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Protective Effects of Vitamin C, Alone or in Combination with Vitamin A, on Endotoxin-Induced Oxidative Renal Tissue Damage in Rats

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KANTER, M., COSKUN, O., ARMUTCU, F., UZ, Y.H. and KIZILAY, G. *Protective Effects of Vitamin C, Alone or in Combination with Vitamin A, on Endotoxin-Induced Oxidative Renal Tissue Damage in Rats*. Tohoku J. Exp. Med., 2005, **206** (2), 155-162 —— This study was designed to investigate the protective effects of vitamin C and vitamin A on oxidative renal tissue damage. Male Wistar rats were given an intraperitoneal injection of 0.5 ml saline (control) or 0.5 ml solution of lipopolysaccharide (10 mg/kg), which caused endotoxemia. Immediately (within 5 min) after the endotoxin injection, the endotoxemic rats were untreated or treated with intraperitoneal injection of vitamin A (195 mg/kg bw), vitamin C (500 mg/kg bw) or their combination. After 24 hours, tissue and blood samples were obtained for histopathological and biochemical investigation. Endotoxin injection caused renal tissue damage and increased erythrocyte and tissue malondialdehyde (MDA) and serum nitric oxide (NO), urea and creatinine concentrations, but decreased the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities compared to the parameters of control animals. Treatment with vitamin C or with vitamins C and A significantly decreased the MDA levels and serum NO, urea and creatinine levels, recovered the antioxidant enzyme activities (SOD, GSH-Px and CAT), and prevented the renal tissue damage in endotoxemic rats. In contrast, vitamin A alone did not change the altered parameters except for creatinine levels. Notably, the better effects were observed when vitamins A and C given together. It is concluded that vitamin C treatment, alone or its combination with vitamin A, may be beneficial in preventing endotoxin-induced oxidative renal tissue damage and shows potential for clinical use. ———— endotoxemia; vitamin A; vitamin C; malondialdehyde; kidney

Endotoxin, released from Gram-negative bacteria, acts as a potent signalling molecule which can elicit a systemic inflammatory response syndrome (SIRS) defined as sepsis. The onset of sepsis is characterised by fever or hypothermia, tachycardia and tachypnea (Cadenas and Cadenas 2002). When sepsis is accompanied by hypotension plus organ dysfunction, the condition

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is known as septic shock. Despite significant progress in understanding the pathophysiology of sepsis and septic shock, these conditions continue to be the most causes of morbidity and mortality in intensive care units (Westphal et al. 2002). Endotoxin has harmful effects on various organs including kidney through the induction of inflammatory mediators (Cadenas and Cadenas 2002; Taniguchi et al. 2003). The occurrence of the functional organ insufficiencies in sepsis depends on the duration of this septic status (Ozdulger et al. 2002).

The common mechanism by which tissues are damaged by the septic response is probably related to widespread vascular endothelial injury and microthrombosis. These, in turn, decrease oxygen and substrate supply to the tissues leading to anaerobic metabolism and the production of free oxygen radicals (Cadenas and Cadenas 2002). Lipid peroxidation is associated with a wide variety of toxicological effects, including decreased membrane fluidity and function, impaired mitochondrial and Golgi apparatus functions, and inhibition of enzymes. Malondialdehyde (MDA) is an end product of lipid peroxidation and is a frequently measured index of these processes (Slater 1984; Comporti 1989). MDA can cross-link with membrane constituents of erythrocyte.

The antioxidant systems such as antioxidant vitamin (Vit) A, C and E, superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px), ceruloplasmin and catalase (CAT) protect the cells against lipid peroxidation, which is the base of many pathologic processes (Williams 1984; Bray and Bettger 1990; Ikeda et al. 2004). Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations without harmful side effects (Cadenas and Cadenas 2002).

The aim of the present study was the evaluation of possible protective effects of Vit A and Vit C, alone or in combination, against oxidative renal tissue damage in experimentally-induced endotoxemic rats.

Materials and Methods

Animals

Fifty healthy inbred male Wistar albino rats, weighing 150-250 g, and averaging 12 weeks old were obtained from the Research and Progress Center, Laboratory Animal Section of Gulhane Military Medical Academy, Ankara, Turkey. The animals were given standard rat pellets (Murat Food Factory, Ankara, Turkey) and tap water ad libitum. The rats were randomly allotted into one of five experimental groups: Control untreated, endotoxemia untreated, endotoxemia treated with Vit A, endotoxemia treated with Vit C, and endotoxemia treated with Vit A and Vit C (n = 10 for each group).

Control group received only an intraperitoneal (i.p.) injection of 0.5 ml normal saline solution. The other groups were administrated single i.p. injection of 0.5 ml endotoxin solution (*Escherichia coli* [*E. coli*] O157: H7, 10^9 bacteria/ml). Purified *E. coli O157*-derived lipopolysaccharide (LPS) was prepared in the Department of Microbiology, Medical School, Erciyes University, Kayseri, Turkey (Hughes et al. 1998). Endotoxemia was induced by a single i.p. injection of LPS (10 mg/kg in 0.5 ml of saline). Immediately (5 min) after the endotoxin injection, rats were treated with i.p. injection of 195 mg/kg bw Vit A (in 0.5 ml) (Ephynal amp., Roche® Industry, Istanbul, Turkey), 500 mg/kg bw Vit C (in 0.5 ml) (Abdi Ibrahim® Pharmaceutical Industry, Istanbul, Turkey) or a combination of Vit A and Vit C. The experiment lasted 24 hours.

Rats were housed in Plexiglas cages under standard laboratory conditions (light period 7.00 a.m. to 9.00 p.m., 21 ± 2°C, relative humidity 55%). The ethics committee of Trakya University approved the design of the experiments, and the protocol conforms to the guidelines of the National Institute of Health (NIH).

Biochemical procedures

At the end of the experiment, rats in all groups were starved overnight for 12 h, and sacrificed under ether anaesthesia. Blood samples were collected by cardiac puncture using heparinised syringe. Blood MDA (mmol/liter) was determined by the double heating method of Draper and Hadley (1990). The principle of the method is spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 100 g/liter trichloroacetic acid (TCA) solution was added to 0.5 ml erythrocyte in each centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water,

the mixture was centrifuged at 1,000 *g* for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/liter TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a Shimadzu UV-1601 (Tokyo) spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex 1.56×10^5 cm^{-1} M^{-1}, and expressed in μmol/g Hb erythrocyte and μmol/g tissue protein.

Harvested kidneys were washed with distilled water prior to homogenisation to remove residual blood. The tissues were homogenised in buffers by means of Ultra Turrax T25 homogenisator. The soluble fraction was prepared by centrifugation at 6,000 *g* for 10 minutes. Tissue SOD and GSH-Px activities were measured by using Ransod and Ransel (Randox Laboratories GmbH, Krefeld, Germany) commercial kits, respectively by using a Shimadzu UV-1601 spectrophotometer. Tissue CAT activity was determined according to Aebi's method (1974). Protein measurements were made according to Lowry's method (1951). Nitric oxide (NO) production (μmol/liter) was measured indirectly using a quantitative, colorimetric assay (Cattell et al. 1990) based on the Griess reaction. Serum urea and creatinine analyses were performed on a Cobas integra 800 automatic analyzer of Roche Diagnostic (MN, USA). Analyses were carried out at 37°C in potassium-EDTA plasma according to the instructions of the manufacturer of the automatic analyzer.

Histopathological procedures

Kidney tissues were harvested from the sacrificed animals, and the fragments from tissues were fixed in 10% neutral formaline solution, embedded in paraffin and then, stained with hematoxylin and eosin (HE). Preparations were evaluated with a bright field microscope and were photographed (Nikon Optiphot 2, Tokyo).

Image analysis

The system used is composed of a PC, hardware and software (Image-Pro Plus 5.0-Media Cybernetics, Georgia, Avenue, Suite, Silver Spring, USA) for image acquisition and analysis, Spot Insight QE (Diagnostic Instruments, Silver Spring, USA) camera and optical microscope. The method requires preliminary software procedures of spatial calibration (micron scale) and setting of color segmentation for quantitative color analysis. Fifty glomeruli from each rat, thus five hundred glomeruli for each group, were chosen randomly. Then the diameter of glomerulus in the renal tissue was measured. The investigator who performed these measurements was unaware of the experiment.

Statistical analysis

The data were expressed as mean ± S.D. and analysed using analysis of variance (ANOVA). Tukey test was used to test for differences among means when ANOVA indicated a significant ($p < 0.05$) F ratio. For the image analysis of glomerules, a nonparametric test (Kruskal-Wallis) was used. Differences were considered statistically significant when the p value was < 0.05.

Results

Biochemical findings

We measured erythrocyte MDA, tissue MDA, SOD, GSH-Px and CAT, and serum NO, urea and creatinine levels for each group (Tables 1-3). Endotoxemia significantly ($p < 0.01$) increased the erythrocyte and tissue MDA levels, serum NO, urea and creatinine and conversely decreased ($p < 0.01$) the antioxidant enzyme activities (SOD, GSH-Px, CAT) compared to control parameters (Tables 1-2). Vit C treatments (alone or in combination with Vit A) significantly ($p < 0.05$) decreased the erythrocyte and tissue MDA levels and serum NO, urea and creatinine levels, and increased ($p < 0.05$) the antioxidant enzyme activities in endotoxemic rats. Vit A treatment did not change the altered parameters ($p < 0.05$) except for creatinine levels ($p < 0.05$). However, the best result was obtained when Vit A and Vit C given together (Tables 1-3).

Histopathological findings

In control untreated group, histology of kidneys was normal (Fig. 1). The most consistent findings in the histologic sections of renal tissues of rats in endotoxemia untreated group were the severe degenerative changes, and shrunken tubules and especially hyalinized glomeruli of the renal cortex. There were also severe mononuclear cell infiltration among glomeruli and tubules in renal cortex (Fig. 2). In endotoxemic rats treated with Vit A, the severity of degenerative changes

TABLE 1. *Erythrocyte MDA (μmol/g Hb) and tissue MDA (μmol/g protein) levels for each group of rats*

Parameters	Control	Endotoxemia			
	Untreated	Untreated	Treated with Vit A	Treated with Vit C	Treated with Vit A and C
Erythrocyte MDA	9.15 ± 2.38[a]	14.92 ± 2.30[b]	14.36 ± 2.10[b]	12.41 ± 1.42[c]	10.80 ± 0.81[a]
Tissue MDA	96 ± 14[d]	134 ± 12[e]	128 ± 21[e]	116 ± 11[f]	103 ± 18[d]

Tukey test was used to test for differences among means when ANOVA indicated a significant ($p < 0.05$) F ratio. Values are expressed as mean ± S.D. ($n = 10$ for each group).

[a] $p < 0.01$ compared to endotoxemia untreated group.
[b] $p < 0.05$ compared to endotoxemia treated with Vit C or endotoxemia treated with Vit A /C group.
[c] $p < 0.05$ compared to endotoxemia treated with Vit A /C group.
[d] $p < 0.01$ compared to endotoxemia untreated group.
[e] $p < 0.05$ compared to endotoxemia treated with Vit C or endotoxemia treated with Vit A /C group.
[f] $p < 0.05$ compared to endotoxemia treated with Vit A /C group.

TABLE 2. *Tissue SOD (U/mg protein), GSH-Px (U/mg protein) and CAT (k/mg protein) levels*

Parameters	Control	Endotoxemia			
	Untreated	Untreated	Treated with Vit A	Treated with Vit C	Treated with Vit A and C
Tissue SOD	22.90 ± 9.71[a]	10.48 ± 1.34[b]	12.23 ± 2.47[b]	18.64 ± 1.95[a]	20.33 ± 1.99[a]
Tissue GSH-Px	0.39 ± 0.04[c]	0.25 ± 0.03[d]	0.26 ± 0.03[d]	0.28 ± 0.03[d]	0.37 ± 0.03[c]
Tissue CAT	0.35 ± 0.03[e]	0.23 ± 0.02[f]	0.24 ± 0.02[f]	0.26 ± 0.02[f]	0.33 ± 0.02[e]

Tukey test was used to test for differences among means when ANOVA indicated a significant ($p < 0.05$) F ratio. Values are expressed as mean ± S.D. ($n = 10$ for each group).

[a] $p < 0.01$ compared to endotoxemia untreated group.
[b] $p < 0.01$ compared to endotoxemia treated with Vit C or endotoxemia treated with Vit A /C group.
[c] $p < 0.01$ compared to endotoxemia untreated group or endotoxemia treated with Vit C.
[d] $p < 0.01$ compared to endotoxemia treated with Vit A /C group.
[e] $p < 0.01$ compared to endotoxemia untreated group or endotoxemia treated with Vit C.
[f] $p < 0.01$ compared to endotoxemia treated with Vit A /C group.

in the glomeruli and especially tubules were less than those in the endotoxemia untreated group (Fig. 3). Although mononuclear cell infiltration was slightly decreased, hyalinized and shrunken glomeruli remained unchanged. In endotoxemic rats treated with Vit C, the severity of degenerative changes, shrunken glomeruli and especially tubules and mononuclear cell infiltration were less than in endotoxemic untreated rats and endotoxemic rats treated with Vit A (Fig. 4). In endotoxemic rats treated with Vit A and Vit C, degenerative changes in the tubules or glomeruli, and mononuclear cell infiltration were not observed. However, slight shrunken tubules and glomeruli were still observed (Fig. 5).

The diameter of glomeruli in the renal tissues was measured. The diameter of the glomeruli was calculated according to these results (Table 4). Endotoxemia significantly ($p < 0.01$) decreased the diameter of glomeruli compared to control. Although Vit C treatments (alone or in combination with Vit A) significantly ($p <$

TABLE 3. *Serum NO (μmol/l), creatinine (mg/100 ml) and urea (mg/100 ml) levels*

Parameters	Control	Endotoxemia			
	Untreated	Untreated	Treated with Vit A	Treated with Vit C	Treated with Vit A and C
Serum NO	4.14 ± 0.56^{a}	7.44 ± 1.14^{b}	7.03 ± 1.12^{b}	5.83 ± 0.72^{c}	4.66 ± 0.64^{a}
Serum creatinine	0.56 ± 0.02^{d}	0.73 ± 0.06^{e}	0.62 ± 0.06^{d}	0.61 ± 0.04^{d}	0.59 ± 0.03^{d}
Serum urea	35 ± 1.41^{f}	47 ± 2.12^{g}	44 ± 1.84^{g}	40 ± 1.63^{f}	37 ± 0.09^{f}

Tukey test was used to test for differences among means when ANOVA indicated a significant ($p < 0.05$) F ratio. Values are expressed as mean ± S.D. ($n = 10$ for each group).

[a] $p < 0.01$ compared to endotoxemia untreated group.
[b] $p < 0.05$ compared to endotoxemia treated with Vit C.
[c] $p < 0.05$ compared to endotoxemia treated with Vit A /C group.
[d] $p < 0.01$ compared to endotoxemia untreated group.
[e] $p < 0.01$ compared to endotoxemia treated with Vit C or endotoxemia treated with Vit A /C group.
[f] $p < 0.01$ compared to endotoxemia untreated group.
[g] $p < 0.01$ compared to endotoxemia treated with Vit C or endotoxemia treated with Vit A /C group.

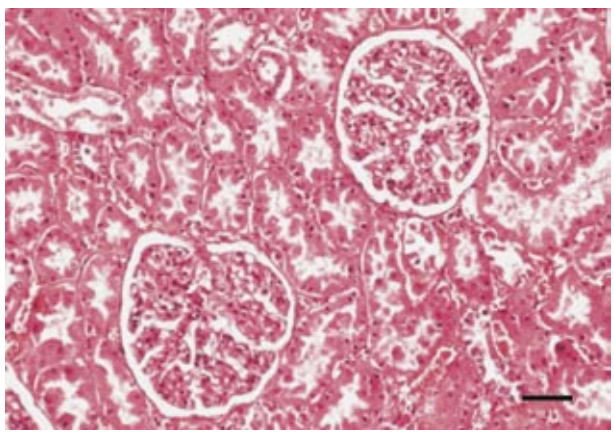

Fig. 1. Kidney histology of control rats (HE; scale bar, 25 μm).

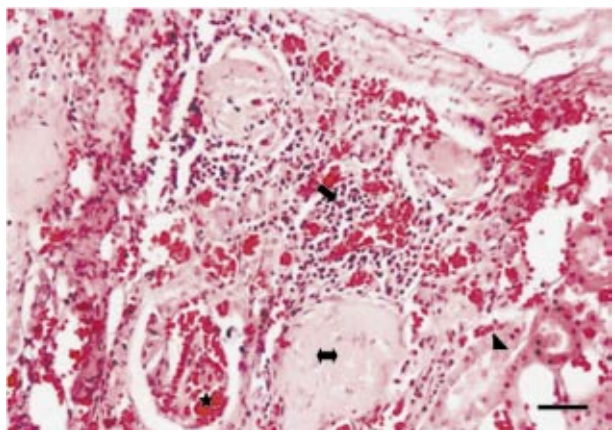

Fig. 2. Kidney histology of endotoxemic rats. Focal, intense interstitial mononuclear cell infiltration (arrow), degenerative epithelial changes of the shrunken tubules (arrow head) and hyalinization of the glomeruli (double arrows) in the renal cortex are seen. Congestion of the vessels is dominant (asterisk) (HE; scale bar, 25 μm).

0.05) increased the diameter of glomeruli in endotoxemic rats, but Vit A treatment did not increase the diameter of glomeruli ($p > 0.05$). However, the best result was obtained when Vit A and Vit C given together.

DISCUSSION

This study demonstrated that single dose of endotoxin injection produced renal damage, increased MDA, serum NO, urea and creatinine concentrations and decreased the antioxidant enzyme activities. The endotoxin lipopolysaccharide is a major component of the outer membranes of Gram-negative bacteria. Host organisms detect the presence of infection by recognizing specific elements on pathogens. These elements are generally known as pathogen-associated molecular patterns. They include diverse bacterial cell wall components, such as lipopolysaccharide,

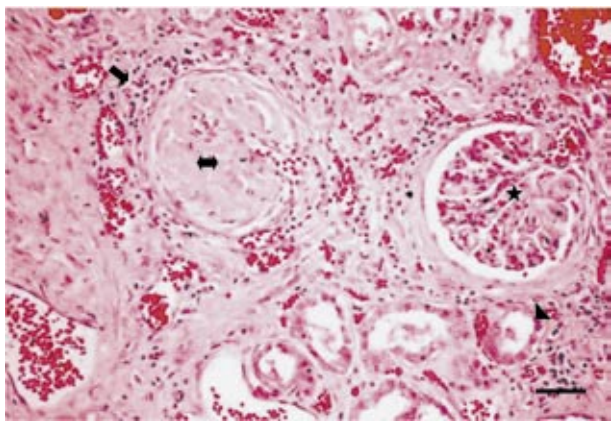

Fig. 3. Kidney histology of endotoxemic rats with Vit A treatment. Slight decreased mononuclear cell infiltration (arrow), hyalinized glomeruli (double arrows), and less degrees of shrinkage in the glomeruli (asterisk), and degenerative changes of the tubules (arrow head) of renal cortex are detected (HE; scale bar, 25 μm).

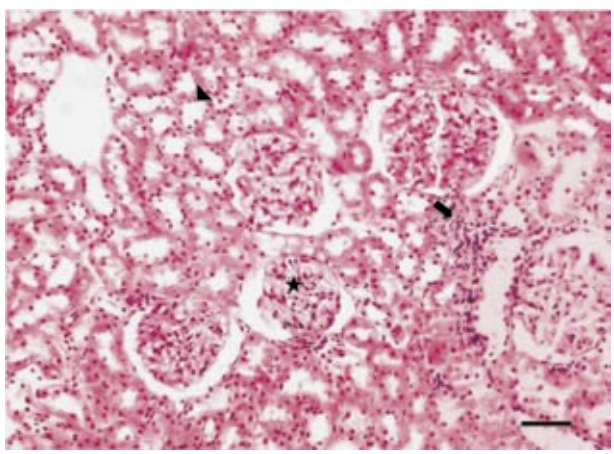

Fig. 4. Kidney histology of endotoxemic rats with Vit C treatment. Decreased mononuclear cell infiltration (arrow) and less degenerative changes, and shrunken tubules (arrow head) and glomeruli (asterisk) of renal cortex are seen (HE; scale bar, 25 μm).

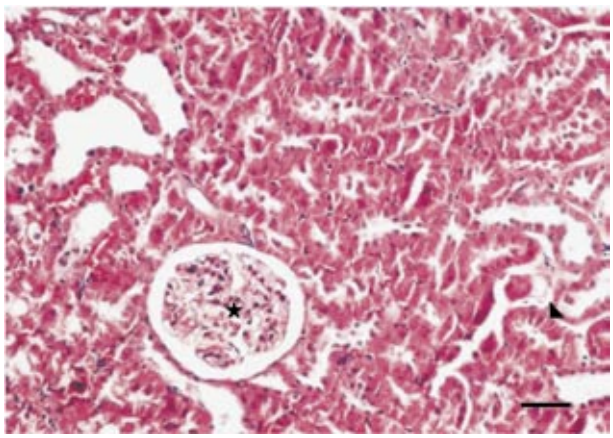

Fig. 5. Kidney histology of endotoxemic rats with Vit A and Vit C treatment. Minimal degeneration of the tubules (arrow head) and glomeruli (asterisk) are seen. Interstitial mononuclear infiltration is not present. However, some slight shrunken tubules and glomerules are still observed (HE; scale bar, 25 μm).

lipopeptides, peptidoglycans and teichoic acids. Their release during infection induces a number of pathophysiological reactions leading to cardiovascular failure and damage to numerous organs such as kidney and liver. The common mechanism by which tissues are damaged by the septic response is related to widespread vascular endothelial injury and microthrombosis. These, in turn, decrease oxygen and substrate supply to the tissues leading to anaerobic metabolism and lipid peroxidation (Cadenas and Cadenas 2002; Sugawara et al. 2003). In a study the interaction of endotoxemia and ischemic organ injury was investigated in a rat model. Animals received lipopolysaccharide to induce endotoxemia and were simultaneously subjected to renal ischemia. If only renal ischemia was induced, moderate azotemia occurred and all animals survived (Maessen et al. 1991). Liu et al. (2002) also indicated that endotoxin increased the BUN and creatinine levels in both normal and cirrhotic rats, with a much higher elevation in the latter group.

The present study also demonstrates that Vit C treatment decreases MDA and serum NO, urea, and creatinine concentrations and increase antioxidant enzyme activities, and also prevents renal tissue damage in experimentally-induced endotoxemic rats. Similar to our results, antioxidant supplementation has proven to be beneficial in decreasing the oxidative stress induced by endotoxin in a variety of tissues. For example, endotoxin administration to guinea pigs increased oxidative damage to liver proteins. This increase is totally prevented in animals supplemented with Vit C, a treatment that

TABLE 4. *Diameter of glomeruli in renal tissues for each group. Kruskal-Wallis test was used for statistical analysis. Values are expressed as mean ± S.D. (n = 10 for each group)*

Parameter	Control	Endotoxemia			
	Untreated	Untreated	Treated with Vit A	Treated with Vit C	Treated with Vit A and C
Mean diameter of glomeruli (μm)	85.82 ± 3.44	43.84 ± 1.23[a]	48.13 ± 1.26[a]	70.13 ± 2.15[b]	77.94 ± 3.46

[a] $p < 0.01$ compared to control group.
[b] $p < 0.05$ compared to control group.

considerably increases liver ascorbate (Cadenas et al. 1998). Vit C is one of the important water soluble vitamins. It is essential for collagen, carnitine and neurotransmitters biosynthesis (Naidu 2003). Most rodents are able to produce Vit C (ascorbic acid), unlike humans and primates (Challem and Taylor 1998). Many health benefits have been attributed to ascorbic acid such as antioxidant, anti-atherogenic, anti-carcinogenic, immunomodulator and prevents cold (Steinbrecher et al. 1990; Anderson et al. 1997; Campbell et al. 1999; Douglas et al. 2000). Vit C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins, which are implicated in chronic diseases including cardiovascular, stroke, cancer, neurodegenerative diseases and cataractogenesis (Halliwell and Gutteridge 1999).

A potential role for the antioxidant micronutrients Vit A and Vit C in modulating oxidative stress generated by restrained stress may determine their clinical usefulness as supplemental nutritional therapeutic agent in disorders affecting the brain free radical metabolism. The treatment of rats both prior to or after stress with these vitamins alone or in combination resulted in an increase in the activities of SOD, glutathione-*S*-transferase, catalase and levels of reduced glutathione with a decrease in lipid peroxidation. Post-vitamin treatments were found more effective in combating stress induced pro-oxidant changes than pre-vitamin treatments. Vit A and Vit C are reported to act as an effective antioxidant of major importance for protection against diseases and degenerative processes caused by oxidative stress (Chaudiere and Ferrari-Illiou 1999; Olas and Wachowiej 2002).

It is concluded that Vit C treatment, alone or in combination with Vit A, may be beneficial in preventing endotoxin-induced oxidative renal tissue damage and, therefore, shows potential for clinical use.

References

Aebi, H. (1974) Catalase. In: *Methods of Enzymatic Analysis,* edited by H.U. Bergmeyer, New York, Academic Press, pp. 673-677.

Anderson, D., Phillips, B.J., Yu, T.W., Edwards, A.J., Ayesh, R. & Butterworth, K.R. (1997) The effects of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with "low" or "high" cholesterol levels. *Environ. Mol. Mutagens,* **30**, 161-174.

Bray, T.M. & Bettger, W.J. (1990) The physiological role of zinc as an antioxidant. *Free Radic. Biol. Med.*, **8**, 281-291.

Cadenas, S. & Cadenas, A.M. (2002) Fighting the stranger-antioxidant protection against endotoxin toxicity. *Toxicology*, **180**, 45-63.

Cadenas, S., Rojas, C. & Barja, G. (1998) Endotoxin increases oxidative injury to proteins in guinea pig liver: protection by dietary vit C. *Pharmacol. Toxicol.*, **82**, 11-18.

Campbell, J.D., Cole, M., Bunditrutavorn, B. & Vella, A.T. (1999) Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. *Cell Immunol.,* **194**, 1-5.

Cattell, V., Cook, T. & Moncada, S. (1990) Glomeruli synthetize nitrite in experimental nephrotoxic nephritis. *Kidney Int.,* **38**, 1056-1060.

Challem, J.J. & Taylor, E.W. (1998) Retroviruses, ascorbate, and mutation, in the evolution of Homo sapiens. *Free Radic. Biol. Med.,* **25**, 130-132.

Chaudiere, J. & Ferrari-Illiou, R. (1999) Intracellular antioxidants: from chemical to biochemical mechanism. *Food. Chem. Toxicol.*, **37**, 949-962.

Comporti, M. (1989) Three models of free radical-induced cell injury. *Chem. Biol. Interact.,* **72**, 1-56.

Douglas, R.M., Chalker, E.B. & Treacy, B. (2000) Vitamin C for preventing and treating the common cold. *Cochrane Database Syst. Rev.,* **2**, CD000980.

Draper, H.H. & Hadley, M. (1990) Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.,* **186**, 421-431.

Halliwell, B. & Gutteridge, J.M.C. (1999) *Free radicals in Biology and Medicine*: Oxford University Press, 3rd ed., Oxford.

Hughes, A.K., Stricklett, P.K. & Kohan, D.E. (1998) Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. *Kidney Int.* **54**, 426-437.

Ikeda, M., Nakabayashi, K., Shinkai, M., Hara, Y., Kizaki, T., Oh-ishi, S. & Ohno, H. (2004) Supplementation of antioxidants prevents oxidative stress during a deep saturation dive. *Tohoku J. Exp. Med.,* **203**, 353-357.

Liu, J.J., Wang, J.Y., Zhang, C., Nilsson, A. & Duan, R.D. (2002) Hepatic cirrhosis increases sensitivity of kidney to endotoxin in rats. *Med. Sci. Monit.*, **8**, 56-60.

Lowry, O.H., Rosebrough, N.J. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J Biol. Chem.,* **193**, 265-272.

Maessen, J.G., Greve, J.W. & Buurman, W.A. (1991) Increased sensitivity to endotoxemia by tissue necrosis. *Surgery,* **109**, 154-159.

Naidu, K.A. (2003) Vitamin C in human health and disease is still a mystery? An overview. *Nutr. J.,* **2**, 7.

Olas, B. & Wachowicz, B. (2002) Resveratol and vitamin C as antioxidant in blood platelets. *Thromb. Res.*, **106**, 143-148.

Ozdulger, A., Cinel, I., Unlu, A., Cinel, L., Mavioglu, I., Tamer, L., Atik, U. & Oral, U. (2002) Poly(Adp-ribose) synthetase inhibition prevents lipopolysaccharide-induced peroxynitrite mediated damage in diaphragm. *Pharmacol. Res.*, **46**, 67-73.

Slater, T.F. (1984) Overview of methods used for detecting lipid peroxidation. *Methods Enzymol.,* **105**, 283-293.

Steinbrecher, U.P., Zhang, H.F. & Lougheed, M. (1990) Role of oxidative modified LDL in atherosclerosis. *Free Rad. Biol. Med.,* **9**, 155-168.

Sugawara, K., Miyata, G., Shineha, R. & Satomi, S. (2003) The lipolytic responsiveness to endotoxin in subcutaneous adipose tissue is greater than mesenteric adipose tissue. *Tohoku J. Exp. Med.,* **199**, 171-179.

Taniguchi, T., Kanakura, H., Takemoto, Y., Kidani, Y. & Yamamoto, K. (2003) Effects of ketamine and propofol on the ratio of interleukin-6 to interleukin-10 during endotoxemia in rats. *Tohoku J. Exp. Med.,* **200**, 85-92.

Westphal, M., Stubbe, H., Bone, H.G., Daudel, F., Vocke, S., van Aken, H. & Booke, M. (2002) Hemodynamic effects of exogenous adrenomedullin in healthy and endotoxemic sheep. *Biochem. Biophys. Res. Com.,* **296**, 134-138.

Williams, R.P.J. (1984) Zinc: what is its role in biology? *Endeavour*, New Series, **8**, 65-70.