A Study on the Effect of Cimetidine on Myoglobinuric Acute Kidney Injury in Male Rats

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Abstract

Introduction: Rhabdomyolysis-induced myoglobinuric acute renal failure accounts for about 10-40% of all cases of acute renal failure (ARF). Iron and Cytochrome P450 are involved in the pathogenesis of myoglobinuric ARF.

The Aim of this Study: To explore the effect of cimetidine as a potent cytochrome P450 inhibitor on myoglobinuric ARF in rat.

Methods: Twenty four rats were divided into 3 groups; Group 1: Control rats. The remaining rats were injected with 50% glycerol (10ml/kg, i.m.) and were divided into 2 groups: Group 2: Untreated myoglobinuric ARF, Group 3: Received cimetidine (150mg/kg i.p) concomitant with glycerol injection. Forty eight hours later blood samples were collected to evaluate BUN and serum creatinine. Kidney specimens were taken to investigate cytochrome P450 in the kidney tissue and for histopathological examination.

Results: Cimetidine treatment significantly decreased serum creatinine and BUN as compared to group 2. Cytochrome P450 was significantly increased in the group treated with cimetidine as compared to group 2. The histological changes confirmed the biochemical findings.

Conclusion: Cimetidine has a protective effect against myoglobinuric renal failure most probably through blocking the loss of cytochrome P450 which is an important source for the injurious catalytic iron.

Key Words: Myoglobinuric acute kidney injury – Catalytic iron – Cimetidine – Cytochrome P450.

Introduction

Rhabdomyolysis is a potentially life-threatening syndrome of skeletal muscle breakdown with leakage of muscle contents. The outcome of rhabdomyolysis is usually good provided that there is no renal failure. Whether traumatic or non-traumatic rhabdomyolysis, acute kidney injury represents the most important life threatening complication.

The true incidence of acute kidney injury in rhabdomyolysis is difficult to establish owing to varying definitions and clinical scenarios. The reported incidence ranges from 13% to approximately 50% [1].

Acute renal failure (ARF) is a syndrome characterized by an acute loss of renal function. Despite the reversibility of the loss of renal function in most patients who survive, the mortality of ARF remains high (over 50%) [2]. Acute tubular necrosis (ATN) is the most common form of ARF [3].

Rhabdomyolysis-induced myoglobinuric acute renal failure accounts for about 10-40% of all cases of acute renal failure [4].

Iron (Fe) has been implicated to play an important role in several models of tissue injury, including myoglobinuric acute kidney injury [5]. In vivo studies suggest that heme Fe causes proximal tubular lipid peroxidation and cytotoxicity, thereby contributing to the pathogenesis of myoglobinuric acute kidney injury [6]. It seems that oxidative stress has a key role in this pathogenesis [7].

Cytochrome P450 is one enzyme that is involved in iron-related acute renal failure. Inhibition of this enzyme may decrease rhabdomyolysis-induced myoglobinuric nephrotoxicity [4].

The present work was designed to study the effect of cimetidine (cytochrome P450 inhibitor) on myoglobinuric ARF in rats with the aim of establishing a mechanism that may aid as prophylactic treatment.

Material and Methods

Experimental animals:

Twenty four adult male albino rats weighting 150-200gm were housed in wire mesh cages at
room temperature. Veterinary care was provided by Laboratory Animal House Unit of Faculty of Medicine, Cairo University from 2013–2014. Rats were housed with normal light and dark cycle and were allowed to acclimatize to their environment for five days before the start of the experiments. All animals were kept under the same environmental conditions and had free access to food and water.

The animals were divided into three groups, 8 rats each:

- **Group 1** (n=8): (Control group), these are normal rats served as normal control reference values for the measurements evaluated. They were injected by normal saline.
- **Group 2** (n=8): (ARF group), rats of this group were injected with 50% glycerol (10mL/kg, i.m.) after 24 hours water deprivation [8] with no further treatment. Glycerol was prepared in the Biochemistry Department Faculty of Medicine, Cairo University.
- **Group 3** (n=8): (ARF + cimetidine group), rats were injected with glycerol (10mL/kg, i.m.) plus cimetidine (150mg/kg, i.p.) concomitant with glycerol injection [1]. Cimetidine was obtained from Sigma Co., Alorich in the form of powder.

Blood samples were withdrawn through retro-orbital route using capillary tubes and serum was separated and stored at –70°C until used. The serum was used for determination of serum creatinine and blood urea nitrogen. Then the animals were sacrificed by cervical dislocation and tissue samples from the right kidneys were dissected and kept frozen at –80°C in liquid nitrogen until used to measure cytochrome P-450 content in the kidney tissue. Tissue samples from the left kidneys were dissected and fixed in 10% formalin-buffered solution for histological examination. Blood and tissue samples were collected 48 hours after glycerol injection.

**Measurement of serum and urine creatinine:**
Creatinine was estimated by Quanti Chrom TM creatinine Assay Kit [9].

**Measurement of blood urea nitrogen:**
Serum urea was estimated by Quanti Chrom TM Urea Assay kit (DIUR-500) [10].

**Measurement of cytochrome P450:**
It was measured by ELISA kit supplied by Uscn Life Science Inc.

**Histological examination:**
At the end of experiment, rats were sacrificed. The histological study was done at the Histology Department, Faculty of Medicine, Cairo University. Sections were taken from the kidney of rats in different groups and fixed in 10% formalin buffered saline solution. Paraffin wax tissue blocks were prepared for sectioning at 5-7 microns using Leica rotator microtome (Germany). The obtained tissue sections were stained by Hematoxylin and Eosin stains for histological examination through the light microscope [11].

**Statistical methods:**
Data were coded and entered using the statistical package SPSS version 15. Data was summarized 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 Kruscal-Wallis test and Mann-Whitney test were used for non normally distributed quantitative variables [12]. p-values less than 0.05 were considered as statistically significant.

**Results**

**The effect of hypertonic glycerol injection and administration of cimetidine on renal functions:**
IM hypertonic glycerol injection resulted in a significant loss of kidney functions as compared to control group. As shown in Table (1), the mean values of serum creatinine and BUN showed significant increases in ARF group (group II) as compared to the values in the control group (group I). Treatment of rats with ARF with cimetidine induced significant decreases in the mean values of serum creatinine and BUN as compared to ARF group (group II), however, the values of these parameters still exhibited significant changes when compared to control animals.

**The effect of hypertonic glycerol injection and administration of cimetidine on cytochrome P450 level in the kidney tissues as compared to the control group:**
As shown in Table (2), the value cytochrome P450 content in the kidney tissue showed a significant decrease in ARF group (group II) as compared to control group (group I). The value of cytochrome P450 content in the kidney tissue was significantly increased in the cimetidine treated group as compared to ARF group (group II), while its mean value remained deviated significantly as compared to the control group (group I).
**Histological Study**

Fig. (1): From control group shows: 1A: Normal glomerular tuft of capillaries and normal Bowman's space. 1B: Normal & regular lining epithelium of tubules with acidophilic staining & vesicular nuclei. (H&E x630).

Fig. (2): From ARF group shows: 2A: Shrinkage of whole size of glomerulus, with massive destruction & shrinkage of glomerular tuft (arrow) with loss of capillary lumen & widening of Bowman's space (*). 2B: Shows loss of tubular lining epithelium (*), with presence of many ballooned apoptotic cells with dark nuclei (arrows), & a large casts in one of the tubules. (H&E x630).

Fig. (3): From ARF + Cimetidine group shows: 3A: Marked preservation of glomerular capillary tuft, with only mild shrinkage & consequent widening of Bowman's space. 3B: Marked preservation of vesicular nuclei of tubules, together with significant congestion (arrows). (H&E x630).
Table (1): Comparison between means ± SD of serum creatinine (mg/dl), and BUN (mg/dl), levels in all the studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group (I)</th>
<th>Group (II)</th>
<th>Group (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine</td>
<td>0.1±0.1</td>
<td>1.3±0.33*</td>
<td>0.62±0.16#</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>28.35±4.05</td>
<td>74.17±8.02*</td>
<td>46.7±5.41 *#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.
*: Statistically significant compared to corresponding value in group (I) (p<0.05).
#: Statistically significant compared to corresponding value in group (II) (p<0.05).

Table (2): Comparison between mean ± SD of cytochrome P450 content in the kidney tissue (nmol/mg ptn) in all the studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group (I)</th>
<th>Group (II)</th>
<th>Group (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450</td>
<td>1.35±0.27</td>
<td>0.24±0.06*</td>
<td>0.58±0.06*#</td>
</tr>
<tr>
<td>content in the kidney</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>tissue (nmol/mg ptn)</td>
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Values are represented as mean ± SD.
*: Statistically significant compared to corresponding value in group (I) (p<0.05).
#: Statistically significant compared to corresponding value in group (II) (p<0.05).

Discussion

Rhabdomyolysis is a well-known clinical syndrome of muscle injury associated with myoglobinuria, electrolyte abnormalities, and often acute kidney injury (AKI). Myoglobin has been identified as the primary muscle constituent contributing to renal damage in rhabdomyolysis [13].

Induction of myoglobinuric acute renal failure (mARF) in the rats that received an intramuscular injection of hypertonic glycerol was demonstrated by the severe deterioration of the renal functions as compared to the control group. Significant increases in the serum creatinine and blood urea nitrogen were noticed as compared to the control group. Histological findings also confirmed the renal injury.

In accordance with our results, Singh et al. and Kaya et al. reported an increase in blood urea and creatinine levels in mARF [14,15].

Additionally our study demonstrated that cytochrome P-450 in kidney tissue was significantly decreased in mARF group as compared to the control group.

In accordance with our results, Baliga et al. demonstrated a marked decrease in the cytochrome P-450 content in a similar animal model [5].

Rhabdomyolysis resulted in acute tubular necrosis and increased labile iron levels in the kidneys of rats [5]. A concomitant reduction in cytochrome P-450 enzymes in the kidney led the investigators to conclude that degradation of these heme-containing mono-oxygenases might have contributed to increased labile iron levels in the kidney [16,17].

Iron plays an important role in the pathophysiology of tissue injury in the absence of systemic iron overload. Critical to iron’s importance in biologic processes is its ability to cycle reversibly between its ferrous and ferric oxidation states. This precise property, which is essential for its functions, also makes it very dangerous because free iron can catalyze the formation of free radicals, which can damage macromolecular components of the cell [18].

Although iron is the most abundant transitional metal in the body, labile, or catalytic, iron, which can be measured by several methods [19], constitutes only a small fraction of the total iron pool. Labile iron was originally defined as a transitional pool between extracellular and cellular iron and was generally associated with low-molecular-weight chelates [18].

From a pathophysiologic standpoint, however, an iron pool that can participate in redox cycling is important and is therefore often referred to as catalytic iron. There are two broad lines of evidence for the role of catalytic iron in the pathophysiology of disease: That it is increased in disease states and that iron chelators provide a protective effect, thereby establishing a cause-effect relationship. This has been demonstrated in a variety of disease states, including acute and chronic kidney disease, acute myocardial infarction, and neurodegenerative disorders. Thus, its role in disease processes seems to be a common theme of cellular injury [20].

On studying the effect of cimetidine treatment on renal functions, ARF + cimetidine group (group 3) showed improvement in the measured renal function parameters as compared to ARF group (group 2). Also, histological examination showed a preservation of renal structure.

Similarly, a study by Najafzadeh et al. [2] showed the protective effect of cimetidine on mARF and the improvement of renal functions in the same rat model.
In an attempt to study the mechanism of this beneficial effect of cimetidine, our study showed a significant increase in the mean values of renal cytochrome P-450 as compared to mARF group (group 2).

Cimetidine has imidazole and cyano groups that inhibit cytochrome P-450 by interacting with the heme moiety [21], which represents an important source for iron during cell and tissue injury [5,22,23]. The effect of cimetidine is specific for cytochrome P-450 as it does not interact with other heme enzymes [24].

In previous studies, it was observed that cimetidine, but not ranitidine, significantly prevented the increase of bleomycin-detectable iron in the kidneys of glycerol-treated rats [8] and that cytochrome P-450 was inhibited by both low (100mg/kg) and high dose (150mg/kg) of cimetidine [2].

Conclusion:

It can be concluded from this study that rhabdomyolysis resulted in deterioration of renal functions and decrease cytochrome P450 in kidney tissues. Cimetidine prophylactic treatment provided partially protective role against mARF. This protective effect might be through blocking the loss of cytochrome P450 which is an important source for injurious catalytic iron.

Future studies are recommended to compare the effect of the use of cimetidine against the effect of the nowadays used guidelines and to explore if inducing this drug to the guidelines would add benefit. Also, transitional studies on human are recommended.

References

21- RENDIC S., KAJFEZ F. and RUF H.H.: Characterization of cimetidine, ranitidine, and related structures \"interaction

