EXPERIMENTAL STUDY

Vitamin C ameliorates high dose Dexmedetomidine induced liver injury

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ABSTRACT

BACKGROUND: We investigated whether vitamin C has protective effects on rat liver tissue treated with different dexmedetomidine doses.

MATERIAL AND METHODS: Thirty wistar albino rats were randomly divided into 5 groups (Control (0.9 % NaCl intraperitoneally (ip)), Dexmedetomidine 5 μg.kg⁻¹ (ip), Dexmedetomidine 5 μg.kg⁻¹ ip plus Vitamin C (100 mg.kg⁻¹), Dexmedetomidine 10 μg.kg⁻¹ ip and Dexmedetomidine 10 μg.kg⁻¹ ip plus Vitamin C (100 mg.kg⁻¹). Histopathological liver injury, superoxide dismutase (SOD) activity and tissue Malondialdehyde levels were investigated.

RESULTS: Hepatocyte degeneration was significantly higher in D10 group than those in other study groups (p < 0.0001, p = 0.002, p < 0.0001, p = 0.005, respectively). Similarly, liver tissue sinusoidal dilatation and hepatocyte necrosis were significantly higher in D10 group than those in other groups (p < 0.0001, p < 0.0001, p = 0.002, p > 0.0001 and p < 0.0001, p = 0.046, p < 0.0001 and p = 0.002, respectively). Tissue MDA levels in D10 group were significantly higher than those in control, D5+Vit C and D10+Vit C groups (p = 0.028, p = 0.004, p = 0.031, respectively). SOD enzyme activity in D10 group was significantly lower than in control, D5+Vit C and D10+Vit C groups (p < 0.0001, 0.023 and p = 0.031, respectively).

CONCLUSION: High dose dexmedetomidine can induce hepatic injury and oxidative stress in rats while pre-treatment with vitamin C may be effective in protecting liver tissue against this newly recognized undesirable dexmedetomidine effect (Tab. 2, Fig. 5, Ref. 30). Text in PDF www.elis.sk.

KEY WORDS: Dexmedetomidine, vitamin C, liver histopathology, MDA, SOD, rat.

Introduction

Dexmedetomidine is a strong alpha-2 agonist that 8 times more selectively binds alpha 2 receptors than clonidine does (1-4). Dexmedetomidine, as a sedative agent, is gaining popularity especially during invasive interventions planned under cooperative sedation and mechanically ventilated patients treated in intensive care units. Dexmedetomidine is being preferred due to low incidence of respiratory depression, delirium, coma and undesirable hemodynamic changes related with drug (5-11). In addition to the benefits listed above various studies showed that dexmedetomidine has protective effects on focal cerebral, cardiac, renal, liver ischemia-reperfusion (IR) injuries (12–16). However dose dependent effects of dexmedetomidine and dexmedetomidine plus vitamin C combination on liver tissue have not been investigated. In this study we aimed to investigate effects of different dexmedetomidine doses on liver tissue and possible protective effects of vitamin C in an experimental rat model.

Materials and methods

This study was conducted in the Physiology laboratory of Kirikkale University upon the consent of the Experimental Animals Ethics Committee of Kirikkale University.

In the study, 35 male Wistar Albino rats (total number = 35) of 250–325 g weight, raised under the same environmental conditions, were used. The rats were kept under 20–21 o C at cycles of 12-hour daylight and 12-hour darkness and had free access to food until 2 hours before the anesthesia.

Thirty wistar albino rats were randomly divided into 5 groups (Control (0.9 % NaCl ip), Dexmedetomidine 5 μg.kg⁻¹ intraperitoneally (ip), Dexmedetomidine 5 μg.kg⁻¹ ip plus Vitamin C (100 mg.kg⁻¹ ip administered 1 hour before dexmedetomidine treatment), Dexmedetomidine 10 μg.kg⁻¹ ip and Dexmedetomidine 10 μg.kg⁻¹ ip plus Vitamin C (100 mg.kg⁻¹ ip administered 1 hour before dexmedetomidine treatment). First study group was
administered low dose dexmedetomidine 5 μg.kg⁻¹ ip and the other study group was given the same amount (10 μg.kg⁻¹) of high dose dexmedetomidine. Thirty minutes after dexmedetomidine administration, all rats were anesthetized with 50 mg.kg⁻¹ ketamine ip and intracardiac blood samples were obtained. Histopathological changes in hepatic tissue were observed. Additionally, tissue MDA levels and SOD activities were measured.

Biochemical analysis

The liver tissues were first washed with cold deionised water to remove blood contamination, and were then homogenised in a homogenisator (Heidolph DIAX900) at 3,000 rpm for 3 min. After centrifugation at 10,000xg for 10 min, the upper clear layer was taken. The amounts of protein and malondialdehyde (MDA) in this supernatant were measured as described by Lowry et al and Van Ye et al, respectively (16,17).

Histological testing

Semiquantitative evaluation technique used by Abdel-Wahhab et al’s (19) was applied for interpreting the structural changes investigated in hepatic tissues of control and research groups. According to this, (−) (negative point) represents no structural change, while (+) (one positive point): mild, (++) (two positive points): medium and (+++) (three positive points): severe structural changes.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 program was used for the statistical analysis. Variations in oxidative state parameters, and histopathological examination between study groups were assessed using the Kruskal–Wallis test. The Bonferroni-adjusted Mann–Whitney U test was used after significant Kruskal–Wallis to determine which groups differed from the others. Results were expressed as mean ± standard deviation (Mean ± SD). Statistical significance was set at a p value of < 0.05 for all analyses.

Results

We found significant differences in terms of hepatocyte degeneration on light microscopical evaluation between study groups. Hepatocyte degeneration in group D10 was significantly higher than in the other groups (C, D5, D5+Vit C and D10+Vit C) (p<0.0001, p=0.002, p<0.0001 and p=0.005 respectively) (Tab. 1, Figs 1–5).

Sinusoidal dilatation in group D10 was significantly higher than in groups C, D5, D5+Vit C and D10+Vit C (p<0.0001, p<0.0001, p=0.002 and p<0.0001, respectively) (Tab. 1, Figs 1–5).

Number of pyknotic bodies in group D10 was significantly higher than those in group C, D5, D5+Vit C and D10+Vit C (p<0.0001, p=0.002, p<0.0001 and p=0.002, respectively) (Tab. 1).

Additional numbers of pyknotic bodies observed in group D5, D5+Vit C and D10+Vit C were significantly higher than that in group C (p=0.0001, p<0.0001 and p<0.0001, respectively) (Tab. 1, Figs 1–5).

Number of cells undergoing necrosis in group D10 was significantly higher than in other study groups (group C, D5, D5+Vit C

Tab. 1. Semiquantitative evaluation results of rat liver tissue between groups (mean ± SD).

|                          | Group C (n=7) | Group D5 (n=7) | Group D10 (n=7) | Group D5+Vit C (n=7) | Group D10+Vit C (n=7) | p**       |
|--------------------------|---------------|---------------|----------------|---------------------|----------------------|-----------|
| Hepatocyte degeneration  | 0.29±0.49*    | 0.50±0.55*    | 2.29±0.49       | 0.58±0.53*          | 1.15±0.69*           | < 0.0001  |
| Sinusoidal dilatation    | 0.43±0.53*    | 1.00±0.58*    | 2.00±0.58       | 0.86±0.38*          | 0.71±0.49*           | < 0.0001  |
| Pyknotic bodies          | 0.00±0.00*    | 0.71±0.49*,+  | 2.14±0.38       | 0.71±0.49*,+        | 1.14±0.38*,+         | < 0.0001  |
| Cells undergoing necrosis| 0.00±0.00*    | 1.14±0.69*,+  | 1.71±0.49       | 0.15±0.38*          | 0.71±0.49*,+         | < 0.0001  |
| MN infiltration in parenchyma | 0.57±0.53* | 1.00±0.58*    | 2.71±0.49       | 0.71±0.49*          | 1.18±0.49*           | < 0.0001  |

p**: p < 0.05 with Kruskal–Wallis test, *p < 0.05: compared with Group D10, +p < 0.05: compared with Group C

Tab. 2. Tissue MDA levels and SOD enzyme activities in rat liver tissue (mean ± SS).

|                          | Group C (n=7) | Group D5 (n=7) | Group D10 (n=7) | Group D5+Vit C (n=7) | Group D10+Vit C (n=7) | p**       |
|--------------------------|---------------|---------------|----------------|---------------------|----------------------|-----------|
| MDA (nmol/mg prot)       | 0.29±0.10*    | 0.33±0.11     | 0.50±0.32       | 0.19±0.06*          | 0.28±0.12*           | 0.039     |
| SOD (IU/mg protein)      | 5.56±1.28*    | 4.32±1.08*    | 1.63±1.19       | 4.23±2.56*          | 3.75±0.79            | < 0.0001  |

p**: p < 0.05 with Kruskal–Wallis test, *p < 0.05: compared with group D10
and D10+Vit C) (p < 0.0001, p = 0.046, p < 0.0001 and p = 0.002, respectively). Also number of cells undergoing necrosis in groups of D5, D5+Vit C and D10+Vit C was significantly higher than in the control group (p = 0.002, p < 0.0001 and p = 0.046, respectively) (Tab. 1, Figs 1–5).

Mononuclear cell infiltration was significantly higher in D10 group than in other groups (C, D5, D5+Vit C and D10+Vit C) (p < 0.0001, p < 0.0001 and p < 0.0001, respectively) (Tab. 1, Figs 1–5).

Tissue MDA levels were significantly higher in D10 group than in C, D5+Vit C and D10+Vit C groups (p = 0.028, p = 0.004 and p = 0.031, respectively) (Tab. 2).

SOD enzyme activity was lower in D10 group than in group C, D5+Vit C and D10+Vit C (p < 0.0001, p = 0.023 and p = 0.031, respectively) (Tab. 2).

**Discussion**

Dexmedetomidine is a relatively new agent that promises less frequent respiratory depression, shorter recovery time with comparable delirium and coma incidence than reported with benzodiazepines and narcotics, minimum hyperalgesia and undesirable hemodynamic changes in a large spectrum from bradycardia to
cardio-pulmonary collapse (6–11). Beside this preferable side effect profile various in vivo studies reported protective effects of dexmedetomidine on cardiac, neurologic, renal and liver IR injury models (12–16). In contrast we firstly described damaging effects of high dose dexmedetomidine on liver tissue while healing effects of vitamin C on liver tissue damage and oxidative stress induced by high dose dexmedetomidine.

In a human study Wang et al (12) investigated whether dexmedetomidine has protective effect on liver IR injury induced by hepatectomy with inflow occlusion protocol. Patients in dexmedetomidine group were treated with a loading dose of 1 μg.kg⁻¹ over 10 minutes followed by a continuous infusion dose of 0.3 μg.kg⁻¹  h⁻¹. Primarily, the serum diamine oxidase (DAO) levels were assessed as an intestinal injury marker. Additionally, kidney, hepatic, intestinal and cardiovascular functions and oxidative state of tissues were measured. The study results showed that DAO activity, D-lactate acid levels, intestinal and liver injury scores in dexmedetomidine treated group were lower than those in control group (0.9 % NaCl administered).

Tufek et al (13) conducted an animal study investigating effects of dexmedetomidine on liver IR injury. In this study a single dose of 100 μg.kg⁻¹ dexmedetomidine (ip) was administered before ischemia and than total oxidative activity (TOA), total antioxidative capacity (TAC), paraoxonase (PON-1), and oxidative stress index (OSI) were measured after a 60 min reperfusion period. They reported that dexmedetomidine was significantly correlated with lower TOA and OSI values and increased TAC and PON-1 values. Also IR induced histopathological injury was ameliorated following dexmedetomidine administration.

Sahin et al (14) showed anti-oxidant effects of low and high doses (10 and 100 mg.kg⁻¹ ip) of dexmedetomidine on hepatic IR injury. In this study tissue MDA levels were lower than those in IR injury group while SOD, catalase, and glutathione levels were higher than those in IR injury group. Also histologic injury scores were lower in dexmedetomidine groups than in the untreated IR injury group. However the authors reported that the histologic injury scores in both dexmedetomidine groups were significantly higher than scores achieved in the control group. The latter result of this study can be interpreted as a confusing result when compared with others because it may imply a liver damage induced by dexmedetomidine administration.

In contrast to the study results presented above our findings showed increased hepatocyte apoptosis and necrosis in addition to sinusoidal dilatation and hepatocyte degeneration with high dose dexmedetomidine treatment. In order to interpret our findings a brief review of mechanisms responsible for drug induced hepatic injury –especially in terms of hepatocyte apoptosis, necrosis and P450 enzyme system induced oxidative damage- is essential. Drug induced hepatocyte apoptosis and necrosis can be driven by Fas ligand, and tumor necrosis factor a (TNF-a), and their receptors (20). Apoptosis process can be dependent on the intracellular energy and redox status of the hepatocyte (21, 22). Another important hepatic injury mechanism is the activation of cytotoxic pathways driven by cytochrome P450 enzymes. These enzymes are the main cellular sites that catalyze oxidation reactions followed by production of active molecules. These products may lead to cellular damage via blocking enzyme functions, protein synthesis and DNA/RNA replication. Thirdly, mononuclear cell mediated injury is an important mechanism. Kupffer cells and/or inflammatory neutrophils and macrophages produce and secrete chemokines, TNF-a, reactive nitrogen products such as nitric oxide and peroxynitrite and oxygen adducts include superoxide anion, hydrogen peroxide, and hydroxyl radical. We can postulate that these injury mechanisms may be responsible for dexmedetomidine induced liver damage that was shown in our study because in adults, there are two main metabolic pathways for dexmedetomidine, direct glucuronidation [5-diphosphoglucuronosyl transferase (UGT1A4 and UGT2B10)] (85 %) and to a lesser proportion – 15 %- cytochrome P450 enzymes (CYP450) mediated (23). Our findings indicate that high dose dexmedetomidin related sinusoidal dilatation, hepatocyte necrosis, apoptosis and increased tissue MDA and decreased SOD levels can be interpreted as cytochrome P450 enzymes induced oxidative and direct hepatic injury. At low doses hepatic tissue may restore dexmedetomidine induced damage by various protective mechanisms including heat shock protein mediated protection and production of anti-inflammatory mediators such as IL-10, IL-6, IL-4, IL-13 and prostaglandin E2, I2 etc. On the other hand high doses of dexmedetomidine may cause high levels of oxidative adducts that couldn’t be cleared by various protective mechanisms in hepatic tissue.

Vitamin C (ascorbic acid) is a well known water-soluble anti-oxidant that is present in various fruits, vegetables and also human and animal cells and body fluids (24–26). Several important roles of vitamin C in metabolic pathways have been identified. Vitamin C is a reactive oxygen species scavenger (24, 25) and essential coenzyme during several important metabolic pathways including collagen synthesis, dopamine and tryptophan hydroxylations (26). Additionally, vitamin C (ascorbic acid) is protective against toxic free radical and ROS induced cellular damage vitamin C neutralizes ROS and limits lipid peroxidation (27). Various studies showed significant benefits of vitamin C on methotrexat (MTX), isoniazid (INH) and carbon tetrachlor (CCl4) induced liver damage (28–30). Similarly our findings indicate protective effects of vitamin C on high dose dexmedetomidine induced liver damage. We suppose that strong anti-oxidant effects of vitamin C are related with low tissue MDA and high SOD levels in dexmedetomidine 10 μg.kg⁻¹ plus vitamin C group when compared with those in dexmedetomidine 10 μg.kg⁻¹ group.

There is a major limitation of this study. More comprehensive evaluation of dexmedetomidine induced liver damage and oxidative stress may be necessary with measuring serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), inflammatory and anti-inflammatory markers (including complement derived peptides, interleukins, kinins etc), tissue derived factors (tissue endothelial nitric oxide synthase, glutathion etc). Nevertheless, we suggest that the results of this study are important for understanding the effects of different dexmedetomidine doses and protective role of vitamin C on liver tissue. Molecular and histologically based more extensive researches in human and
animals can help to clarify the different results with dexmedetomidine effects on hepatic tissue.

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