Protective Action of Antioxidants on the Remote Effects of Renal Ischemia-Reperfusion Injury

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Abstract: Many studies indicate that the production of reactive oxygen species (ROS) after renal ischemia / reperfusion (I/R) may initiate a cascade of cellular injury locally and in remote organs. It has been demonstrated that preconditioning by natural antioxidants (garlic, vitamin E, Vitamin A + Selenium) may attenuate the damage induced by ROS to remote organs. However, the effect of any of these natural antioxidants differed from the others. On the basis of these results it may be postulated that the combination of these three antioxidants may provide synergistic protection.

Key words: Antioxidants, renal ischemia, renal reperfusion, Catalase, Superoxide dismutase.

INTRODUCTION

Renal ischemia-reperfusion (I/R) contributes to the development of ischemic acute renal failure (ARF) and acute tubular necrosis in renal transplantation where I/R of the kidney directly influence graft and patient survival (Shirli, 2003). However, studies in humans and animal models have demonstrated that acute kidney injury (AKI) has a significant effect on the function of extra-renal organs (Grigoryev, 2008).

Multi-factorial processes are involved in the development and progression of renal I/R injury. The generation of reactive oxygen species, (Rah, 2007) nitric oxide and peroxynitrite (Yu, 1994) and the decline of endogenous antioxidant protection play major roles, (Turgut, 2008) leading to dysfunction, injury and death of the cells of the kidney. Concerning remote affects, inflammation products, involving cytokine/ adhesion molecule cascades with recruitment, activation, and diapedesis of circulating leukocytes added to ROS, NOS were also implicated (Meldrum, 2002). However, the cellular mechanisms involved in the development of renal I/R injury have been targeted by several pharmacological interventions by antioxidants anti-inflammatory agents and others (Chatterjee, 2007). The Aim of this experimental study was to verify the protective effect of natural antioxidants (garlic, Vitamin E and Vitamin A + Se2−) on the remote effects of renal I/R on liver and heart as regards the oxidant-antioxidant status of these organs.

Materials:

Five groups of male albino rats (200-240g.) were the material of the present study. Each group was formed of 10 rats. All rats were maintained under standard laboratory conditions and were allowed to have food (formed of wheat and milk) and had access to water and libitum.

Group I:

Sham operated control group.

Group II:

Renal (left kidney) ischemia – reperfusion (I/R) was performed as previously described: (Walker et al. 2001) the left renal pedicle was clamped by non traumatic clamp for 60 minutes, followed by right kidney nephrectomy under pentobarbital sodium (20 mgKg−1) intraperitoneally (I/P).
After clamp removal, the muscles and skin were sutured and the rats were allowed to recover. After 24hs rats were sacrificed by cardiac paracenthesis under ether anesthesia. Blood was collected in heparinized centrifuge tubes.

**Group III:**
Rats received in diet 80 mg kg\(^{-1}\) garlic powder (Sekum Co, Cairo – Egypt) daily for 1 month before I/R as group II.

**Group IV:**
Rats received Vitamin E (6.5 mg Kg\(^{-1}\)) fed with diet for 1 month before I/R.

**Group V:**
Rats received a combination of Vitamin A (7.9 mg Kg\(^{-1}\)) and Selenium (Se\(^{2+}\)) (50 Mg Kg\(^{-1}\)) for 1 month before I/R.

Plasma was separated, the heart and liver were excised, decapsulated, washed with ice cold saline and then homogenized in phosphate buffered saline (PBS).

Urea and plasma creatinine were determined for all rats using available commercial kits. The plasma and tissue homogenates were used also for the measurement of oxidative stress parameters:
1. Lipid peroxidation end products: Malondialdehyde (MDA) using thiobarbituric acid reaction (9).
2. Reduced Glutathione (GSH) (Griffith, 1980).
3. Oxidized glutathione (GSSG) (Griffith, 1980).

Also antioxidant enzymatic activities were assayed.
5. Glutathione peroxidase (GP-x) (Flohe, 1984).
7. Protein concentration in tissue homogenates was also determined (Lowry, 1951).

**Statistical Analysis:**
Data were analyzed using SPSS program for windows version 9. Unpaired t-test was used for comparison between each two studied groups. The results were considered significant at p<0.05. Also Pearson coefficient for correlation studies was used to find the significance of correlation between the parameters (Nie, 1995).

**Methods:**
Five groups of male albino rats were classified as follows.
1. Sham-operated group.
2. I/R group
3. Garlic group (garlic powder administered in diet for one month before I/R)
4. Vitamin E group like group 2 but adding vitamin E.
5. Vitamin A + Se\(^{2+}\) group, like group 2 but vitamin A + Se\(^{2+}\) were added to diet.

Rats were sacrificed 24 hours after I/R. Blood, liver and heart tissues were taken to determine: Blood urea, plasma creatinine, Malondialdehyde (MDA), reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations, and the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) enzymes in plasma, liver and heart homogenates and redox potential of plasma (RP).

**Results:**
Ischemic reperfused (I/R) animals demonstrated marked renal injury manifested by deterioration of renal function. Remote injury (liver, heart) shown by marked oxidative stress, increased liver and heart enzymes, MDA in plasma. MDA was also increased in tissue homogenates accompanied by decrease in tissue antioxidant enzymes and GSH concentration. Pretreatment of animals with garlic, vitamin E or vitamin A + Se\(^{2+}\) attenuated the oxidative stress and decreased blood urea and plasma creatinine. However the effect of each antioxidant on the studied parameters was not similar.
1. Renal function as measured by blood urea and plasma creatinine after I/R was markedly increased compared with sham operated rats (2.13 folds for the first parameter and 4.57 folds for the second). MDA was markedly increased in plasma, liver and heart for I/R groups compared with sham operated groups (2.1 folds for plasma, 1.5 for liver and 14.8 folds for heart). However garlic, Vitamin E Vitamin A + Se\(^{2+}\)
partially ameliorated the affected kidney function and oxidative stress marker “MDA” in plasma (Table I). Administration of garlic did not however, affect the MDA concentration of heart, also Vitamin E and Vitamin A + Se²⁺ did not significantly decrease the MDA of liver (Table II, III).

2. I/R also injured the liver and heart cells as manifested by increased AST, ALT, LDH and CKMB enzyme activities. However, the natural antioxidants given, partly improved the cell injury of these organs. (Tables II, III).

3. I/R increased GSSG concentration and GSH-Px activities in plasma, heart and liver compared to that in sham operated groups. These dates were accompanied with decrease of GSH concentration, enzyme activities of catalase, GR enzymes of plasma, liver and heart and insignificant change of SOD activity.

4. Depending on glutathione levels, the redox state of the plasma was also affected. This latter was calculated as redox potential (RP) by Nernest equation, it showed deviation towards oxidizing environment in I/R group. (Table I).

5. The natural antioxidants (garlic, Vitamin E, & Vitamin A + Se²⁺) ameliorated this oxidant derangement manifested in plasma, liver and heart. However the effect of these antioxidants on the oxidant – antioxidant markers was not equal (Tables I-III, Fig 5-7).

6. Correlation studies showed significant positive one between MDA and creatinine concentration in plasma of I/R group $r = 0.708, P=0.022$). Also there was negative correlation between blood GSH and blood urea concentrations in I/R group ($r =-0.724, p=0.018$), also negative correlation ($r =-0.657, p=0.039$) between GSH-Px and LDH of plasma in I/R group, and between GSH-Px and GR of the liver of I/R group ($r =-0.703, p=0.023$) (Fig 1-4).

**Fig. 1:** Correlation between MDA and creatinine concentrations in plasma of IR groups.

**Fig. 2:** Correlation between blood GSH and blood urea in IR groups.
Fig. 3: Correlation between LDH and GSH-Px in plasma of IR groups.

Fig. 4: Correlation between GR and GSH-Px in liver of IR groups.

Fig. 5: The effect of I/R on the level of malonaldehyde (MDA) nmole/ml in the plasma of the studied group.

*1. Data are presented as mean ± S.D.
2. See tables for significance.
3. N = 10 for each group.
Fig. 6: The effect of I/R on the level of malonaldehyde (MDA) nmole/ml in the liver of the studied group.

1. Data are presented as mean ± S.D.
2. See tables for significance.
3. N = 10 for each group.

Fig. 7: The effect of I/R on the level of malonaldehyde (MDA) nmole/mg protein in the heart of the studied group.

1. Data are presented as mean ± S.D.
2. See tables for significance.
3. N = 10 for each group.

Table 1. The effect of natural antioxidants on renal I/R as reflected in plasma antioxidant status of rats

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control</th>
<th>I/R</th>
<th>I/R + garlic</th>
<th>I/R + Vit E</th>
<th>I/R + Vit A and Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea mg/dl</td>
<td>18.6 ± 1.1</td>
<td>52.6 ± 1.0</td>
<td>55.9 ± 0.60</td>
<td>59.9 ± 0.89</td>
<td>49.9 ± 0.27</td>
</tr>
<tr>
<td>Plasma creatinine mg/dl</td>
<td>0.49 ± 0.06</td>
<td>3.55 ± 0.03</td>
<td>1.19 ± 0.039</td>
<td>0.74 ± 0.006</td>
<td>1.43 ± 0.04</td>
</tr>
<tr>
<td>MDA nmole/ml</td>
<td>1.51 ± 0.067</td>
<td>3.18 ± 0.309</td>
<td>2.08 ± 1.204</td>
<td>2.36 ± 0.724</td>
<td>2.06 ± 1.208</td>
</tr>
<tr>
<td>GSH nmole/ml</td>
<td>3.75 ± 0.076</td>
<td>2.48 ± 0.794</td>
<td>1.28 ± 0.704</td>
<td>1.26 ± 0.694</td>
<td>1.29 ± 0.704</td>
</tr>
<tr>
<td>GSHS nmole/ml</td>
<td>0.21 ± 0.006</td>
<td>0.35 ± 0.013</td>
<td>0.24 ± 0.008</td>
<td>0.18 ± 0.006</td>
<td>0.24 ± 0.008</td>
</tr>
<tr>
<td>GSH-Pt /mml</td>
<td>14.3 ± 0.523</td>
<td>31.4 ± 0.889</td>
<td>7.04 ± 2.468</td>
<td>7.0 ± 0.799</td>
<td>14.5 ± 0.59</td>
</tr>
<tr>
<td>GR unit</td>
<td>3.63 ± 0.132</td>
<td>9.70 ± 0.875</td>
<td>3.35 ± 0.187</td>
<td>3.4 ± 0.193</td>
<td>3.02 ± 0.10</td>
</tr>
<tr>
<td>CAT Unit</td>
<td>0.7 ± 0.08</td>
<td>0.17 ± 0.008</td>
<td>0.33 ± 0.041</td>
<td>0.25 ± 0.006</td>
<td>0.44 ± 0.008</td>
</tr>
<tr>
<td>SOD Unit</td>
<td>934 ± 583</td>
<td>934.19 ± 131</td>
<td>937 ± 218</td>
<td>934.84 ± 138</td>
<td>961 ± 962</td>
</tr>
<tr>
<td>FR nmol/ml</td>
<td>154 ± 564</td>
<td>132 ± 389</td>
<td>141 ± 556</td>
<td>146 ± 74</td>
<td>146 ± 739</td>
</tr>
</tbody>
</table>

Data presented as mean ± S.D. *P < 0.05 for each group.
**Discussion:**

In the present study animals that were subjected to I/R, exhibited significant increase in blood urea and creatinine compared to sham operated rats, denoting some degree of renal dysfunction. These results are in agreement with previous work (Sener, 2005). Treatment with natural antioxidants (garlic, Vitamin E and Vitamin A + Se⁵) decreased both urea and creatinine concentrations in blood. Tissues subjected to a period of ischemia undergo morphological and functional changes that increase during reperfusion phase (Singh, 2004; Kadkhodaei, 2004). Among many other factors, excessive production of reactive O₂ species (ROS) outstripping endogenous antioxidant defense mechanisms has been implicated in oxidant stress process in which these latter, oxidize biological macromolecules such as DNA, proteins, carbohydrates and lipids (Lien, 2003). I/R injury can affect any organ exposed to it as for example, the kidney, myocardium, nervous system, liver, bowel, testis and lung (Singh, 2004; Kaiserova, 2006; Li, 1999). Also, studies in humans and animals have demonstrated that acute I/R of kidney has a significant injurious effects not only on the kidney but also on other extra-renal organs as for example the liver (Fadillioglu, 2008). Similarly I/R of skeletal muscle and lower extremities cause both local damage and serious dysfunction to remote organs including lungs, kidneys and liver respectively (Cowled, 2008; Yassin, 2004). Intestinal I/R also produced increase of MDA (an oxidative marker), decreased GSH and histo-pathological changes in the intestine and other major organs (lungs, liver, and kidneys) (Lai, 2000). Also induced oxidative injury of the skin produced remote effects on liver, kidneys, lung, stomach and ileum (Sener, 2006).In the present study MDA was increased after renal I/R in the plasma, liver and heart. Liver and heart enzymes (AST, ALT, LDH and CKMB) were also markedly increased denoting that renal I/R produced remote injurious effects on the liver and heart added to its local effects on the kidney. Also oxidative stress markers GSSG, GSH-Px were increased in the plasma added to decreased GSH concentration and antioxidant enzyme activities of GR, catalase and SOD.

### Table II. The effect of natural antioxidants on renal I/R as reflected on liver function, antioxidant-antioxidant status of rats liver.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control</th>
<th>I/R</th>
<th>I/R + garlic</th>
<th>I/R + Vit. E</th>
<th>I/R + Vit. A and Se⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/g tissue)</td>
<td>0.2 ± 0.009</td>
<td>0.42 ± 0.019*</td>
<td>0.2 ± 0.008*</td>
<td>0.3 ± 0.015*</td>
<td>0.2 ± 0.009*</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>28.6 ± 3.0</td>
<td>39.7 ± 4.0</td>
<td>32.1 ± 7.9</td>
<td>31.4 ± 3.7</td>
<td>31.3 ± 3.5</td>
</tr>
<tr>
<td>GSH (mmol/L)</td>
<td>2.5 ± 0.5</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.5</td>
<td>2.2 ± 0.5</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>CAT (IU/L)</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>SOD (IU/L)</td>
<td>6.2 ± 0.2</td>
<td>7.0 ± 0.4</td>
<td>6.9 ± 0.5</td>
<td>6.1 ± 0.4</td>
<td>6.0 ± 0.3</td>
</tr>
</tbody>
</table>

*P < 0.05 for each group

### Table III. The effect of natural antioxidants on renal I/R as reflected on the integrity and antioxidant-antioxidant status of rats heart.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control</th>
<th>I/R</th>
<th>I/R + garlic</th>
<th>I/R + Vit. E</th>
<th>I/R + Vit. A and Se⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/g tissue)</td>
<td>0.1 ± 0.009</td>
<td>2.65 ± 0.152*</td>
<td>0.36 ± 0.016*</td>
<td>2.42 ± 0.14*</td>
<td>0.73 ± 0.029*</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td>0.3 ± 0.05</td>
<td>0.16 ± 0.07</td>
<td>0.26 ± 0.06</td>
<td>0.22 ± 0.07</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>312.4 ± 4.6</td>
<td>522.6 ± 4.66</td>
<td>514.5 ± 11.44</td>
<td>516.6 ± 12.96</td>
<td>510 ± 10.9</td>
</tr>
<tr>
<td>GSH (mmol/L)</td>
<td>8.05 ± 0.15</td>
<td>7.07 ± 0.088</td>
<td>7.49 ± 0.13*</td>
<td>7.84 ± 0.098*</td>
<td>7.85 ± 0.123*</td>
</tr>
<tr>
<td>GSH-Px (μmol/L)</td>
<td>14.9 ± 0.63</td>
<td>128.6 ± 5.63</td>
<td>10.7 ± 0.116</td>
<td>19.6 ± 0.013</td>
<td>19.6 ± 0.047</td>
</tr>
<tr>
<td>CR (IU/L)</td>
<td>4.6 ± 0.26</td>
<td>0.39 ± 0.06</td>
<td>0.37 ± 0.12</td>
<td>0.38 ± 0.08</td>
<td>0.37 ± 0.12</td>
</tr>
<tr>
<td>CAT (IU/L)</td>
<td>3.05 ± 0.12</td>
<td>2.06 ± 0.06</td>
<td>2.05 ± 0.12</td>
<td>2.09 ± 0.06</td>
<td>2.07 ± 0.06</td>
</tr>
<tr>
<td>SOD (IU/L)</td>
<td>9.2 ± 0.02</td>
<td>95.7± 0.24*</td>
<td>97.4 ± 0.35</td>
<td>95.3 ± 0.179</td>
<td>95.8 ± 1.14</td>
</tr>
</tbody>
</table>

*P < 0.05 for each group
These results are in agreement with those of previous authors (Sener, 2005; Lai, 2000; Yokozawa, 1998; Davies, 1995). Correlation studies showed positive MDA correlation with creatinine concentration in plasma of I/R group and negative correlation between plasma GSH and blood urea concentration of I/R group, negative one with GSH-Px and LDH enzyme activities in plasma and GR of liver, which reflects the antioxidant effect of these enzymes in the present study. Ischemia of renal tissue results in dissociation of oxidative phosphorylation which results in univalent reduction of O2 to “O” catabolism of ATP into hypoxanthine and uric acid (Harpey, 1989). Hypoxanthine causes the generation of highly reactive free radicals during reperfusion (Harpey, 1989). Superoxide radicals and its reduction products (H2O2, and OH- radicals) cause injury via lipid peroxidation of cell plasma and mitochondria membranes locally and in remote organs (Hermestima, 1991). Therefore, many studies had attributed the tissue damage from I/R to energy depletion, accumulation of toxic metabolites and disturbance of electrolyte homeostasis especially that of Ca2+ (Tucci, 2005; Weinberg, 1991). ROS, however, has been considered a major deleterious factor especially in the reperfusion phase (Singh, 2004). Cellular defense against free radical injury is provided by GSH and catalase, SOD, GR and GSH-Px enzymes (Weinberg, 1991; Grace, 1999). The endogenous antioxidant defense mechanism of SOD enzyme is to remove superoxide anion from the surrounding, catalase and GSH-Px to inactivate H2O2 and tryptophan, histidine, Vitamin E, A, C and Se2+ to scavenge hydroxyl radicals (OH) (Singh, 2004; Kadkhodaei, 2004; Weinberg, 1991; Feri, 1999). In case of natural compounds (Vitamin E, Vit A + Se2+ and garlic) administrated to rats in the present study, their antioxidant property can also be explained on the basis that they can stimulate constitutive nitric oxide synthase (NOs) activity to increase NO levels which can decrease LDL oxidation and counteract the vasoconstrictive effect of ROS (Grace, 1999; Feri, 1999).

It may be added also that Vitamin E is known that it can stabilize polyunsaturated lipids against auto-oxidation, as free radicals react with these polyunsaturated fatty acids in cell membranes resulting in cellular destruction. It seems that Vitamin E reacts with ROS preventing free radical chain reactions to attack membranes However, the store of endogenous antioxidants decreases gradually while reacting with (Kadkhodaei, 2004) free radicals resulting in cell injury (Kadkhodaei, 2004). This last assumption is confirmed by reports about the gradual reduction of antioxidants after renal I/R in tissues such as the brain and heart, in which water soluble antioxidants including ascorbate, GSH, and SOD are first oxidized to protect the cell membrane integrity (Grace, 1999). In this regard the lipid soluble antioxidants are also consumed. Thus tissue Vitamin E, A and Se2+ many in addition to being an acceptable index of lipid oxidation, their administration to I/R animals may be important to replenish the depleted defense barrier, against the local and remote I/R insult. Sulphur containing compounds from garlic (allicin diallyl and disulphide constituents) has also and antioxidant property (Mikhail, 2001). In the present study it is apparent that plasma, liver and heart stores of GSH are depleted. GSH being an important intracellular antioxidant which acts by directly scavenging ROS, also by being a cofactor for GSH-PX catalyzed reactions that degrades H2O2 (Grace, 1999). Depletion of GSH therefore renders the cells susceptible to oxidative stress. In accordance with this we found a significant inhibition of antioxidant enzymes, GR, CAT and SOD activities which were decreased in plasma, liver and heart after I/R and which were partly corrected after administration of natural antioxidants (Vitamin E, A, Se2+ and garlic) for one month before I/R in the given doses. Also the redox potential RP in plasma which was decreased in I/R group was also partially improved. However, the present study showed that the effect of these natural antioxidants on the parameters studies in plasma; liver and heart were similar on some of them and different on others. In conclusion it may be said that ROS plays a casual role in renal I/R injury both locally and remotely on liver and heart. The preconditioning of the animals with natural antioxidants may be useful in protecting the animals from I/R injury. As the effect of these three natural antioxidants is different on the tissue, therefore the administration of these three natural antioxidants together to animals (or human) may bemoore beneficial than giving one of them only.

REFERENCES


