Protective Role of L-Carnitine and Tocopherol Against Cold Restraint Stress Induced Gastric Lesions In Streptozotocin–Diabetic Rats

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ABSTRACT

In the present study, the influence of cold restraint stress (CRS)-induced gastric damage in diabetic rats, in relation to the antioxidative system was investigated. Male albino rats were used in the study, they were divided into 5 groups: i): non stressed group, ii): control CRS group, iii) diabetic CRS group (the rats of this group were injected with streptozotocin (STZ) 70 mg/kg i.p. and used 4 weeks after induction of diabetes with blood glucose levels of >350 mg/dl), iv): STZ L-carnitine pretreatment group (STZ-induced diabetic rat of this group were given L-carnitine 500 mg/kg 30 min before CRS) and v): STZ-vitamin E pretreatment group (STZ-induced diabetic rat of this group were given vitamin E 60 mg/kg body wt three weeks before CRS). The last four groups were exposed to CRS, at the end of each experiment, gastric damage was observed macroscopically. CRS induced gastric lesion, that was markedly exacerbated in STZ diabetic rats, but this aggravation was significantly suppressed by pretreatment with either L-carnitine or tocopherol (vitamin E) pretreatments. Diabetic rat stomachs showed significantly less glutathione peroxidase (GPX) activity as well as reduced glutathione (GSH) content than normal rat stomachs. In addition, the deleterious influence of diabetes on the gastric ulcerogenic response to CRS was significantly mitigated by decreasing lipid peroxidation by pretreatment with either L-carnitine or vitamin E. These results suggest that the gastric mucosa of diabetic rats is more vulnerable to cold restraint–induced injury, and the mechanism may be partly accounted for by impairment of the antioxidative system associated with a reduced GPX activity and GSH content. Based on these data, the beneficial effects of L-carnitine and vitamin E on CRS-induced mucosal injury especially in diabetics may be attributed to their antioxidative effects.

INTRODUCTION

Peptic ulcer is one of the common diseases affecting man. Stress, ingestion of alcohol, aspirin are predisposing factors. Diabetes mellitus is a chronic disease characterized by hyperglycemia and by complications that include microvascular diseases and a variety of neuropathies. Experimental studies show that prolonged diabetic conditions have deleterious influences on various functions in the gastrointestinal tract(1,2). Indeed, recent studies showed an increased mucosal susceptibility to various ulcerogenic stimuli in STZ–induced diabetic rats, an accepted model of insulin dependent diabetes(3).
However, the mechanism underlying the increased mucosal susceptibility in diabetic rats has not yet been elucidated. Reactive oxygen species (ROS), such as superoxide radical and hydroxyl radical, are known to directly or indirectly cause tissue damage\(^4\). These molecules are also involved in the pathogenesis of gastric lesions observed after CRS\(^5\). Several studies showed that the persistence of hyperglycemia causes increased production of ROS, through glucose autooxidation and nonenzymatic glycation, suggesting an increased oxidative stress in diabetic animals\(^6\). It has also been shown that the increased oxidative stress in diabetic conditions is caused not only by an accelerated production of ROS but also, by a decreased scavenging ability of those molecules\(^7\). L-carnitine is a small water-soluble molecule important in mammalian fat metabolism. It is essential for normal oxidation of fatty acids by the mitochondria and it is involved in trans-esterification and excretion of acyl-CoA esters, the oxidation of branched chain \(\alpha\)-ketoacids, and removal of potentially toxic acyl carnitine esters from mitochondria\(^8\). It is known that L-carnitine and its derivatives prevent the formation of ROS and protect cells from preoxidative stress\(^9\)\(^-\)\(^11\).

Vitamin E is a naturally occurring antioxidant in the biological system. It was postulated that vitamin E is more mobile and less restricted in its interaction with lipid radicals in the membrane than other antioxidants\(^12\). The biological activity of vitamin E is believed to be due to its antioxidant action to inhibit lipid peroxidation in biological membrane by scavenging the peroxyl chain reaction\(^13\).

The present study aims at demonstrating the increased susceptibility of gastric mucosa to CRS-induced gastric damage in diabetic rats, and investigating the underlying mechanism of protection exerted by L-carnitine and vitamin E, especially in relation to the endogenous anti-oxidative system.

**MATERIALS & METHODS**

**Animals**

Adult albino rats weighing 200-250 g were used throughout the present study. Rats were housed at room temperature with 12/12 hr light-dark cycle, and left for two weeks to acclimatize to the laboratory conditions. The animals were fed standard rat chow and tap water *ad libitum*. Rats were randomly classified into the following groups, (8 rats each):

I- Non-stressed control group: in which rats were left freely wandering in their cage at room temperature.

II- Cold Restraint Stressed Group (CRS): in which each rat was restraint by fixing the four limbs to a wooden board and placed in a refrigerator at 4° C for three hrs, the door of the refrigerator was opened every 15 min for inspection and oxygenation\(^14\).

III- Cold Restraint Stressed Diabetic Group: in which the rats were given streptozotocin (70 mg/kg body weight i.p. in citrate buffer, 0.05 M pH 4.5), after overnight fasting\(^15\). Successful induction of diabetes was confirmed by testing
blood glucose levels with Accu-Check active glucose strips three days after STZ injection. Only diabetic rats having fasting blood glucose level >350mg/dl were included in this study. Four weeks later, rats were subjected to CRS.

IV- L-carnitine Pretreatment Diabetic CRS group: in which STZ-diabetic rats were treated with L-carnitine 500 mg/kg intragastrically 30 minutes prior to induction of CRS(16).

V- Vitamin E Pretreatment Diabetic CRS group: in which diabetic rats were subjected to oral supplement of vitamin E at 60 mg/kg body weight for three weeks prior to CRS induction(17).

Three hours after CRS exposure, rats were decapitated, their stomachs were removed, opened along their greater curvature. Each stomach was rinsed in ice-cold saline and scored for macroscopic mucosal lesions. Gastric mucosa was then scrapped over ice and stored at -20º C till used for determination of nitrates, lipid peroxides, reduced glutathione and glutathione peroxidase.

Measurements and Assays:

I. Assessment of gastric mucosal lesion: this was expressed in term of ulcer index (UI) according to the method of Robert et al(18).

II. Determination of gastric mucosal lipid peroxide: Gastric mucosal malonaldehyde (MDA), as an index of lipid peroxidation, was assayed by the Thiobarbituric acid described by Okhawa et al(19).

III. Determination of nitric oxide in gastric mucosa: Nitric oxide in gastric mucosa was determined using enzyme immunoassay kits for total nitric oxide assay for the quantitative determination of total nitrates NO3-(20).

IV. Determination of reduced glutathione in gastric mucosa: this is performed by colorimetric method described by Beutler et al(21).

V. Determination of glutathione peroxidase (GPX) in gastric mucosa: Gastric mucosa GPX was determined using Cayman chemical GPX assay kits(22).

All chemicals were purchased from Sigma, St. Louis, USA.

Statistical analysis of data:

Data were presented as means ± SE of mean (M±SEM) and were analyzed using Student's "t" test. The statistical significance between two means was considered significant at p value ≤ 0.005, (23).

RESULTS

Blood glucose levels in STZ-diabetic rats

Blood glucose levels under non-fasting conditions were increased after STZ injection, reaching significantly high at one week (384.5±7.9 mg/dl) as compared to basal Values (141.5±5.2 mg/dl) and remained significantly elevated for 4 weeks thereafter. Normal rats receiving saline showed stable blood glucose levels during the test period. Subsequent treatment of STZ-CRS group with either L-carnitine 500 mg/kg or vitamin E (60 mg/kg body weight) produced insignificant changes in blood glucose level, compared to SZT-induced diabetic rats, Table (1).
Table 1: Changes in blood glucose levels in STZ-diabetic rats

<table>
<thead>
<tr>
<th>Group/parameter</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control non stressed</td>
<td>141±5.2</td>
</tr>
<tr>
<td>Cold restraint stressed</td>
<td>120±5.8</td>
</tr>
<tr>
<td>STZ cold restrained</td>
<td>385±7.0º</td>
</tr>
<tr>
<td>L-carnitine STZ cold restrained</td>
<td>380±6.9</td>
</tr>
<tr>
<td>Vit.E STZ cold restrained</td>
<td>382±8.1</td>
</tr>
</tbody>
</table>

Data represent means ± SEM of observation from 8 rats per group.
STZ: Streptotozocin
º: Significant difference from cold restraint group P≤0.05

Effect of L-carnitine and vitamin E on gastric mucosal lesions development induced by CRS in STZ diabetic rats:

CRS induced ulcerative lesions in rats achieving an UI of 21.25 in CRS. The UI was more aggravated in STZ rats achieving an UI 22.6.
Pretreatment of STZ diabetic rats with either L-carnitine 500 mg/kg or vitamin E (60 mg/kg body weight) attenuated ulcerative lesion induced by CRS and attained UI of 16.5 and 13.29 respectively, Table (2).

Table (2): Effect of L-carnitine and vitamin E on ulcer profile in STZ diabetic rats exposed to CRS

<table>
<thead>
<tr>
<th>Group /Parameters</th>
<th>% incidence</th>
<th>MSS</th>
<th>MUS</th>
<th>UI</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>--</td>
</tr>
<tr>
<td>Non diabetic CRS</td>
<td>100</td>
<td>3.5</td>
<td>7.75</td>
<td>21.25</td>
<td>....</td>
</tr>
<tr>
<td>STZ- CRS</td>
<td>100</td>
<td>3.6</td>
<td>9.00</td>
<td>22.6</td>
<td>-36.97</td>
</tr>
<tr>
<td>L-carnitine STZ-CRS</td>
<td>100</td>
<td>2.0</td>
<td>4.5</td>
<td>16.5</td>
<td>22.35</td>
</tr>
<tr>
<td>Vitamin E STZ-CRS</td>
<td>100</td>
<td>1.29</td>
<td>2.0</td>
<td>13.29</td>
<td>27.34</td>
</tr>
</tbody>
</table>

STZ: Streptozotocin induced diabetes CRS: cold restraint stress
MSS: Mean Severity Score MUS: Mean Ulcerative Score
UI : Ulcer Index PI : Preventive Index

Effect of L-carnitine and vitamin E on gastric mucosal GSH & GPX content in STZ diabetic rats exposed to CRS:

Gastric mucosal GSH levels were significantly decreased in all rats subjected to CRS compared to control group (P≤0.05). Either L-carnitine 500 mg/kg or vitamin E (60 mg/kg body weight) pretreatment prevented the CRS-induced reduction GSH level of both normal and STZ diabetic rats. Gastric mucosal GPX levels were also, significantly decreased in all rats subjected to CRS compared to control group (P≤0.05). Either L-carnitine 500 mg/kg or vitamin E (60 mg/kg body weight) pretreatment prevented the CRS-induced reduction GPX level of STZ diabetic rats (P≤0.05), Table (3).
Table (3): Effect of L-carnitine and vitamin E on gastric mucosal GSH & GPX content in STZ diabetic rats exposed to CRS:

<table>
<thead>
<tr>
<th>Group/parameters</th>
<th>GM-GSH (mg/g tissue)</th>
<th>GM-GPX (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control non stressed</td>
<td>26.1±1.3</td>
<td>7.09±0.68</td>
</tr>
<tr>
<td>Cold restraint stressed</td>
<td>19.3±0.7º</td>
<td>5.22±0.22º</td>
</tr>
<tr>
<td>STZ cold restrained</td>
<td>23.5±1.2º</td>
<td>4.22±0.49º</td>
</tr>
<tr>
<td>L-Carnitine STZ CRS</td>
<td>24.6±0.6•</td>
<td>6.84±0.36•</td>
</tr>
<tr>
<td>Vit. E STZ cold restrained</td>
<td>25.1±1.2•</td>
<td>6.85±0.40•</td>
</tr>
</tbody>
</table>

Data represent means ± SEM of observation from 8 rats per group. STZ: Streptotozocin. GM: gastric mucosa. GSH: reduced glutathione. GPX: glutathione peroxidase. º Significant difference from normal control group P≤0.05. • Significant difference from STZ diabetic group P≤0.05.

Effect of L-carnitine and vitamin E on gastric mucosal nitrate & MDA content in STZ diabetic rats exposed to CRS:

Gastric mucosal nitrate levels were significantly decreased in all rats subjected to CRS compared to control group (P≤0.05). Either L-carnitine 500 mg/kg or vitamin E (60 mg/kg body weight) pretreatment prevented the CRS-induced reduction of nitrate level of STZ diabetic rats (P≤0.05). Gastric mucosal MDA levels were significantly increased in all rats subjected to CRS compared to control group (P≤0.05). Either L-carnitine 500 mg/kg or vitamin E (60 mg/kg body weight) pretreatment prevented the CRS-induced increase MDA level of STZ diabetic rats (P≤0.05), Table (4).

Table (4): Effect of L-carnitine and vitamin E on gastric mucosal nitrate & MDA content in STZ diabetic rats exposed to CRS:

<table>
<thead>
<tr>
<th>Group/parameters</th>
<th>GM-nitrate levels (mg/g tissue)</th>
<th>GM-MDA (pg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control non stressed</td>
<td>31.8±1.5</td>
<td>45.5±4.1</td>
</tr>
<tr>
<td>Cold restraint stressed</td>
<td>20.3±1.9º</td>
<td>53.7±5.2º</td>
</tr>
<tr>
<td>STZ cold restrained</td>
<td>19.5±0.8º</td>
<td>60.7±3.2º</td>
</tr>
<tr>
<td>L-Carnitine STZ CRS</td>
<td>21.4±1.0•</td>
<td>50.1±2.2•</td>
</tr>
<tr>
<td>Vit. E STZ cold restrained</td>
<td>29.5±1.9•</td>
<td>48.6±1.9•</td>
</tr>
</tbody>
</table>

Data represent means ± SEM of observation from 8 rats per group. STZ: Streptotozocin. GM= gastric mucosa. MDA= Malondialdehyde. º Significant difference from normal control group P≤0.05. • Significant difference from STZ diabetic group P≤0.05.
DISCUSSION

CRS has been generally used for evaluation of anti ulcer activities in rats because of its reproducibility\(^\text{[14]}\). The pathogenic mechanism responsible for stress-induced gastric mucosal lesion depends mainly on reduced gastric blood flow and subsequently induces the production of free radicals\(^\text{[24]}\). This toxic effect was evidenced by the increase in gastric mucosal lipid peroxides (MDA) and decreased in the antioxidant defense system (GSH, GPX) as found in the present study.

The present study showed that CRS induced gastric lesions were aggravated in STZ-induced diabetic rats, a finding which is in agreement with previous findings of Tashima et al.\(^\text{[25]}\) that diabetes increases the mucosal susceptibility to ulcerogenic stimuli and predisposition to gastric ulceration. The present study, also, showed that the aggravation of these lesions in STZ-diabetic rats is associated with a depressed antioxidative system, including a decrease in GPX activity and GSH content in the gastric mucosa.

STZ is known to possess diabetogenic properties and cause selective destruction of pancreatic β-cells. As expected, all STZ-treated animals developed a persistent hyperglycemia, which was observed four weeks after STZ injection. Although, CRS provoked hemorrhagic damage in both CRS-control rats and STZ-diabetic rat stomachs, the severity was much greater in the latter. The exacerbation of these lesions in diabetic rats could be attributed to a decrease in gastric mucosal blood flow with subsequent decrease in the production of NO\(^\text{[26]}\) and increased production of free radicals as illustrated in the present results.

The present work showed that L-carnitine restored the decreased NO in response to CRS of STZ-diabetic rats which is in accordance with the previous results\(^\text{[27&28]}\). Also, the current results showed that vitamin E supplementation reversed the decreased NO in STZ-diabetic rats exposed to CRS which is in agreement with the previous investigations\(^\text{[29&30]}\).

Oxygen-derived free radicals are known to play a major role in the pathogenesis of CRS-induced damage in the gastrointestinal tract\(^\text{[31]}\). This contention is mainly based on the observation that free radicals scavengers such as GPX and GSH attenuate the micro vascular damage associated with stress exposure and play a role in maintaining the mucosal integrity by counteracting oxygen-derived free radicals\(^\text{[32]}\). On the other hand, several studies showed that the persistence of hyperglycemia causes increased production of ROS, though glucose auto oxidation and nonenzymatic glycation, suggesting an increased oxidative stress in diabetic animals\(^\text{[33]}\). The increased oxidative stress in diabetic conditions may be caused not only by an accelerated production of ROS, but, also, by decreased scavenging ability of those molecules\(^\text{[7,27&34]}\).

Indeed, it has been reported that GPX activity and GSH levels were decreased in various organs of diabetic animals, such as kidney,
Moreover, Goldin et al. \( ^{36} \) reported that the occurrence of gastric lesions in STZ-diabetic rats after starvation was related to the mucosal GSH depletion.

The current study showed that CRS–induced gastric lesions were markedly aggravated in STZ-diabetic rats. This aggravation in diabetic rats was significantly antagonized by either oral L-carnitine or vitamin E pretreatment. It could be assumed that aggravation of CRS-induced gastric lesions in diabetic rats is, at least partly, due to the impairment of the antioxidative system. Indeed, a marked reduction in mucosal GPX activity and GSH content was observed in STZ-diabetic rat stomachs. One explanation as to why the decreased antioxidative system is impaired in diabetic rat stomachs might be the enhanced non-enzymatic glycation of antioxidant protein and the high consumption of antioxidants caused by increased oxidative stress in diabetic conditions\(^ {37} \).

The relationship between reduced GPX activity and increased ulcerogenic response to CRS was, also, supported by the experiment performed by Tashima et al\(^ {25} \) who reported that the inhibition of superoxide dismutase (SOD) by diethyldithiocarbamate significantly reduced the mucosal SOD activity and markedly worsened the gastric ulcerogenic response to ischemia/reperfusion in normal rats.

Carnitine is a vitamin like substance that is structurally similar to amino acids. Most carnitine is obtained from diet. It can be synthesized endogenously by skeletal muscle, heart, liver, kidney, and brain from the amino acids glycine and methionine. L-carnitine and its derivatives have several important intracellular functions\(^ {8} \). Since the carnitine system, which consists of carnitine, carnitine esters, several specific intracellular enzymes and membrane transporters, plays an important role trafficking of short-, medium-, and long-chain fatty acids. Indeed, the carnitine system is involved in many functions: 1) utilization of substrate for energy production; 2) lipid peroxidation at peroxisomal level; 3) acylation and deacylation of protein at endoplasmic reticulum level; 4) membrane phospholipids turnover and 5) maintenance of cell osmotic balance\(^ {8} \).

The antioxidative and/or free radicals scavenging of L-carnitine have been proven in many previous studies\(^ {96,10} \). The present study was undertaken to evaluate the antiulcer activity of L-carnitine. The gastroprotective effect of L-carnitine observed in the current study could possibly be mediated through its well-known antioxidant potential. The present study demonstrated that L-carnitine attenuated CRS-induced gastric mucosal injury in STZ diabetic rats, and significantly inhibited the increase in MDA production which is an index of lipid peroxidation.

The results of the present study also, demonstrated that pretreatment of rats with vitamin E markedly reduced gastric mucosal damage induced by stress. The high gastric MDA content in the stressed non diabetic and STZ diabetic stomachs supports the hypothesis that stress-
induced injury is mediated by lipid peroxidation process. This indicates that ROS and lipid peroxidation is important in the pathogenesis of gastric mucosal injury induced by stress. The present investigation, also, showed that vitamin E decreased the breakdown of gastric mucosal barrier by reducing the product of lipid peroxidation (MDA) and increasing the antioxidant defense system (GPX & GSH). The reduced MDA levels accompanying the improved gastric lesion in these groups suggest that vitamin E probably reduced injury by retarding the lipid peroxidation process.

In conclusion, the present results demonstrated that diabetic conditions increased the vulnerability of the gastric mucosa to CRS-induced damage. The current data revealed that the phenomenon was attributed, at least, in part to a loss of GPX activity as well as GSH content in the gastric mucosa. It is assumed that diabetic conditions may cause an impairment of the antioxidative defense mechanism, leading to an increase of the mucosal susceptibility to oxidative stress injury as induced by CRS. The protective effect of L-carnitine and vitamin E is related to a decrease in lipid peroxidation and prevention in gastric GSH and GPX activity reduction produced by the harmful effect of stress. Hence, L-carnitine and vitamin E may be promising antiulcer drugs in peptic ulcer therapy especially in patients who are diabetics and get peptic ulceration.

REFERENCES


دور حماية ماتي آل-كارنيتيون و فيتامين ه ضد آفة الغشاء المخاطي لمعدة الفئران البيضاء المستحثة تجاربًا بعقار الستيرويودوتين بعد تعرضهم للأجهزة بالبريد مع التثبيت

د/ صلاح الدين عزیز السيد و/ و د/ مصمسى السفقر
قسم الفسيولوجى والكيمياء الحيوى - كلية الطب - جامعة المنيا

يفيد البحث أنه دراسة دور حماية ماتي آل-كارنيتيون و فيتامين ه من آفة الغشاء المخاطي لمعدة الفئران البيضاء المصابة بالبلاو الرأسي (المستحث تجارب) بعقار الستيرويودوتين 20 مجم/كلج من وزن الجسم عند تعرضهم للأنواع بالبريد. لقد تم اختبار الفئران المصابة بالبلاو الرأسي التي وصل مستوي السكر في الدم إلى أكثر من 300 مجم/كلج. لقد تم قسم الفئران إلى خمسة مجموعات: مجموعة ضابطة لم تتعرض لشيء، مجموعة تعرضت إلى الأجهزة بالبريد مع التثبيت، مجموعة تعرضت إلى الأجهزة بالبريد من غيرها، مجموعة تعرضت إلى الفئران التي تعرضت للبريد وتم إعطاؤها مادة آل-كارنيتيون 50 مجم/كلج، و مجموعة تعرضت إلى الفئران التي تعرضت للبريد وتم إعطاؤها فيتامين ه 10 مجم/كلج من وزن الجسم عن طريق الفم لمدة ثلاثة أسابيع قبل تعرض للأجهزة بالبريد مع التثبيت. أحدث الأجهزة بالبريد مع التثبيت إلى زيادة ذات دالة إحصائية لآفة الغشاء المخاطي لمعدة الفئران المصابة بالبلاو الرأسي مقترنة بفقدان المجموعة الضابطة وقد تضاعفت هذه الزيادة في متوسط المجموعة الضابطة إذا تم إعطاء أيا من ماتي آل-كارنيتيون أو فيتامين ه للفئران المصابة بالبلاو الرأسي. قبل تعرضهم للأجهزة بالبريد مع التثبيت. إن مستوي الأزم الدماغي للأكسدة (الجولوناتون والروتيناتون براكسيداز) في الغشاء المخاطي لمعدة الفئران المصابة بالبلاو الرأسي كانت أقل من نظريةها في المجموعة الضابطة بنسبة ذات دالة إحصائية. إن إعطاء أيا من ماتي آل-كارنيتيون أو فيتامين ه للفئران المصابة بالبلاو الرأسي قد أضعف التأثير الضار للمرض أو الجلود بالبريد مع التثبيت عن طريق تقليل الأمد الفوقية للدهنات. يمكن أن يعتبر هذا التأثير على الغشاء المخاطي لمعدة الفئران المصابة بالبلاو الرأسي معرفة للجرح عند تعرضها للأجهزة بالبريد مع التثبيت. وهذا يعود إلى ضعف الجهاز المناعي للأكسدة في مادة تصل في مستوي الجولوناتون والروتيناتون براكسيداز. ذلك يتيح أن يكون كلا من ماتي آل-كارنيتيون و فيتامين ه في مهارة الغشاء المخاطي ضد الأجهزة بالبريد مع التثبيت خاصة عند الأساندة نداء البلاو الرأسي يرجع إلى تأثيرهما المحاذ.