THE PROTECTIVE EFFECTS OF DEXMEDETOMIDINE ON THE LIVER AGAINST OBSTRUCTIVE JAUNDICE IN RATS


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ABSTRACT

Aims: Dexmedetomidine is a highly selective and potent α-2 adrenergic agonist with sedative, analgesic, anxiolytic, sympatholytic, hemodynamic, and diuretic properties. In recent years, investigations have shown that dexmedetomidine possesses secondary antioxidant and also anti-inflammatory effects. For this reason, we aimed to determine the possible hepatoprotective effects of dexmedetomidine in experimental obstructive jaundice.

Materials and methods: Thirty Wistar-Albino male rats were randomized and divided into 3 groups of 10 animals. Group I, sham-operated; Group II, ligation and division of the common bile duct (BDL); Group III, BDL followed by daily intraperitoneal injection of 25 µg/kg dexmedetomidine. The animals were sacrificed on postoperative day 10 by high dose diethyl ether inhalation. Blood and liver samples were taken for biochemical and histopathological evaluation.

Results: In this study, biochemical and pathological parameters were significantly better in the BDL+dexmedetomidine group when compared with the BDL group. Liver MDA (p=0.001), MPO (p=0.021) and total-SH (p=0.001) were found to be significantly different between the BDL+ dexmedetomidine and the BDL groups. Plasma total-SH (p=0.027) and MDA (p=0.012) values were also statistically different between these groups. Statistical analyses of histological activity index (HAI) scores showed that the histopathological damage in the BDL+ dexmedetomidine group was significantly less than the damage in the control group (p<0.05 for all pathological parameters).

Conclusion: The results of this study show that dexmedetomidine had a significant hepatoprotective effect on the detrimental effects of obstructive jaundice. We concluded that these effects might be due to its sympatholytic, antioxidant and anti-inflammatory activities.

Key words: Dexmedetomidine, obstructive jaundice, antioxidant, anti-inflammatory.

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Introduction

Cholestasis is a liver function disorder, caused by cessation of bile flow through bile ducts into the small intestine[1]. Patients with jaundice due to extrahepatic biliary obstruction still experience a high rate of postoperative complications and death. Many complications are infectious in nature or related to defects in host defence, whereas others are systemic. The major complications of untreated obstructive jaundice are cholangitis, coagulation defects and liver damage progressing to biliary fibrosis and cirrhosis[2]. Surgical procedures for relief of obstructive jaundice are associated with high mortality and morbidity rates, mainly due to postoperative complications such as hepatic failure, sepsis, bleeding, renal failure and pulmonary dysfunction[3]. Although the mechanisms of liver damage associated with cholestasis are complex and multifactorial, bile acid–mediated hepatotoxicity certainly plays a pivotal role in the pathogenesis of the disease. Cholestasis initiates an inflammatory
response in the liver, although its mechanism is not known. Numerous experimental studies have proved more intense oxidative stress and increased intensity of lipid peroxidation in the plasma and liver tissue in animals with experimentally induced cholestasis.\(^4\)

Dexmedetomidine, which was approved by the US Food and Drug Administration in 1999 for sedation of patients hospitalized in an intensive care setting, is a potent \(\alpha_2\)-adrenergic agonist with sedative, analgesic, sympatholytic, hemodynamic, and diuretic properties.\(^5\) It acts by binding to G-protein coupled \(\alpha_2\)-adrenergic receptors, which are found in the central, peripheral, and autonomic nervous systems and also in various vital organs and blood vessels throughout the body. It has been increasingly used in clinical practice for anxiolysis, analgesia, sedation, and anesthetic sparing.\(^6\) Both in vivo and in vitro studies have demonstrated that dexmedetomidine has a protective effect against ischemia-reperfusion (I/R) injury of the heart, kidney, brain, and testis in animal models.\(^7\) Preclinical studies on oxidative stress and free radical formation, prophylactic administration of dexmedetomidine in various experimental I/R injury models has been found to protect tissues against the formation of free radicals after reperfusion. Moreover, preclinical studies have shown that dexmedetomidine could decrease systemic inflammation and increase the survival rate following sepsis caused by endotoxins.\(^8\) Whole studies have demonstrated that apart from its anesthetic property, dexmedetomidine possesses anti-inflammatory, anti-oxidant and anti-apoptotic effects.

In the light of all these features of dexmedetomidine, the aim of this study was to investigate the effects of dexmedetomidine on oxidative stress parameters and the histomorphology of liver tissue in an obstructive jaundice model. To the best of our knowledge, the effect of dexmedetomidine on obstructive jaundice has not been previously investigated in literature.

Materials and methods

Animals

Thirty Wistar-Albino male rats, weighing 250±25g, were allowed to adapt to laboratory conditions for 1 week before experimental use. The animals had free access to water and standard laboratory chow. They were housed under constant temperature (21±2°C) individually in wire cages under a 12-hour light-dark cycle. Twelve hours before anesthesia, the animals were deprived of food but had free access to water until 2 hours before anesthesia. No enteral or parenteral antibiotics were administered at any time. Rats that died during the experiment were excluded from the study and no new rats were included. The procedures in this experimental study were performed in accordance with the National Guidelines for The Use and Care of Laboratory Animals and the Animal Ethics Committee of Ankara Education and Research Hospital granted approval for the study.

Study groups

The rats were randomized and divided into 3 groups of 10 animals. Group I, sham-operated; Group II, ligation and division of the common bile duct (BDL); Group III, BDL followed by daily intraperitoneal injection of 25 \(\mu\)g/kg dexmedetomidine. The animals were sacrificed on postoperative day 10 by high dose diethyl ether inhalation. Blood and liver samples were taken for biochemical and histopathological evaluation.

Operative procedure

The animals were anesthetized by intramuscular injection of 30 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Istanbul, Turkey) and 5 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey). A midline laparotomy was performed under sterile conditions. In the sham-operated group (Group I) the common bile duct (CBD) was freed from the surrounding soft tissue and was manipulated without ligation and transection. In groups II and III, the CBD was identified in each rat, double ligated with 5-0 silk sutures, and divided between the ligatures. The same surgeon performed all the procedures. The abdominal incisions were closed in 2 layers with continuous 3-0 silk sutures. The animals were allowed to feed after the operation.

Evaluation of biochemical parameters

The biochemical evaluation was performed in the Biochemistry Department of Ankara Education and Research Hospital. Total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin and direct bilirubin levels were measured by an autoanalyser (Olympus AU640, Japan) for biochemical evaluation of liver functions.
Evaluation of oxidative stress

The evaluation of oxidative stress parameters was performed in the Biochemistry Department of Ankara Education and Research Hospital. Tissues were stored at – 80°C until the assays. Tissue malondialdehyde (MDA), total-SH (sulphydryl) levels and myeloperoxidase (MPO) enzyme activities were measured. Plasma MDA and total SH levels were also evaluated.

MDA levels were calculated by the fluorometric method, as described by Wasowicz et al. [9]. After the reaction of thiobarbituric acid (TBA) with MDA, the reaction product was extracted in butanol and was measured spectrophotometrically at wavelengths of 525 nm for excitation and 547 nm for emission. 0.5 μmol/L 1,1′,3,3′-tetraethoxypropane solution was used as standard.

For the measurement of tissue MDA levels; 50 μL of homogenate was added and introduced into 10 mL glass tubes containing 1 mL of distilled water. After the addition of 1 mL of the solution containing 29 mmol/L TBA in acetic acid and mixing, the samples were placed in a water bath and heated for 1 h at 95-100°C. The samples were then cooled, 25 μL of 5 mol/L hydrochloric acid (HCL) was added and the reaction mixture was extracted by agitation for 5 min with 3.5 mL n-butanol. After separation of the butanol phase by centrifugation at 1500 x g for 10 min, the fluorescence of the butanol extract was measured with a fluorometer (HITACHI F-2500) at wave-lengths of 525 nm for excitation and 547 nm for emission. 0.5 μmol/L 1,1′,3,3′-tetraethoxypropane solutions were used as standard. MDA levels were given as μmol/g wet tissue[10].

Total SH groups were measured spectrophotometrically using the method of Sedlak and Lindsay[10]. Aliquots of 250 μL of the supernatant fraction of the tissue homogenate were mixed in 5 mL test tubes with 750 μL of 0.2 M Tris buffer, pH 8.2, and 50 μL of 0.01 M 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). The mixture was brought to 5 mL with 3950 μL of absolute methanol. A reagent blank (without sample) and a sample blank (without DTNB) were prepared in a similar manner. The test tubes were stoppered with rubber caps, the color was developed for 15 min and the reaction mixtures were centrifuged at approximately 3,000g at room temperature for 15 min. The absorbance of supernatant fractions was read in a spectrophotometer at 412 nm[10].

MPO activity was assayed spectrophotometrically by determining the decomposition of hydrogen peroxide using o-dianisidine as the hydrogen donor. Tissue samples of approximately 50 mg were taken, weighed and homogenized three times for 30 s at 4°C in 1 mL of ice-cold 0.5% hexade-cyltrimethylammonium bromide in 50 mmol/L phosphate buffer (pH 6). The homogenate was subjected to 3 freeze/thaw cycles and centrifuged for 15 min at 40,000 x g. MPO activity was determined by the addition of 0.1 mL of the supernatant to 2.9 mL of 50 mmol/L phosphate buffer containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm over a 5 min period was measured at 25°C. The data were expressed as the change in absorbance/min/g wet weight[11].

Histopathological examination

The histopathological analyses were performed in the Pathology Department of Harran University Faculty of Veterinary Medicine. For light microscopy analyses, the samples were obtained from the liver and fixed in 10% neutral buffered formalin solution for 2 days. The tissues were washed in running water and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96%, 100%). After dehydration, the specimens were put into xylene to obtain transparency and were then infiltrated with and embedded in paraffin. The embedded tissues were cut into 5-μm thick sections using a Leica RM 2125 RT microscope and stained with hematoxylin&eosin. Histopathological examinations were performed with a light microscope (Olympus, BX51TF) by a pathologist blinded to the study design. Inflammatory activities and fibrosis were evaluated semiquantitatively according to modified histological activity index (HAI) described by Ishak K[12,13].

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 15.0 for Windows (SPSS Inc, Chicago, IL). All variables were normally distributed about the mean. Data were presented as mean±SD. Differences between the groups were evaluated by one-Way analysis of variance (ANOVA) or Kruskal-Wallis variance analysis, whichever was appropriate. When the P values from the variance analysis were statistically significant, the Tukey honestly significant difference (HSD) or Kruskal-Wallis multiple
comparison test was used to determine which group was different from the others. A value of \( p < 0.05 \) was considered to be statistically significant.

**Results**

**General**

All rats were sacrificed on postoperative day 10. A total of 3 rats died during the early postoperative period probably due to anesthesia; 2 from Group II (BDL group) and 1 from Group III (BDL + dexmedetomidine group). These rats were excluded from the study and no new rats were included.

**Biochemical results**

Total protein, albumin, ALT, AST, LDH, GGT, ALP, total bilirubin and direct bilirubin levels were significantly better in the BDL+dexmedetomidine group than in the control group (\( p < 0.05 \) for all parameters). All biochemical parameters were statistically different in the sham group when compared with the control and BDL+dexmedetomidine groups (\( p < 0.05 \)) (Table 1).

Table 1: Mean liver function test values of the groups. There is significant difference between BDL+dexmedetomidine group and control group (\( p < 0.05 \) for all parameters). All biochemical parameters are statistically different in the sham group when compared with the control and BDL+dexmedetomidine groups (\( p < 0.05 \)).

**Oxidative stress parameters**

The mean levels of the oxidative stress parameters of the liver (MDA, MPO, and total-SH) and plasma (MDA and total-SH) are summarized in Table 2. Liver MDA (\( p = 0.001 \)), MPO (\( p = 0.021 \)) and total-SH (\( p = 0.001 \)) are significantly better in the BDL+dexmedetomidine group than the BDL group. Plasma total-SH (\( p = 0.027 \)) and MDA (\( p = 0.012 \)) values are also statistically different between these groups.

Table 2: Oxidative stress parameters of liver and plasma. Liver MDA (\( p = 0.001 \)), MPO (\( p = 0.021 \)) and total-SH (\( p = 0.001 \)) are significantly better in the BDL+dexmedetomidine group than the BDL group. Plasma total-SH (\( p = 0.027 \)) and MDA (\( p = 0.012 \)) values are also statistically different between these groups.

**Histopathological results**

The mean scores of the histological activity index (HAI) of the groups are given in Table 3.

Table 3: Mean pathological scores of the groups. †, Significantly different, sham vs BDL group; ‡, Significantly different, BDL vs BDL+dexmedetomidine group; ¥, Significantly different, sham vs BDL+dexmedetomidine group.

When statistical analyses of the HAI scores were performed, it was found that the histopathological damage in the BDL+dexmedetomidine group was significantly less than the damage in the control group (\( p < 0.05 \) for all pathological parameters). The HAI scores of the sham group were significantly

better than those of the control (p<0.05 for all parameters) and BDL+dexmedetomidine groups (p<0.05 for all parameters except for focal inflammation, bile duct proliferation, congestion and sinusoidal dilatation) (Table 3). Pathological abnormalities are presented in Fig. 1 and 2.

**Discussion**

Cholestasis syndrome includes liver function disorder due to the obstruction of bile drainage into the intestine, with the consequent retention of bile constituents in the liver and their regurgitation in the blood\(^\text{[4]}\). Both increased concentrations of bilirubin and bile salts in serum, and lack of bile in the gut lumen, are harmful during biliary obstruction. Obstructive jaundice, caused by either benign or malignant disease, is associated with a high rate of postoperative complications and mortality.

Sepsis, bleeding, renal failure, and impaired wound healing are common\(^\text{[14]}\). Hyperbilirubinemia, which is an integral part of cholestasis syndrome, leads to liver function damage, dysfunction of gastrointestinal barrier, immunodeficiency, coagulation disorders and disorders in detoxification, accompanied by impeded wound healing\(^\text{[15]}\). Although the mechanism of the bile-salt-induced damage has not been fully elucidated, inflammatory cell infiltration, accumulation of hydrophobic bile acids, endotoxemia, changes of the mitochondrial permeability transition and the deleterious effect of oxygen free radicals are possible factors responsible for cholestatic liver injury\(^\text{[15]}\).

Cholestasis initiates an inflammatory response, the mechanism of which is unknown. Allen et al.\(^\text{[16]}\) demonstrated two mechanisms. Firstly, activation of Toll-like receptor 4, either by bacterial lipopolysaccharide or by damage-associated molecular pattern molecules released from dead hepatocytes, triggers an inflammatory response. Secondly, bile acids act as inflammagens, and directly activate signal pathways in hepatocytes that stimulate production of proinflammatory mediators. In addition to the reticuloendothelial system dysfunction, a hypersensitivity to endotoxin challenge also exists, which leads to an exaggerated proinflammatory response in jaundiced animals. This response is marked by an increased production of tumor necrosis factor alpha, interleukin-1, and interleukin-6 in common bile duct ligated versus sham animals, and is associated with increased markers of end-organ injury and death\(^\text{[17]}\). Inflammatory features of obstructive cholestasis include portal tract edema, neutrophil infiltration in the portal tracts, proliferation of the biliary epithelial cells and portal tract fibrosis\(^\text{[18]}\).

The liver, the largest reticuloendothelial organ, is affected by obstruction of bile ducts. When biliary obstruction occurs, the stationary macrophages in the sinusoids of the liver, the Kupffer cells, do not work\(^\text{[19]}\). Although the responsible pathophysiological mechanisms are not fully understood, it is generally thought that the removal of bile from the gastrointestinal tract promotes bacterial overgrowth and increased translocation of endotoxin to the liver, thus serving to inhibit hepatic macrophage (Kupffer cell) function in these patients\(^\text{[20]}\).

Bile acids cause oxidative damage by stimulating the generation of oxygen free radicals from mitochondria, as well as promoting their release from neutrophils and macrophages\(^\text{[21]}\).
Padillo et al.\textsuperscript{(22)} found that bile duct obstruction induced intense oxidative stress with depletion of different molecules and enzymes with antioxidant properties in experimental cholestasis.

Oxidative stress is a process of tissue injury caused by the effect of free radicals. The mechanism of reactive oxygen species (ROS)-induced cell killing during inflammation involves the promotion of mitochondrial dysfunction through an intracellular oxidant stress in hepatocytes leading mainly to necrosis and to a lesser degree to apoptosis\textsuperscript{(4)}.

Reactive intermediates produced under conditions of oxidative stress cause the oxidation of polyunsaturated fatty acid in the membrane lipid bilayers, leading eventually to the formation of aldehydes\textsuperscript{(23)}. Severe oxidative stress produces ROS and induces uncontrolled lipid peroxidation. The products of oxidative stress, such as malondialdehyde (MDA), have been found in in the blood of patients with cholestasis. These products are extremely cytotoxic and damage cell membranes and intracellular macromolecules\textsuperscript{(24)}.

Dexmedetomidine, an $\alpha$-2 adrenergic agonist, acts by binding to G-protein coupled $\alpha$-2 adrenergic receptors, which are found in the central, peripheral, and autonomic nervous systems and also in various vital organs and blood vessels throughout the body. In the intensive care setting, it has been effectively used in postoperative analgesia and sedation of high risk and complex surgical patients, and during transition from other conventional sedatives. The activation of post-synaptic $\alpha$-2 receptors leads to sympatholysis and results in hypotension and bradycardia, thus helping to attenuate the stress response. Dexmedetomidine also offers good perioperative hemodynamic stability and an intraoperative anesthetic-sparing effect. Therefore, it is used as anesthetic adjuvant during surgery\textsuperscript{(25-28)}.

The attenuation of noradrenaline release in the circulation by dexmedetomidine may prevent potential destructive effects of excess metabolism caused by noradrenaline by means of prohibiting increased free oxygen radical production\textsuperscript{(29)}. Tüfek et al.\textsuperscript{(30)} showed that dexmedetomidine was a protective agent against the oxidative alterations in hepatic ischemia-reperfusion injury on the liver and remote organs, when given before induction of ischemia. Previous studies have demonstrated that dexmedetomidine may lessen systemic inflammation and increase survival rates in sepsis and endotoxin-induced shock in rats\textsuperscript{(4)}.

In a study by Sun et al.\textsuperscript{(31)}, it was reported that the protective effects of dexmedetomidine on ischemia/reperfusion-induced lung inflammation, capillary barrier dysfunction, tissue edema, and injury were similar to those of the steroid dexamethasone. The studies on rats supported that it could decrease systemic inflammation and increase the survival rate following sepsis caused by endotoxins\textsuperscript{(32,33)}.

Free radicals can react with lipids in the cell and mitochondrial membranes and initiate lipid peroxidation\textsuperscript{(29)}. MDA is the end product of lipid peroxidation and is widely used as a marker of oxidative activity\textsuperscript{(34)}. In a study by Kurt et al.\textsuperscript{(35)}, the inhibition of acute ischemia/reperfusion damage by dexmedetomidine in rat ovarian tissue was investigated and microscopic findings, such as very severe edema, very severe vascular congestion, hemorrhage, and leucocyte infiltration, were found to be present in the ovarian tissue with elevated MDA and these histological observations indicated that the increase in MDA was related to the intensity of the tissue injury. Gideroglu et al.\textsuperscript{(36)} also reported that ischemic insult resulted in high MDA levels in an inferior epigastric artery skin flap as a flap I/R injury model. Studies of various tissues have shown that dexmedetomidine prevented an increase in MDA levels, thus resulting in a simultaneous decrease in lipid peroxidation\textsuperscript{(3,8,29,33)}. In the present study, MDA level was measured to evaluate lipid peroxidation and tissue damage. Liver and plasma MDA levels were higher in the control group than in the sham and dexmedetomidine groups. These results show that dexmedetomidine reduced tissue injury and lipid peroxidation.

MPO, which is a member of the heme peroxidase-cyclooxygenase superfamily, is used an enzyme marker for the degree of neutrophil infiltration. Many reports have shown that activated neutrophils are able to produce oxygen metabolites or protease, and these neutrophil-derived cytotoxic agents cause endothelial cell injury and result in tissue damage. Over-reaction of neutrophils may be responsible for organ failure in various pathological conditions. MPO is abundant in granules of human inflammatory cells such as activated neutrophils, macrophages and monocytes. In the current study, MPO activity was used to evaluate the degree of neutrophil infiltration. Tissue MPO activity was high in the control group when compared with the sham and dexmedetomidine groups.
In other words, the treatment with dexmedetomidine reduced the tissue MPO activity and this process might be considered as less tissue injury and less neutrophil infiltration. It has been consistently proven that dexmedetomidine decreased tissue MPO in studies carried out by Uysal et al.\(^{(29)}\) and Kılıc et al.\(^{(30)}\)

Glutathione (GSH) is a cysteine-containing tripeptide that is abundant in most eukaryotic cells. GSH helps to maintain cellular sulhydryl residues in a reduced state. GSH also reacts with free radicals generating glutathionyl. GSH is involved in DNA synthesis, the repair of injured DNA portions, metabolic functions, inactivation of toxic substances and the prevention of possible damage caused by free radicals\(^{(38)}\). Although the physiological significance of protein glutathiolation has not been fully assessed, it is currently believed that the addition of GSH to protein sulphydryls prevents excessive oxidation and thereby preserves protein integrity and function under conditions of oxidative stress. GSH and total-SH levels have been found to be lower in injured tissues when compared with normal tissues. GSH has been measured to assess the defense mechanism against the hazardous effects of reactive oxygen species, and to roughly estimate the degree of injured tissues. In the current study total-SH levels were low in the control group and high in the dexmedetomidine group. To date, published papers have supported that dexmedetomidine increases the GSH level\(^{(35,39)}\).

In the present study, liver MDA (p=0.001), MPO (p=0.021) and total-SH (p=0.001) were found to be significantly different between the BDL+ dexmedetomidine and the BDL groups. Plasma total-SH (p=0.027) and MDA (p=0.012) values were also statistically different between these groups.

The statistical analyses of the histological activity index (HAI) scores showed that the histopathological damage in the BDL+ dexmedetomidine group was significantly less than the damage in the control group (p<0.05 for all pathological parameters) (Table 3). These results demonstrated that treatment with dexmedetomidine ameliorated the negative effects of obstructive jaundice on liver histology and oxidative stress parameters.

This study is just an experimental model on rats. The number of rats used in this study is low and there may be some possible alterations related to the anesthetic and surgical technique. These are the main weak points of the current study. Thus, prospective randomized clinical studies are needed for the use of dexmedetomidine in clinical practice for treatment of the patients with obstructive jaundice.

In conclusion, the results of this study demonstrate that dexmedetomidine possessed a significant hepatoprotective effect in the experimental obstructive jaundice model. It may be beneficial to improve the prognosis of patients with obstructive jaundice. It can be concluded that these effects of dexmedetomidine might be due to its antioxidant and anti-inflammatory activities, but further studies are needed to evaluate the exact mechanism of the hepatoprotective effect of dexmedetomidine.

References


