A Study on the Effect of L-Carnitine on Myoglobinuric Acute Kidney Injury in Male Rats

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Abstract

Background: Rhabdomyolysis is frequently observed in patients admitted to the ICU. Rhabdomyolysis-induced myoglobinuric acute renal failure is one of the most common causes of death in this condition. Free radicals and nitric oxide are involved in the pathogenesis of myoglobinuric ARF.

Aim of the Study: To study the effect of L-carnitine as an antioxidant on mARF in rats.

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: Group 2: Myoglobinuric ARF, group 3: Received L-carnitine (200mg/kg, i.p.) concomitant with and 24 hours after glycerol injection. Forty eight hours later blood samples were collected to evaluate BUN, creatinine, NO and glutathione levels. Kidney specimens were taken for histological examination.

Results: The group treated by L-carnitine showed significantly decreased levels of serum creatinine and BUN as compared to ARF group. NO was significantly decreased, while renal glutathione was significantly increased in the group treated with L-carnitine as compared to ARF group. The histological changes confirmed the biochemical findings.

Conclusion: L-carnitine has a partial protective effect against myoglobinuric renal failure. This protective effect may be due to the antioxidant functions of L-carnitine.

Key Words: Rhabdomyolysis – Acute kidney injury – Oxidative stress – L-carnitine.

Introduction

ACUTE kidney injury (AKI) is a clinical syndrome of acute loss of renal functions. Despite its increasing incidence and high morbidity and mortality, no specific treatment is currently available [1]. One of the important causes of AKI is rhabdomyolysis which may result from trauma, exertion, body temperature changes, electrolyte disorders, some genetic metabolic disorders, some drugs and toxins, infections and sometimes idiopathic [2].

To understand the pathophysiology of rhabdomyolysis induced AKI, an animal model using intramuscular glycerol injection is studied [3]. Although not fully clear, experimental data demonstrated renal VC as a characteristic feature. Direct heme-protein cytotoxicity and myoglobin casts are involved as well. Myoglobin is a scavenger of NO and generates reactive oxygen species resulting in VC [2].

L-Carnitine is an anti-oxidant and prevents the accumulation of end-products of lipid peroxidation [4].

The important role of reactive oxygen species in toxic acute renal failure may provide therapeutic opportunities for preventing or treating acute renal failure in humans.

The present work was designed to study the effect of L-carnitine (antioxidant) on myoglobinuric ARF in rats with the aim of establishing a mechanism that may aid as prophylactic treatment.

Material and Methods

Twenty four adult male Wistar albino rats weighting 150-200gm were housed singly in wire mesh cages at room temperature (25 °C with 55% relative humidity). 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 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. All groups were water deprived 24 hours before saline (control group) or glycerol (group 2&3) injection [4].

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 (10mL/kg, i.m.).

Group 2 (n=8): (ARF group), rats of this group were injected with 50% glycerol *(1 0mL/kg, i.m.) with no further treatment.

Group 3 (n=8): (ARF+L-carnitine group), rats were injected with glycerol (10mL/kg, i.m.) plus L-carnitine (200mg/kg, i.p.) concomitant with and 24h after glycerol injection [4].

*Glycerol was prepared in the Biochemistry Department Faculty of Medicine, Cairo University.

L-carnitine was obtained from Mepaco Co., Egypt in the form of ampoules, each ampoule contains 1g/5ml.

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, blood urea nitrogen, glutathione level and NO level. Then animals were sacrificed and 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.

Biochemical study:

The biochemical study was held at the Biochemistry Department Faculty of Medicine, Cairo University.

Creatinine was estimated by QuantiChromTM creatinine Assay Kit [5]. Serum urea was estimated by QuantiChromTM Urea Assay kit (DIUR-500) [6]. Nitric oxide was determined in serum according to the method of Miranda et al., [7]. Glutathione was determined in serum according to the method of Beutler et al., [8].

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 [9].

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 [10]. p-values less than 0.05 were considered as statistically significant.

Results

Study of renal functions in the experimental groups:

IM Hypertonic glycerol injection induced a deterioration of kidney functions as compared to control group. Table (1) demonstrates that the value of serum creatinine and BUN showed significant increases in ARF group (group II) as compared to control group (group I).

Treatment of rats with ARF with L-carnitine induced significant decreases in the value 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.

Study of oxidative stress in the experimental groups:

Table (2) presents that the value of serum NO level showed a significant increase while glutathione level showed significant decreases in ARF group (group II) as compared to control group (group I). The value of serum NO level showed a significant decrease while glutathione level was significantly increased in the L-carnitine treated group as compared to ARF group (group II), while the value of these parameters remained deviated significantly as compared to control group (group I).
Table (1): Comparison between mean ± SD of serum creatinine (mg/dl) and BUN (mg/dl) in all 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>0.66±0.13 *#</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>28.35±4.05</td>
<td>74.17±8.02*</td>
<td>48.22±4.03*#</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).

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 shrinkage & of capillary tuft & obliteration of lumen (arrow), marked widening of Bowman's space (*), & large cast in tubule. (2B): Shows loss of tubular architecture (*), with presence of many ballooned apoptotic cells with dark nuclei (arrows). (H&E x630).

Fig. (3): From ARF + L carnitine group shows: (3A): Preservation of size of glomerulus, with partial preservation of the capillary tuft (arrow). (3B): Preservation of organization and vesicular nuclei of tubules despite vacuolations (arrows). Size of casts remaining in tubules is also markedly reduced (*). (H&E x630).

Table (2): Comparison between mean ± SD of serum NO (umol/ml) and glutathione level (mmol/ml) in all 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 NO (umol/ml)</td>
<td>0.18±0.03</td>
<td>1.01±0.21 *</td>
<td>0.64±0.1 *#</td>
</tr>
<tr>
<td>Glutathione level</td>
<td>53.29±2.51</td>
<td>22.74±5.09*</td>
<td>34.27±3.46*#</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).
Discussion

In the present study, rats of the ARF group (group II) received an intramuscular injection of hypertonic glycerol (50%) 10ml/kg body weight equally divided into both hind limbs after 24 hours of water deprivation. Then, 48 hours after glycerol injection, blood samples were collected by retro orbital route. Renal functions were severely deteriorated in this group as compared to the control group. A significant increase in the serum creatinine and blood urea nitrogen was noticed as compared to the control group (group I).

The increase in blood urea and creatinine levels in ARF group is consistent with previous studies performed on this model [11-14].

On studying the effect of L-carnitine on renal functions, we found ARF+L-carnitine group (group 3) showing a significant decrease in BUN and serum creatinine as compared to ARF group while the level of these parameters remain significantly higher as compared to control group (group I).

Similar to our study, Aydogdu et al., and Ustandag et al., stated that L-carnitine treatment ameliorated renal functions (serum urea and creatinine) as compared to glycerol induced myoglobinuric acute kidney injury [4,15].

Also, rats of the myoglobinuric acute renal failure group showed a significant decrease in serum GSH level as compared to control group. Similar to our results, a significant decrease in GSH has been reported in experimental studies on mARF [4,16].

However, no significant decrease in GSH level was noted by Oktay et al., studying the same animal model, and, as they reported, this may be a result of low number of samples studied due to high mortality in this group [14].

Hanly et al., reported that GSH depletion induces LPO and ultimately cell lyses. Replenishing the GSH level is, therefore, necessary for the maintenance of the overall thiol status in the cell [17]. Indeed, GSH concentration closely correlated with the degree of renal failure [18]. Depletion in GSH by itself could contribute to the progression of uremia because it has been demonstrated that GSH depletion in rats leads to an acute renal failure [19].

In addition, our study demonstrated that there was a significant increase in the serum NO level in mARF group as compared to the control group. Other studies showed no significant difference in NO levels [14]. Gök found a decrease in renal NO levels studying the same animal model [20].

Various animal studies have demonstrated that induction of inducible nitric oxide synthase (iNOS) leads to excessive production of nitric oxide (NO) and subsequent formation of peroxynitrite that causes renal injury [22-24].

On the otherhand, our study demonstrated that administration of L-carnitine in group 3 induced a significant decrease in NO, and a significant increase in serum GSH as compared to untreated mARF group (group 2).

It is well accepted that oxidative stress is one of the mechanisms involved in the pathogenesis of acute renal injury [28]. L-carnitine (β-hydroxy-γ-N-trimethylammonium-butyrate) is a vital component for the production of ATP through the β-oxidation of long-chain fatty acids in lipid metabolism. L-carnitine has been shown to have antioxidant and protective effects against oxidative damage in different organs or tissues, including the kidney [15,26-30].

Conclusion:

L-carnitine has a partial protective effect against myoglobinuric renal failure. This protective effect may be due to the antioxidant functions of L-carnitine.

Future studies are recommended to check the effect of the use of L-carnitine-added to or replacing some of the nowadays used guidelines—would add benefit. Also, transitional studies on human are recommended.

References


Hassan Eissa, et al.


المختصر العربي

مقدمة البحث: كثيراً ما لوحظ زيادة الميoglobين في الدم في المرضى الذين يتم إدخالهم إلى وحدة العناية المركزية. الفشل الكلوي الحاد الناجم عن زيادة الميoglobين في الدم في واحدة من أكثر الأسباب شيوعًا للوفاة في هذه الحالة. وتشترك أسباب الأكسجين الحرّة وأكسيد النتريك في السبب في الفشل الكلوي الحاد الناجم عن زيادة الميoglobين في الدم.

الهدف من هذه الدراسة: دراسة تأثير L-كارنيتين (مضاد للأكسدة) على الفشل الكلوي الحاد الناجم عن زيادة الميoglobين في الدم.

طريقة إجراء البحث: تم تقسيم أربع عشرين فأرا إلى 3 مجموعات.

- مجموعة 1: الفئات الضابطة.

- مجموعة 2: مجموعة الفشل الكلوي الحاد الناجم عن زيادة الميoglobين في الدم دون علاج.

- مجموعة 3: الفئات المضادة لـ L-كارنيتين (100 ملغ/كجم) بالتزامن مع 48 ساعة بعد حقن الجليسرول.

بعد ثمانية وأربعين ساعة تم جمع عينات الدم لقياس مستويات الالوريا، والكوارتئين، وأكسيد النتريك والجلوتاتثين في الدم. كما أخذت عينات من الكلى لفحص النسيج.

النتائج: أظهرت المجموعة المعالجة بالـ L-كارنيتين انخفاضاً ملحوظاً بشكل كبير في مستويات الالوريا والكوارتئين في الدم بالمقارنة مع مجموعة الفشل الكلوي الحاد الناجم عن زيادة الميoglobين في الدم دون علاج. تم انخفاض أكسيد النتريك بشكل ملحوظ في جميع المجموعات التي تلقى العلاج بالـ L-كارنيتين بالمقارنة مع مجموعة الفشل الكلوي الحاد الناجم عن زيادة الميoglobين في الدم دون علاج. وأدت النتائج السجية تناقص الكيمياء الحيوية.

الخلاصة: L-كارنيتين لعب دوراً مهمًا في تقليل التأثيرات السامة لزيادة السمنتار في الدم. هذه النتائج تقدّم راحة إلى وظائف L-كارنيتين الضامة للأكسدة.