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A study on the effect of cimetidine and L-carnitine on myoglobinuric acute kidney injury in male rats

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ABSTRACT

Myoglobinuric acute renal failure is the most important life threatening complication of rhabdomyolysis. Iron, free radicals, nitric oxide and cytochrome p450 are involved in the pathogenesis of mARF.

The aim of this study is to compare the effect of cimetidine, L-carnitine and both agents together on mARF in rats.

Forty rats were divided into 5 groups; group I: control rats, group II: myoglobinuric ARF rats, group III: mARF rats received L-carnitine (200 mg/kg, i.p.), group IV: mARF rats received cimetidine (150 mg/kg i.p.) and group V: mARF rats received both agents together. 48 h after glycerol injection, systolic blood pressure was measured. Urine and blood samples were collected to evaluate urine volume, GFR, BUN, creatinine, K, Na, serum creatine kinase, NO and glutathione levels. Kidney specimens were taken to investigate renal cytochrome p450 and for histological examinations.

Cimetidine treatment significantly decreased creatinine, BUN, K, Na, SBP and creatine kinase and increased GFR and urine volume compared to group II. L-carnitine exerted similar changes except for the effect on K and GFR. NO was significantly decreased, while renal glutathione and cytochrome p450 were significantly increased in groups treated with L-carnitine or cimetidine as compared to group II. Combined treatment further improved renal functions, creatine kinase, oxidative stress parameters and SBP as compared to each therapy alone. The histological changes confirmed the biochemical findings.

Cimetidine and L-carnitine have protective effects – almost equally – against mARF. Using both agents together, minimises the renal injury.

Introduction

Rhabdomyolysis is a common cause of ARF, especially at times of conflict or after major disasters when crush injuries are frequent. The outcome of rhabdomyolysis is usually good provided that it doesn't result in myoglobinuric acute renal failure (mARF) [1].

The pathophysiology of mARF has been studied extensively in animal model of glycerol-induced ARF. The main pathophysiological mechanisms are renal vasoconstriction, intraluminal cast formation, and direct heme-iron-induced cytotoxicity [2]. Oxidative stress has a key role in this pathogenesis [3].

Iron (Fe) has been implicated to play an important role in myoglobinuric acute kidney injury [4]. In vivo studies suggest that heme Fe causes proximal tubular lipid peroxidation and cytotoxicity, without invoking release of free iron, and this process is due to redox cycling of the heme group from ferrous to ferric and to ferryl oxidation states [5]. L-Carnitine is an anti-oxidant and prevents the accumulation of end-products of lipid peroxidation [6].

Studies indicate that cytochrome p450 may also be an important source of the catalytic iron. Inhibition of this enzyme by the use of cimetidine may decrease rhabdomyolysis-induced myoglobinuric nephrotoxicity [7]. Cimetidine binds to cytochrome p450 and forms a stable complex [8], through the binding of the imidazole ring structure of cimetidine to the haem moiety of cytochrome p450 [9].

This collective body of evidence suggests an important role for reactive oxygen metabolites in toxic acute renal failure and may provide therapeutic opportunities for the prevention or treatment of mARF in human.

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The present work was designated to study the effect of cimetidine (cytochrome p450 inhibitor), L-carnitine (antioxidant) and both agents together on myoglobinuric ARF in rats aiming to establish a mechanism that may aid as a prophylactic treatment.

Materials and methods

This study was carried out in strict accordance with the approved guidelines. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Faculty of Medicine Cairo University.

The present study was carried out in Physiology Department, Faculty of Medicine, Cairo University on forty adult male Wistar Albino rats weighting 150–200 g. They were housed singly in wire mesh cages at room temperature (25 °C with 55% relative humidity), normal light and dark cycle and were allowed to acclimatise to their environment and to the blood pressure measurement procedures every day for 5 days before the beginning of the experiment. Veterinary care was provided by the Laboratory Animal House Unit of Faculty of Medicine, Cairo University. All animals had a free access to laboratory chow[1] and tap water.

The animals were divided into five groups, eight rats each. **Group I, (Control group)** served as normal control reference values for the measurements evaluated. They were injected with normal saline (10 ml/kg, i.m.). **Group II, (ARF group)** were injected with 50% glycerol[2] (10 ml/kg, i.m.) with no further treatment. **Group III, (ARF + L-carnitine group)** were injected with glycerol (10 ml/kg, i.m.) and L-carnitine[3] (200 mg/kg, i.p.) concomitant with, and 24 h after glycerol injection [6]. **Group IV, (ARF + cimetidine group)** were injected with glycerol (10 ml/kg, i.m.) and cimetidine[4] (150 mg/kg, i.p.) concomitant with glycerol injection [7]. **Group V,** (ARF + L-carnitine + cimetidine group) were injected with glycerol (10 ml/kg, i.m.) and L-carnitine (200 mg/kg, i.p.) concomitant with and 24 h after glycerol injection, with cimetidine (150 mg/kg, i.p.) concomitant with glycerol injection. All groups were water deprived 24 h before saline (control group) or glycerol (groups 2–5) injection [6].

Urine was collected separately for each rat, using special cages similar to those described by Demirkan and Melli [10], for 24 h, starting 24 h after glycerol injection. Forty-eight hours after glycerol injection, rat tail systolic blood pressure was measured by the Power Lab at Physiology Department, Faculty of Medicine, Cairo University. This system is an electronic version of the traditional sphygmomanometer cuff method [11].

The rats were anaesthetised and blood samples were withdrawn through retro-orbital route using capillary tubes [12] and serum was separated and stored at −70 °C until used. The serum was used for determination of serum creatinine, blood urea nitrogen, Na, K, creatine kinase, glutathione (GSH), and nitric oxide (NO) levels. At the end of experiment, rats were culled using chloroform inhalation and tissue samples from the right kidneys were dissected and kept frozen at −80 °C in liquid nitrogen until they were used to measure cytochrome p450 content in the kidney tissue. Tissue samples from the left kidneys were dissected and fixed in 10% formalin—buffered solution for histological examinations. Blood and tissue samples were collected 48 h after glycerol injection.

[1] Laboratory chow ingredients (g/kg): Carbohydrates: Corn starch 480, Sucrose 100, Fats: Soybean oil 50, animal fat 120, Protein: Casein 190.
[2] Glycerol was prepared in the Biochemistry Department Faculty of Medicine Cairo University.
[3] L-Carnitine was obtained from Mepaco Co., Egypt in the form of ampoules, each ampoule contains 1 g/5 mL.
[4] Cimetidine was obtained from Sigma Co., Alorich in the form of powder.

Biochemical studies

The biochemical studies were held at the Biochemistry Department, Faculty of Medicine, Cairo University.

Creatinine was estimated by QuantiChromTM creatinine Assay Kit [13]. Determination of GFR was done by calculating the creatinine clearance using the following equation:

$$\text{Creatinine clearance} = \frac{u}{p} \times v$$

where u = urinary concentration of creatinine (mg/100 ml); p = plasma concentration of creatinine (mg/100 ml); v = urine volume (ml/min).

Serum urea was estimated by QuantiChromTM Urea Assay kit (DIUR-500) [14]. Serum Na and K were estimated by Sodium and Potassium Enzymatic Assay Kit (Liquid Stable) [15,16]. Serum creatine kinase was estimated by Enzy ChromTM Creatine Kinase Assay Kit (ECPK-100) [17]. Nitric oxide was determined in serum according to the method of Miranda et al. [18]. Glutathione was determined in serum according to the method of Beutler et al. [19]. Cytochrome p450 in kidney tissues was measured by ELISA kit supplied by Uscn Life Science Inc.

Histological examination

The histological studies were done at the Histology Department, Faculty of Medicine, Cairo University. Sections were taken from the kidneys of the different groups' rats and fixed in 10% formalin buffered saline solution. Paraffin wax tissue blocks were prepared for sectioning at 5–7 μm using Leica rotator microtome (Germany). The obtained tissue sections were stained by Hematoxylin and Eosin stains for histological examination through the light microscope [20].

To evaluate renal damage, counting the affected glomeruli and the affected tubules was done in 10 serial non overlapping fields (at magnification of ×200) for each specimen for all the experimental groups. Values were calculated as percent of total of 100 glomeruli, or 200 cortical tubules for each specimen. Results were tabulated and statistically analyzed.

Glomeruli were counted as affected according to presence of: segmentation of capillary tuft, narrowing or obliteration of Bowman's space, mesangial expansion, proliferation of parietal layer of Bowman's capsule, distortion, or shrinkage of glomerulus.

Tubules were counted as affected according to presence of: abnormal staining or cytoplasmic vacuolation of lining epithelium, darkened nuclei, detached epithelium, or presence of casts inside lumen.

Statistical methods

Data were coded and entered using the statistical package SPSS version 15. Data was summarised using mean, standard deviation and range for the quantitative variable. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non-parametrical Kruskal–Wallis test and Mann–Whitney test were used for non-normally distributed quantitative variables [21]. *P*-values less than 0.05 were considered as statistically significant.

Results

Study of Renal functions in the experimental groups

Intra muscular hypertonic glycerol injection induced a deterioration of glomerular and tubular kidney functions as compared to

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