes in the DEX10 and DEX20 groups were not significant compared with the control group but they were significantly lower compared with the colistin group. TAS values in the DEX10 group were significantly lower than in the control group. Apoptotic activity was significantly higher in the colistin group compared with the control group, but there was no significant difference in terms of caspase-3 staining activity when DEX10 and DEX20 groups were compared with the control group.

Conclusion: Oxidative damage and apoptosis played roles in colistin nephrotoxicity, and colistin nephrotoxicity could be prevented by treatment with dexmedetomidine.

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Dexmedetomidina

Dexmedetomidina impede a nefrotoxicidade da colistina?

Resumo

Justificativa: Neste estudo, buscamos investigar o efeito da dexmedetomidina sobre a nefrotoxicidade da colistina em ratos.

Métodos: Trinta e dois ratos Wistar albinos foram alocados em quatro grupos: o grupo controle recebeu 1 mL.kg^{-1} de solução salina intraperitoneal (ip); o grupo colistina recebeu 10 mg.kg^{-1} de colistina ip; o grupo DEX10 recebeu 10 mcg.kg^{-1} de dexmedetomidina ip 20 minutos antes da injeção de 10 mg.kg^{-1} de colistina ip; o grupo DEX20 recebeu 20 mcg.kg^{-1} de dexmedetomidina ip 20 minutos antes da administração de 10 mg.kg^{-1} de colistina ip. Estes tratamentos foram continuados duas vezes ao dia durante sete dias. As amostras foram colhidas no oitavo dia. BUN, Cr, KIM-1, TAS e TOS foram examinados nas amostras de sangue e caspase-3 foi examinada nas amostras de tecido renal.

Resultados: Os valores de BUN, Cr e TOS foram significativamente maiores no grupo colistina que no grupo controle. As alterações em BUN, Cr e TOS nos grupos DEX10 e DEX20 não foram significativas em comparação com o grupo controle, mas foram significativamente menores em comparação com o grupo colistina. Os valores de TAS no grupo DEX10 foram significativamente menores que no grupo controle. A atividade apoptótica foi significativamente maior no grupo colistina em comparação com o grupo controle, mas não houve diferença significativa em termos de atividade na coloração da caspase-3 quando os grupos DEX10 e DEX20 foram comparados com o grupo controle.

Conclusão: O dano oxidativo e a apoptose desempenharam papéis na nefrotoxicidade da colistina, e a nefrotoxicidade de colistina pode ser prevenida pelo tratamento com dexmedetomidina.

Introduction

Nosocomial pneumonia is one of the most frequent infections in intensive care units. Approximately one quarter of the all intensive care infections involve nosocomial pneumonia.[1] Nosocomial pneumonia seen in patients depending on mechanical ventilators is defined as Ventilator-Associated Pneumonia (VAP) and the rate of incidence varies between 7 and 70 percent.[2] VAP caused by multi-drug resistant *Acinetobacter baumannii* is the most common infection first contracted in the ICU.[3] This microorganism is resistant to many antibiotics including carbapenems. Therefore, although colistin has been avoided in recent years due to its neurotoxic and nephrotoxic effects, its use is now being considered again.[4]

One of the important side-effects restricting the usage of colistin is its nephrotoxicity. The degree of nephrotoxicity depends on the usage period and dosage of colistin. In renal failure caused by colistin, it is thought that the proximal tubules are affected. It has been shown in various experimental studies that oxidative stress and apoptotic activity could be responsible for this development of nephropathy and that it is recoverable.[5,6] Positive effects of various pharmacologic agents such as ascorbic acid, melatonin, and vitamin E have been demonstrated on nephropathy induced by colistin.[7-9] Dexmedetomidine, is a selective α2-agonist. Alpha 2-adrenoceptors are the instigators of renal functions. α2-receptor stimulation causes diuresis and natriuresis. It decreases the secretion of vasopressin and antagonizes the effect of renal tubules.

Alfa 2-receptor stimulation also inhibits the release of renin, which increases the Glomerular Filtration Rate (GFR) by causing afferent arterial dilation. In parallel, α2-receptor stimulation also increases GFR by causing atrial natriuretic peptide secretion.[10,11]

The primary aim of our study was to investigate the effect of dexmedetomidine in nephrotoxicity induced by colistin in rats. The secondary purpose was to investigate the mechanism of colistin nephrotoxicity.

Our hypothesis was that dexmedetomidine would show a protective effect against nephrotoxicity induced by colistin due to renoprotective effects.

Methods

This study was carried out in the Erciyes University Hakan Çetinkaya Experimental and Clinical Research Center with approval n° 14/82 dated 14.05.2014 from the Erciyes University Medical Faculty Animal Studies Ethics Committee and was supported by the Erciyes University Scientific Research Projects Unit (TTU-14-5306). Thirty-two adult (over 8 weeks) male Wistar-albino rats whose weights ranged between 180 and 280 g were used in the study. The rats were kept in the same environment in standard plastic cages and fed with standard rat food. Tap water was used as drinking water. The room temperature was maintained at 22 °C, with 12 h of darkness and 12 h of illumination each day. The experimental animals were divided into four groups consisting of eight rats each. Before the start of the experiment, the rats

were weighed in order to calculate the drug doses to be applied.

Group S (control group, $n = 8$); 20 min after intraperitoneal (ip) injection of $1\,mL.kg^{-1}$ 0.9% NaCl solution, a further $1\,mL.kg^{-1}$ of ip saline was injected.

Group COL (colistin group, $n = 8$); 20 min after intraperitoneal (ip) injection of $1\,mL.kg^{-1}$ 0.9% NaCl solution, $10\,mg.kg^{-1}$ ip colistin (Colimycin 150 mg im/iv KoçakFarma Medicine) was injected.

Group DEX10 (Colistin-dexmedetomidine $10\,mcg.kg^{-1}$ group, $n = 8$); 20 min after intraperitoneal (ip) injection of $10\,mcg.kg^{-1}$ dexmedetomidine (Precedex 200 mcg/2 mL; Hospira, Rocky Mount, NC, USA), $10\,mg.kg^{-1}$ ip colistin was injected.

Group DEX20 (Colistin-dexmedetomidine $20\,mcg.kg^{-1}$ group, $n = 8$); 20 min after intraperitoneal (ip) injection of $20\,mcg.kg^{-1}$ dexmedetomidine ip, $10\,mg.kg^{-1}$ ip colistin was injected.

The injections detailed above were administered to each rat two times each day with an 8 h gap between injections via insulin injection from the left bottom quadrant. In order to exclude intravenous injection, each injection was first gently aspired. The treatment continued for seven days. On the eighth day the rats were sedated using $50\,mg.kg^{-1}$ ip pentobarbital before blood samples were taken. Intracardiac blood samples were taken with the aid of an injector. By performing laparotomy, both kidneys were resected and placed in 10% formal dehyde solution. At the end of the experiment, the rats were sacrificed by cervical dislocation.

On the final day, in the serum solved in the room temperature, analysis of BUN (blood urea nitrogen), Cr (creatine), KIM-1 (kidney injury molecule-1), TOS (total oxidative stress), and TAS (total antioxidative stress) was performed. BUN and Cr levels were measured in a Sweeden Roche-Cobas device. The levels of KIM-1, TAS, TOS were measured using appropriate kits according to the manufacturer's instructions.

Kidney and tissue samples of a thickness of 0.5 cm were taken and fixed in 10% formol solution. Paraffin blocks were prepared using routine procedures for processing of tissue. In order to show Caspase-3 immunoreactivity, rat specific anti-caspase-3 antibodies (RB-1197-R7, Thermo Scientific, Fremont, CA) were used. Preparations were painted manually by a pathologist and a cell count was performed.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 21 program. The Shapiro–Wilk Normality test was used to assess whether the data was normally distributed. For the variables showing normal distribution, one-way analysis of variance (ANOVA) was used to test for differences between groups. Tukey tests were used for multiple comparisons in the groups that there was a difference. For the evaluation of non-normally distributed data, the Kruskal–Wallis test, a non-parametric test, was used. Where there were significant differences the groups were subjected to pairwise comparisons to determine within which groups the statistical differences lay; $p < 0.05$ was considered statistically significant.

Results

Biochemical results

BUN and Cr values were found to be significantly higher in the COL group compared with the S group ($p < 0.001$). In the DEX10 and DEX20 groups, BUN and Cr values were significantly lower than in the COL group ($p < 0.001$). There was no significant difference between the values in the DEX10 and DEX20 groups and the control group ($p > 0.05$), and also no difference between the DEX10 and DEX20 groups ($p > 0.05$) (Table 1).

The KIM-1 level was lower in the DEX10 group than the COL group. In the COL group, KIM-1 was higher than the control; however this difference was not statistically significant (Table 1).

A significant difference in TAS values was found between the S Group and the DEX10 Group. In the COL Group, the values of TAS were low compared with the control group; however a statistically significant difference was not found. In the DEX20 Group, TAS was higher than in the COL Group; however this difference was not statistically significant (Table 1). The values of TOS in all three groups were significantly lower than in the COL Group (Table 2).

İmmunohistochemical results

Analysis of caspase-3 staining in tissue sections showed apoptotic activity in the kidneys of the rats. The percentage of cells were showing caspase-3 staining in all groups is shown in Table 2. In the COL Group, caspase-3 activity was found to be significantly higher than in the control group. In the DEX10 and DEX20 groups there was no significant difference in caspase-3 staining activity compared to the control group.

Discussion

In this experimental study, colistin negatively affected kidney function and increased serum BUN, Cr, KIM-1 levels, while co-administration of dexmedetomidine prevented this nephrotoxic effect.

In a study by Ozyilmaz et al.,[12] the effect of N-acetyl cysteine (NAC) on colistin nephrotoxicity in rats, was measured by assessing plasma BUN and Cr values. In the colistin group, the BUN and Cr values were significantly increased compared with controls. In the NAC/colistin treatment group, it was reported that there was no significant change in BUN and Cr.

In many studies, it has been shown that the colistin nephrotoxicity depends on the applied dose and the possible damage due to nephrotoxicity occurred in the proximal tubules. PCysC appears to be more reliable than pCr and uNGAL seems to be the most sensitive factor of colistin nephrotoxicity.[13] We measured serum KIM-1 values because we thought could be used as an early diagnosis marker in colistin nephrotoxicity. But, KIM-1 is not a frequently used marker practical use.

Yousef et al.[9] investigated the effect of ascorbic acid on colistin nephrotoxicity in rats. The colistin was applied over several days resulting in a cumulative dose of $36.5\,mg.kg^{-1}$.

Table 1 Comparisons of BUN, Cr, KIM-1, and TAS levels in the different treatment groups.

	Group S (n = 8)	Group COL (n = 8)	Group DEX10 (n = 8)	Group DEX20 (n = 8)	p
BUN (mg.dL^{-1})	17.4 ± 2.9	40.3 ± 3.9[a]	20.7 ± 3.3[b]	21.9 ± 1.7[b]	<0.001
Cr (mg.dL^{-1})	0.4 ± 0.04	0.6 ± 0.08[a]	0.3 ± 0.03[b]	0.3 ± 0.04[b]	<0.001
KIM-1 (ng.dL^{-1})	0.74 ± 0.1	1.02 ± 0.2	0.71 ± 0.2b	0.94 ± 0.2	0.018
TAS (μmoL.L^{-1})	373.4 ± 19.7	322.3 ± 29.5	306.5 ± 63.4[c]	336 ± 54.4	0.04

BUN, blood urea nitrogen; Cr, creatinine; KIM-1, kidney injury molecule-1; TAS, total antioxidant capacity/stress.
The values are given as mean ± standard deviation ($X ±$ SD); $p < 0.05$ significant.
  [a] Significantly high compared with the S group.
  [b] Significantly low compared with the COL group.
  [c] Significantly low compared with the S group.

Table 2 Comparisons of TOS values and caspase-3 staining rates in the different treatment groups.

	Group S (n = 8)	Group COL (n = 8)	Group DEX10 (n = 8)	Group DEX20 (n = 8)	p
TOS (μmoL/H$_2$O$_2$/eq.L^{-1})	3.2 (2.4–6.7)[b]	17.7 (14.4–23.0)	3.3 (2.9–6.8)[b]	3.9 (2.9–6.8)[b]	<0.001
Caspase-3 staining percentage	10 (5–10)	10 (10–20)[a]	10 (10–20)	10 (5–10)	0.021

TOS, total oxidant capacity/stress.
The values are given as median (minimum–maximum).
  [a] Significantly high compared with the S group.
  [b] Significantly low compared with the COL group.

Urinary NAG (N-acetyl β-ᴅ-glucosaminidase) values and plasma Cr values were measured in order to detect proximal tubule damage. In the colistin group, Cr and urinary NAG was high, while in the group treated with ascorbic acid and colistin, these values remained low. It was shown in hematoxylin and eosin (H & E) stained sections that tubular damage occurred in the colistin group, and apoptotic activity increased in cell culture. Application of ascorbic acid decreased this effect. The same researchers gained positive results using melatonin in order to prevent colistin nephrotoxicity in rats in another study.[8]

Taken together, these studies have shown experimentally that colistin nephrotoxicity can be decreased by using antioxidants such as ascorbic acid and melatonin.

Ghlissi et al.[7] reported that in their studies on the effect of vitamin E on colistin nephrotoxicity in rats, vitamin E improved the values for malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GSH). Nephrotoxicity of colistin is thought to be caused by oxidative damage and the protective effect of Vitamin E against nephrotoxicity may be due to its antioxidant effect.

The best method for treatment or prevention of colistin nephrotoxicity is still not known as its mechanism is not fully understood, although many studies investigating the nephrotoxicity mechanism have been carried out. Ozkan et al.[6] researched reduction of the effect of renal damage via the antiapoptotic effect of Grape Seed Extract (GSPE) and the role of apoptosis in the colistin nephrotoxicity. In their study[6] they investigated caspases 1 and 3, calpain1, iNOS, eNOSve and TUNEL involvement via histopathological evaluation as well as making plasma BUN and Cr measurements. In the colistin group, caspase-3 was higher than in the control group, and was decreased in the colistin + GSPE group. Based on these findings, it was reported that caspase-dependent apoptotic activity could be responsible for the colistin damage.

As a primary objective of this study was to investigate the mechanism of colistin nephrotoxicity and caspase-3 was used as a marker to evaluate apoptotic activity. It was found that the caspase-3 staining rate was significantly higher in the colistin group compared with the control group. This result shows that the apoptosis may be effective in colistin nephrotoxicity and that the caspase-3 pathway may be involved.

Alfa 2-receptor stimulation causes diuresis and natriuresis. It decreases vasopressin secretion and antagonizes the effect of renal tubules. In addition, it has been reported that α2-receptor stimulation decreased renal vasopressin secretion, and increased Atrial Natriuretic Factor (ANF) secretion and GFR.[11,14]

Bayram et al.[15] evaluated renal function in patients who had undergone percutaneous nephrolithotomy by applying intraoperative dexmedetomidine infusions. They reported that dexmedetomidine significantly decreased the levels of renin. The same researchers reported that dexmedetomidine could prevent increases in plasma and renal endothelin-1 in a double-blind randomized study in pediatric patients undergoing cardiac angiography, and renal damage was decreased.[16] Based on this study, dexmedetomidine is considered to have a protective effect against colistin nephrotoxicity. In our study we employed two different doses of dexmedetomidine in order to find an efficient dose. Dexmedetomidine causes hypotension and bradycardia via its symphatolytic effect. This effect is more evident at high doses and can damage renal perfusion.[17] The higher values of KIM-1 in the DEX20 group, compared with the DEX10 group can be attributed to this effect. There is a need for further studies to determine the ideal dose of dexmedetomidine conveying a renoprotective effect.

It has been shown in various studies that dexmedetomidine has an antiapoptotic effect.[18,19] Caspase-3 can be used to detect apoptosis in renal damage.[20] In our study, we evaluated the apoptotic activity via caspase-3 immunostaining. In the DEX10 and DEX20 groups, the caspase-3 staining rate was found to be lower compared with the colistin group although the difference was not statistically significant. We can say that dexmedetomidine has an antiapopitotic effect on the caspase-3 pathway, but there is a need for more comprehensive studies that investigate the effect of dexmedetomidine on apoptosis in various doses and evaluating the affected pathways.

As a limitation of this study, the uNGAL level could be re-evaluated at this dose, but we have not been able to provide enough urine collection from rats because of technical deficiency.

In our study, the nephrotoxic effect of colistin resulted in proximal tubule dysfunction. It is thought that oxidative damage and apoptosis could be mechanisms of nephrotoxicity. Dexmedetomidine can prevent the nephrotoxicity caused by colistin in rat but we do not know yet in what extension these results can be transposed to humans.

Conclusion

All applications were realized under the veterinarian control in accordance with the Universal Declaration of International Animal Rights after receiving approval of Erciyes University Experimental Animal Ethicd Committee (date: 14/05/2014 n° 14/82).

Conflicts of interest

The authors declare no conflicts of interest.

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