Effect of Erythropoietin on Ischemia-Reperfusion Injury in Experimentally Induced Uremic Cardiomyopathy

Thesis
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by

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Introduction

Chronic heart failure and chronic kidney disease are serious medical conditions; emerging evidences indicate that the functions of these two organ systems are affected by each other in a complex interplay **(Zolty et al., 2008)**. Clinically, cardiac disease due to renal failure is called uremic cardiomyopathy and it is directly responsible for the extremely high morbidity and mortality seen in patients with end stage renal disease **(Kennedy et al., 2006)**.

Many factors have been proposed to be involved in the pathogenesis of uremic cardiomyopathy like the extracellular volume expansion, hyperparathyroidism, hypertension, and anemia (**Middleton et al., 2001)**. There is increasing evidence pointing to the important contribution of "nontraditional" risk factors in the etiology of cardiovascular diseases in chronic renal failure, including oxidative stress and inflammation. Chronic kidney disease is associated with a chronic imbalance of prooxidant and antioxidant factors that result in a state of chronic inflammation **(Pai & Conner, 2008)**.

However, the exact molecular basis of this uremic cardiomyopathy is still poorly understood **(Kennedy et al.,**

2006) and remains to be a target for investigation aiming to reach better therapeutic approach.

Recently, the discovery that erythropoietin (EPO) receptor is, also, expressed on non-haematopoietic cells such as endothelial cells, vascular smooth muscle cells, cardiac myocytes and neuronal cells, highlighted the role of EPO beyond haematopoiesis **(Paschos et al., 2008)**.

In vitro administration of erythropoietin to isolated perfused hearts confers cardio-protective effect during ischemia-reperfusion injury **(Wright et al., 2004)**, also, reduction in infarct size, apoptosis, oxidative stress, and inflammation have been reported (**Burger et al., 2009).** Besides, animal models of heart failure have shown that EPO can potentially reverse cardiac remodeling **(Prunier et al., 2007)** and improve myocardial contractility and relaxation **(Lipsic et al., 2008)**. Also, the increase in angiogenesis and the reduction in arrhythmias have been implicated in the cardio-protective effects of erythropoietin **(Burger et al., 2009)**.

It was therefore worthwhile to investigate the cardiovascular effects of erythropoietin on experimentally induced uremic cardiomyopathy.

Aim of the work

The present work was planned to study the cardiovascular functions in renal failure and the response of hearts with uremic cardiomyopathy (UCM) to ischemia-reperfusion injury. Also, to elucidate the possible role that could be played by erythropoietin in the protection against and/or treatment of uremic cardiomyopathy as a trial to alleviate cardiac consequences and complications associated with chronic renal failure. In addition, the possible underlying mechanisms of such effects could be highlighted.

Materials and Methods

Animals:

The present study was performed on 135 adult Wistar albino rats of both sexes initially weighing 160-220g. The rats were purchased from *Vacsera Animal House (Helwan)* as well as *Research Institute of Ophthalmology (Giza)* and were maintained in the *Physiology Department Animal House* under standard conditions of boarding and feeding. The given diet consisted of bread, milk and green vegetables with free access to water.

Experimental protocol:

1-To induce experimental cardiomyopathy, rats were subjected to 5/6 nephrectomy and were studied either after 6 weeks **(Smith et al., 2009)** or 12 weeks **(Ulu et al., 2013)**.

2-Therefore, rats included in the present study were allocated into:

I. 6 weeks Model:

Rats in this model were divided into the following groups:

a) 6 week sham-operated control group (n= 20)

Rats of this group were subjected to all surgical procedures of 5/6 nephrectomy except for removal of the kidney. This group was studied after 6 weeks.

b) 6 week uremic cardiomyopathy group (UCM) (n= 25)

Rats of this group were subjected to 5/6 nephrectomy for experimental induction of uremic cardiomyopathy **(Kennedy et al., 2006)** and were studied after 6 weeks.

c) 6 week uremic cardiomyopathy erythropoietin-treated group (UCM-Epo) (n=19)

Rats of this group were subjected to 5/6 nephrectomy for experimental induction of cardiomyopathy. Immediately after the operative intervention, rats of this group received intraperitonial (i.p.) injection of erythropoietin to explore the possible preventive or protective effect of Epo on the pathogenesis and development of cardiomyopathy. Rats of this group were studied after 6 weeks.

II. 12 weeks Model:

Rats in this model were further divided into the following groups:

a) 12 week sham-operated control group (n= 26)

Rats of this group were subjected to all surgical procedures of 5/6 nephrectomy except for removal of the kidney. This group was studied after 12 weeks.

b) 12 week uremic cardiomyopathy group (UCM) (n= 28)

Rats of this group were subjected to 5/6 nephrectomy for experimental induction of uremic cardiomyopathy and were studied after 12 weeks.

c) 12 week uremic cardiomyopathy erythropoietin-treated group (UCM-Epo) (n=17)

Rats were subjected to 5/6 nephrectomy for experimental induction of uremic cardiomyopathy. Six weeks after operative intervention and induction of cardiomyopathy, rats in this group received i.p. injection of Epo for another 6 weeks to clarify possible therapeutic effect of Epo on uremic cardiomyopathy, thus rats of this group were studied after 12 weeks.

Surgical Procedures

Five-sixths nephrectomy technique:

The procedure for 5/6 nephrectomy was done according to the technique described by **Addis and Lew (1940),** as modified by **Muchaneta-Kubara et al. (1995)** in the form of one stage operation**.**

Anaesthesia

On the day of the operation, anaesthesia was induced by placing the rat in a large jar containing cotton sprinkled with ether (VWR International Ltd, England). Throughout the whole period of the operation, anaesthesia was maintained using a mask containing cotton pad moistened with ether.

Steps:

1. After induction of anesthesia, the rat was fixed on the dissecting board in the prone position, and a dorsal midline incision about 2 cm long, extending from the tenth thoracic to the third lumbar vertebra, was made.
2. The skin was retracted laterally and a small hole through the thin muscle, just anterior to the upper pole of the

kidney, was made with a fine-pointed forceps. The forceps was opened to enlarge the hole.

3. The left kidney was delivered by pressure at the side of the wound. The adrenal gland was displaced upward and detached from upper pole of the kidney.

4. A loop was made, using a 2-0 chromic catgut suture, and positioned around each of upper and lower renal poles. The loop was strongly tightened till the renal pole is sectioned in a well defined plane, to provide hemostasis and minimize bleeding. Then, each pole was removed surgically by scissors.

5. Hemostasis was ensured by compression of the remnant part of the kidney using a piece of cotton wool for 2 minutes after surgical removal of the renal poles.

6. Then, a loop of chromic catgut was made around the pedicle of the right kidney and tightened, and then, the right kidney was excised.

7. The muscle wound was closed with one or two stitches, using 2-0 chromic catgut thread and a curved needle. The skin was closed with two or three stitches with silk sutures.

8. Asepsis was maintained during the operation and baneocin antibiotic powder (Bacitracin + Neomycin, Pharco pharmaceuticals Co., Egypt) was applied to the wound and daily till healing occurred.

In the sham-operated control group, rats were exposed to the same surgical manipulation except for 5/6 nephrectomy.

Erythropoietin treatment

Erythropoietin was supplied as Aranesp® (Amegen, Europe B.V.) containing the long acting analogue darbepoietin alfa (20µg in 0.5 ml). Stock solutions were prepared by diluting 0.1 ml of Epo in 9.9 ml distilled water making final concentration of 0.4 µg/ml. Epo was administered by i.p. injection in a dose of 0.4 µg/ kg/ 3 weeks(Each rat received 2 doses separated by 3 weeks) **(Lipsic et al., 2008).**

Experimental Studies:

Rats in all the studied groups were subjected to the following studies:

1- Measurement of body weight and body mass index.

2- Measurement of arterial blood pressure and heart rate.

3- Recording of the electrocardiogram ‘ECG’.

4- Collection of blood samples for determination of:

a- Hemoglobin content and hematocrite value
b- Plasma creatinine and urea
c- Plasma total antioxidant capacity

d- Plasma malondialdehyde level

5- In vitro study of isolated hearts perfused in a Langendorff preparation to record:

a- Intrinsic activity of the heart under baseline condition.

b- Responses of the heart during 30 minutes of reperfusion following 30 minutes of total global ischemia

6- Determination of cardiac weights

7- Assessment of cardiac tissue for:

a- Glutathione peroxidase enzyme activity

b- Malondialdehyde level

Experimental Procedure:

At the start of the study, the initial body weight and body mass index were determined in rats of all the studied groups.

On the day of sacrifice, overnight fasted rats were weighed and subjected to measurement of arterial blood pressure. Then injected intraperitoneally with 5000 IU/Kg B.W heparin sodium (Nile Company, Egypt). Fifteen minutes later, the rats were anaesthetized with i.p. injection of thiopental sodium (Sandoz, Austria), in a dose of 40 mg/kg B.W. When the stage of surgical anaesthesia had been reached as judged by loss of withdrawal

reflexes, the animal was placed on its back and fixed on the dissecting board and the length of the anaesthesized rat was measured.

Measurement of Arterial Blood Pressure and Heart Rate:

Determination of rat arterial blood pressure (systolic, diastolic and mean) as well as heart rate, was performed using the non invasive small animal tail blood pressure system (NIBP200A, Biopac systems Inc; USA).

Steps:

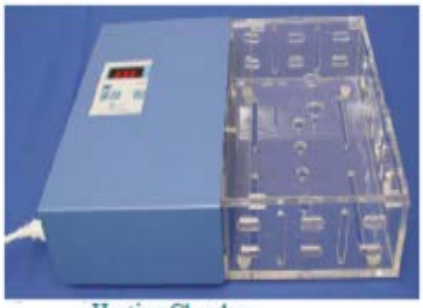
1- The animal heating chamber was switched on and the temperature was set to warm the animal tail to 32°C, a step needed for accurate non- invasive blood pressure measurement.

2- The animal was put inside the restrainer (animal holder) suitable to its size, with its tail outside. The length was adjusted to obtain a position where the animal had limited movement.

3- The restrainer with the animal were placed in the heating section of the animal heating chamber for 30 minutes in order that the animal reaches the selected temperature then removed.

4- The suitable IR SENSOR was selected and connected to the tail of the animal inside the restrainer, the IR SENSOR fitting to the tail between the midpoint of the tail and tail end.

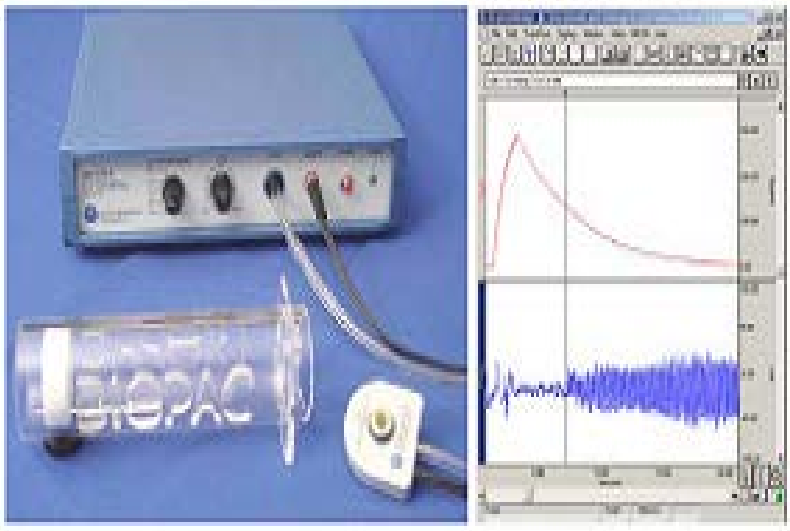
5- Measurements were not started till the animal was relaxed and inactive.



Heating Chamber

Restrainer Animal Holders

Tail Cuff/Sensor



Picture (5): Small animal tail blood pressure system (NIBP200A)

Recording

1- After launching the BIOPAC software, the IR SENSOR was calibrated for the pressure (A1) and pulse (A2) measurement.

2- By pressing the start button on the front panel of NIBP200A, the IR SENSOR pumped up the cuff automatically.

3- When the cuff pressure on A1 reached 30mmHg, the cuff pressure and tail pulse signals were generated, then the recording stopped automatically after 24 seconds.

4- Serial measurements were recorded and mean values were calculated.

Analysis

Calculation of systolic, diastolic and mean blood pressure

1- The A1 channel and the cursor I were selected, and then an area from the graphical display was selected, starting from the point of first pulse to the point of the maximum wave.

2- The maximum value (systolic pressure), minimum value (diastolic pressure) and the mean blood pressure (mean value) were reviewed from the label on the top of the A1 panel.

Calculation of heart rate

1-The A2 channel and the cursor I were selected, and then the maximum points of the peaks of the A2 pulse waveform were chosen.

2- The heart rate value was reviewed from the label on the top of the A2 panel.