Egyptian Journal of Anaesthesia xxx (xxxx) xxx–xxx

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Egyptian Journal of Anaesthesia

journal homepage: www.elsevier.com/locate/egja

Research article

Dexmedetomidine protects against myocardial ischaemia/reperfusion-induced renal damage in rats

Osama M. Assad[a,*], Dina A. Aly Labib[b], Laila Ahmed Rashed[c]

[a] Department of Anesthesiology, Faculty of Medicine, Cairo University, Egypt
[b] Department of Medical Pharmacology, Faculty of Medicine, Cairo University, Egypt
[c] Department of Medical Biochemistery and Molecular Biology, Faculty of Medicine, Cairo University, Egypt

ARTICLE INFO

Keywords:
Dexmedetomidine
Ischaemia reperfusion
Anti-oxidant
Kidney
Rats

ABSTRACT

Background: Myocardial ischaemia/reperfusion (MI/R) may induce renal damage. Our aim was to investigate the effects of dexmedetomidine (DEX) administration at two different timings either before or after ischaemia on renal damage induced by MI/R.

Methods: MI/R injury was induced in a rat model. we ligated the left anterior descending coronary artery for 30 min (ischaemic period), then reperfusion occurred for 2 h (reperfusion period). A single dose of DEX (100 μg/kg) was given intraperitoneally, either 30 min before myocardial ischaemia or 5 min after reperfusion. With the end of reperfusion period, rats were sacrificed, then we collected the blood and removed both kidneys quickly for biochemical and histopathological analysis.

Results: MI/R caused an elevation in serum urea and creatinine, significant elevation in malondialdehyde (MDA) release and decrease in superoxide dismutase (SOD) activity in the rat kidney. There were also higher levels of serum tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). Treatment with dexmedetomidine, 30 min before induction of myocardial ischaemia, succeeded to improve all the tested parameters. The valuable changes in these biochemical parameters were linked with similar enhancement in the histopathological appearance of the kidney. Meanwhile, DEX given 5 min after reperfusion improved serum urea and creatinine only.

Conclusion: These findings imply that MI/R plays a fundamental role in kidney damage through increased production of oxygen radicals or deficiency in antioxidants, and DEX given before ischaemia exerts reno-protective effects probably by its radical scavenging antioxidant activity and anti-inflammatory mechanism.

1. Introduction

Among the common causes of death occurring perioperatively are the renal or cardiac injuries following cardiac surgery [1]. Lipid peroxidation, inflammatory reaction or oxidative stress following myocardial ischaemia/reperfusion (MI/R) may be leading causes of distant organs' damage after myocardial ischaemia [2]. An organ as the heart if exposed to severe ischaemia and then reperfused can affect a distant organ that was not exposed to the initial ischaemic event or cause multiple organ damage [3]. Because of its anatomical and unique structure, the kidney is considered a very sensitive organ affected easily by ischemia–reperfusion (I/R) [4]. Though, benefits of coronary revascularization or related techniques as thrombolysis or angioplasty may be life-saving from irreversible renal necrosis, still it is a double-edged sword because reperfusion may even augment renal damage [5].

The mechanisms behind the decline in renal function following coronary ischaemia then revascularization is most probably multifactorial and can be explained by decrease in renal blood flow, absence of perfusion in pulsatile manner, rupture of traumatized red blood cells (RBCs) or inflammatory reaction [6]. Also, apoptosis share in the pathophysiology of I/R insult. There is meaningful increase in the oxygen free radicals (FR) and reactive oxygen species (ROS) in the kidney. FR in turn initiate an inflammatory response. The most affected structures by the ROS are proteins, membrane lipids, and deoxyribonucleic acids. The endogenous antioxidant system includes enzymes as superoxide dismutase (SOD) and catalase which act to minimize the I/R insult [7]. Ongoing researches and studies are being developed to introduce new agents to alleviate organs' reperfusion-mediated insult. The anesthetic agents have impact on endogenous antioxidant systems and formation of free oxygen radical formation [8].

Dexmedetomidine (DEX) is a strong alpha 2 agonist. It is an anxiolytic which can be used pre-operatively as a preanaesthetic agent to

Peer review under responsibility of Egyptian Society of Anesthesiologists.
* Corresponding author at: Department of Anesthesiology, Faculty of Medicine, Cairo University, Kaseralini, Cairo, Egypt.
E-mail addresses: osamaasaad72@yahoo.com (O.M. Assad), labibd5@gmail.com (D.A. Aly Labib), Lailaahmedrashed@gmail.com (L. Ahmed Rashed).

http://dx.doi.org/10.1016/j.egja.2017.09.005
Received 14 August 2017; Received in revised form 9 September 2017; Accepted 28 September 2017

help reduction of the dose of anaesthetics [9]. It has anti-inflammatory potentials and cardioprotective effects [10]. Previous studies have demonstrated that dexmedetomidine could alleviate direct organ damage secondery to exposure to I/R in different animal groups and can decrease the deleterious effects of I/R insult [11,12]. But, no studies have investigated the effects of dexmedetomidine on indirect renal damage developping after myocardial I/R. The objective of our study was to evaluate if DEX can improve the remote kidney damage following myocardial ischaemia reperfusion and clarify its potential protective effects on MI/R-induced renal damage, and investigate whether the timing of its administration, 30 minutes before and 5 minutes after ischaemia play a role or not. Several parameters were assessed including biochemical measurements of kidney function (serum urea and creatinine) and assessment of its anti-oxidant effect using biochemical markers as tissue malondialdehyde (MDA) and SOD and investigated its anti-inflammatory effect by measuring serum TNF-α and IL-1β. Changes in the heart rate were recorded during different periods and histopathological examination of the renal tissue was also done.

2. Material and methods

2.1. Animal grouping

A total of 40 healthy male wistar rats weighing between 200–250 gm were housed in separate cages in temperature- adjusted room with 12 h. light/dark cycle. They were adapted to the new atmosphere for one week before experiment and all animals had free access to water. The study protocol was permitted by the Institutional Reviewer Board of Faculty of Medicine, Cairo University and the animal experiments were done in agreement with the ethical guidelines of animal welfare. We randomly divided them into five groups, 8 rats in each group. Group I: control group, received normal saline. Group II: sham-operated, where isolation of left anterior descending (LAD) was performed but with no ligation and the rats received only normal saline. Group III: (I/R, untreated) in which myocardial I/R was induced after thoracotomy, by ligating LAD for 30 min, then followed by deligation and reperfusion for 2 h. Group IV (DEX before): in which Dexmedetomidine (Precedex 200 μg/2 ml, Hospira®, Illinois, USA) was injected at a dose of 100 μg/Kg by intraperitoneal (I.P.) route 30 minutes earlier than induction of ischaemia [13,14]. Group V (DEX after): in which rats received DEX at a dose of 100 μg/Kg I.P after ischaemia (5 minutes from the beginning of reperfusion). The timing of giving DEX 5 min after reperfusion was taken from a previous study investigating its effect on renal ischaemia by Gonullu et al. [14].

2.2. Experimental design

2.2.1. Myocardial ischaemia reperfusion

Rats were anesthetized with 100 mg/kg of ketamine hydrochloride (sigma-Aldrich, Inc, Canada) I.P. A cannula was introduced in the trachea for positive-pressure ventilation using room air. All animals were artificially ventilated with a standard tidal volume ventilation protocol. After shaving the chest, it was opened through a midline incision, the pericardium was incised and a loose 6/0 braided prolene suture was placed around the left anterior descending coronary artery (LAD) for 30 min in groups III, IV and V to induce ischaemia. The ends of the suture were threaded through a propylene tube to form a snare, to facilitate the successive removal of the suture to start reperfusion for 120 min. The body temperature was maintained throughout the experiment by using a heating pad and heat lamps. Subcutaneous electrocardiogram (ECG) leads (Suzuken, Kenz - ECG-102) placed in the rat's limbs to allow measurement of heart rates [15].

2.2.2. Biochemical studies

Following 2 hours of reperfusion, blood was withdrawn. The whole blood was centrifuged at 3500 rpm for 15 min, then we separated the serum and stored it at -20 °C for further biochemical studies to measure urea, creatinine, TNF α and IL-1β. Rats were then sacrificed. The left kidney was instantly fixed with 10% neutral buffered-formalin solution for 2 h at 20–25 °C, dehydrated, then embedded in paraffin for further histopathological analysis. The right kidney was snap frozen at -80 °C and used for determination of tissue MDA and SOD.

2.2.2.1. Measurement of serum urea and creatinine. Serum urea and creatinine levels were estimated using a commercial kit in an autoanalyzer. The results were expressed as mg/dl.

2.2.2.2. Measurement of serum TNF-α and IL-1β. Serum levels of IL-1β and TNF-α were evaluated using enzyme-linked immunosorbent assay [ELISA] Kit (Biomed, Diepenbeek, Belgium), based on the manufacturer's instructions and the values were presented as pg/ml.

2.2.2.3. Measurement of tissue MDA. MDA levels in the renal tissue homogenate were determined spectrophotometrically according to the protocol of Van Ye et al. [16] using thiobarbituric acid reactive substances (TBARS) assay kit from Zepto Metrix Inc. (USA). Values were expressed as nmol/mg protein.

2.2.2.4. Measurement of tissue SOD. The activities of SOD in the renal tissue homogenate were determined spectrophotometrically as previously described by Xie et al. [17], with the use of commercial SOD assay kits (Nanjing jiancheng Bioengineering, China). The results were expressed as U/mg protein.

2.2.3. Histopathological examination

Kidney tissue samples were kept in 10% neutral buffered formalin, embedded in paraffin, sectioned and lastly stained by hematoxylin and eosin (H & E), according to Bancroft et al. [18]. The EGTI (Endothelial, Glomerular, Tubular, and Interstitial) scoring system is created exactly for animal research on renal tissue in the setting of injury (Table 1). The scoring system entails histological damage in four discrete components: Endothelial, Glomerular, Tubular, and Interstitial. EGTI scoring was applied in both the intact and injured parts of the renal cortex [19].

Table 1
The EGTI histology scoring system.

Tissue type	Damage	Score
Tubular	• No damage	0
	• Loss of Brush Border (BB) in less than 25% of tubular cells • Integrity of basal membrane	1
	• Loss of BB in more than 25% of tubular cells, Thickened basal membrane	2
	• (Plus) Inflammation, Cast formation, Necrosis up to 60% of tubular cells	3
	• (Plus) Necrosis in more than 60% of tubular cells	4
Endothelial	• No damage	0
	• Endothelial swelling	1
	• Endothelial disruption	2
	• Endothelial loss	3
Glomerular	• No damage	0
	• Thickening of Bowman capsule	1
	• Retraction of glomerular tuft	2
	• Glomerular fibrosis	3
Tissue type	Damage	Score
Tubulo/Interstitial	• No damage	0
	• Inflammation, Hemorrhage in less than 25% of tissue	1
	• (Plus) necrosis in less than 25% of tissue	2
	• Necrosis up to 60%	3
	• Necrosis more than 60%	4

EGTI: Endothelial, Glomerular, Tubular, and Interstitial.