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The Effects of Combined Vitamin C and Vitamin E in Streptozotocin-Induced Diabetic Rat Kidney

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Abstract: Diabetic nephropathy is a serious complication of diabetes mellitus. Oxidative stress has been suggested to play a key role in the pathogenesis of diabetic nephropathy. Vitamins C and E play important roles in the antioxidant defense system. It is likely that both vitamins act in a synergistic manner, with vitamin E primarily being oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vitamin C. The purpose of the study was to determine the effects of supplementation of Vitamins C and E (VCE) on diabetic rat kidney. Adult female Wistar rats were used in the study. The animals were divided into three groups. Group I: the control group; Group II: diabetic group, streptozotocin (40 mg kg^{-1}) was administered group; Group III: diabetes + VCE group, received a diet containing a combination of ascorbic acid and dl-α-tocopheryl acetate per kg of feed. Rats were killed on 21st day and renal tissues were taken and fixed in 2.5% glutaraldehyde solution for electron microscopic examination. When compared with the control group, congestion of the glomerular capillaries, increased mesangial cells and distinct mesangium, shortened podocyte processes and disappearance of filtration slit pore of diabetic rat kidney were observed. In the group treated with VCE, glomerular changes were less distinct than the diabetic group. Lengths of the pedicles were similar to the control group. VCE reduced the changes in the glomerular structures due to diabetes.

Key words: Diabetes, kidney, vitamin C and E, ultrastructure, renal, tissue, oxidized

INTRODUCTION

Diabetic nephropathy is one of the leading causes of chronic renal failure in the western European countries, USA and Japan. Renal injury is observed in some 35% of patients with type I and II diabetes. Renal disorders observed in type I and II diabetes are similar. Long term hypoglycemia, genetic factors, race, sex and hypertension have been implicated in the development of diabetic nephropathy (Monhart, 2008; Rychlik, 2008). Diabetic nephropathy presents itself with ischemic nephropathy, nodular glomerulosclerosis and renal failure. Clinically, 30-300 mg day^{-1} or 20-200 μg min^{-1} microalbuminuria indicates diabetic nephropathy (Ritz, 2006). Diabetes is an important etiopathological factor in oxidative stress (Punithavathi *et al*., 2008). As a result of lipid and protein oxidation, the levels of Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (CAT) increase in kidneys (Prakasam *et al*., 2005; Je *et al*., 2001; Yildirim and Buyukbingol, 2003). Various studies have reported protective effects of antioxidants such as melatonin (Oktem *et al*., 2006), ginkgobiloba (Welt *et al*., 2007), Circumin (Murugan and Pari, 2006), Groundnut oil (Ramesh *et al*., 2006), taurin (Wang *et al*., 2008), herbal medications (Yokozawa *et al*., 2008), soybean oil (Sena *et al*., 2008), naringin, a flavonoid glycoside which gives the bitter taste of grapefruit juice (Punithavathi *et al*., 2008), vitamin E (Minamiyama *et al*., 2008; Hamdy *et al*., 2008; Ruperez *et al*., 2008) and vitamin C (Ruperez *et al*., 2008; Wu *et al*., 2007a; Fadupin *et al*., 2007) against oxidative damage of diabetes.

The levels of vitamin C and E in plasma and renal tissues are significantly reduced in diabetic patients (Wu *et al*., 2007b; Kashiba *et al*., 2002; Peerapatdit *et al*., 2006). A decrease in Vitamin C causes hyperlipidemia and hypertension (Wu *et al*., 2007a; Chen *et al*., 2005). Epidemiologic studies showed that certain fruits and vegetables are important to prevent or alleviate the complications of diabetes and that Vitamins C and E had complication-reducing effects (Harding *et al*., 2008; Villegas *et al*., 2008). Vitamins C and E not only reduce the risk of thromboembolism in patients with diabetes-related

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hypertension (Haidara *et al*., 2004) but also exert favorable effects on wound healing (Musalmah *et al*., 2005). Vitamins C and E have been shown to prevent the teratogenic effects in diabetic rats and autoimmunity of β-cells in babies (Uusitalo *et al*., 2008; Cederberq and Erikssan, 2005). Vitamins C and E can be used as antioxidants separately or in combination. Both vitamins act synergistically (Naziroglu *et al*., 2004; Kutlu *et al*., 2005). Majority of the studies demonstrated the antioxidant effects of Vitamins C and E while electron microscopic studies are scarce. In the present study, the effects of Vitamin C, a hydrophilic antioxidant and Vitamin E, a lypophilic antioxidant, on structural changes in renal tissue were investigated by feeding the experimental diabetes-induced rats with a combination of these vitamins.

MATERIALS AND METHODS

Fifteen adult female Wistar rats weighing 200-220 g were obtained from Experimental Research Center of Firat University Faculty of Medicine (FUTDAM). The rats were housed at 22-24°C and were exposed to alternate cycles of 12 h light and darkness. All animal care and handling procedures conformed to the Guidelines set by the Association for Assessment and Accreditation of Laboratory Animal Care and approval was obtained from the Local Ethics Committee for Animal Studies. The animals were divided into three groups:

Group I: Control group (n = 5). Control rats were given intraperitoneal citrate buffer only (0.1 M, pH = 4.5).

Group II: Diabetic group (n = 5). Streptozotocin (STZ, Serva GmbH, Heidelberg, Germany) was administered intraperitoneally at a dose of 40 mg kg^{-1} body weight dissolved in citrate buffer (29).

Group III: VCE group (n = 5). Rats were fed with VCE (Vitamin C and E) supplemented diet for 15 days prior to induction of diabetes.

VCE supplemented and unsupplemented food compositions were homogenized using a mixer and pellets were prepared in laboratory by heating below 45°C for 2 days. The VCE supplemented diet contained a combination of 1 g vitamin C (ascorbic acid, F. Hoffman La Roche, Istanbul, Turkey) and 600 mg vitamin E (di-α-tocopheryl acetate, F. Hoffman La Roche, Istanbul, Turkey) per kg of feed (Naziroglu *et al*., 2004).

At the end of the experiment, renal tissues were taken under Rompun (5 mg kg^{-1}) and Ketamin (60 mg kg^{-1}) anesthesia. Renal tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer and postfixed with 2% osmium tetraoxide in sodium phosphate buffer. Dehydration was accomplished by gradual ethanol series and tissues were embedded in epoxy resin. Ultrathin sections were stained with Uranly acetate and lead citrate. Sections were then viewed and photographed with a Zeiss 9 EM.

RESULTS

Thin parts of the renal proximal tubules and glomeruli of the control group looked normal. Podocytes and cytoplasmic extensions, infiltration slits were evenly distributed (Fig. 1).

Capillary congestion, activation of and increase in the number of mesangial cells and shortened podocyte processes were observed in the renal glomeruli secondary to STZ. Basement membrane was thickened in certain regions (Fig. 2a and b). The invaginations on the basal regions of the proximal tubules were irregular and collagen fibers were clustered in the areas between the tubules (Fig. 3). Compared to the control group, mesangial accumulation, shortened podocyte processes and obscured filtration slits could clearly be identified in the diabetic group. Obscureness of the infiltration slits were not diffuse (Fig. 4a and b).

Degeneration of the glomerular endothelia, diffuse or nodular glomerulosclerosis, apoptosis in the podocytes and hyalinization beneath the basal lamina were not observed in this group. Structural changes in the glomeruli of the diabetic rats fed with VCE were milder

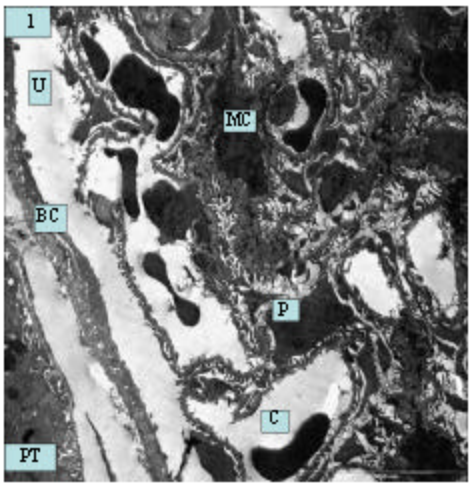

Fig. 1: A glomerulus from the control group. Proximal Tubule (PT), Bowman's Capsule (BC), Capillaries (C), Podocyte (P), Urinary space (U) and Mesangial Cells (MC)

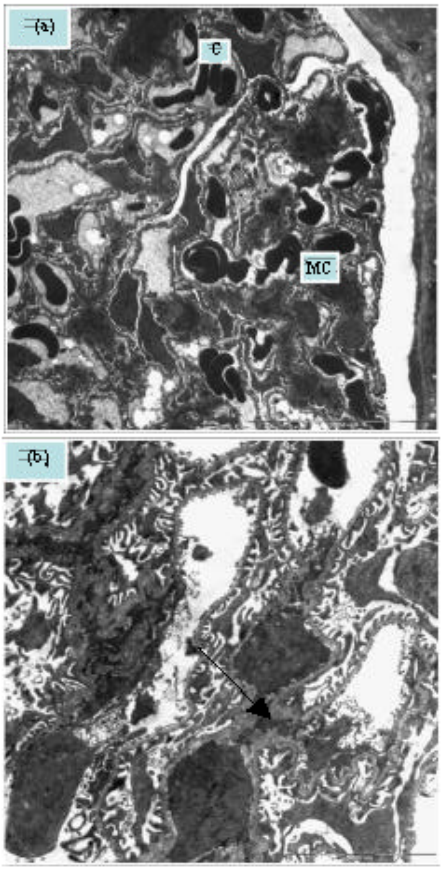

Fig. 2: STZ-induced diabetic rat kidney. a) Capillary congestion and increase in mesangial cells can be observed, b) Occasional basement membrane thickenings can be observed under higher magnification (arrow)

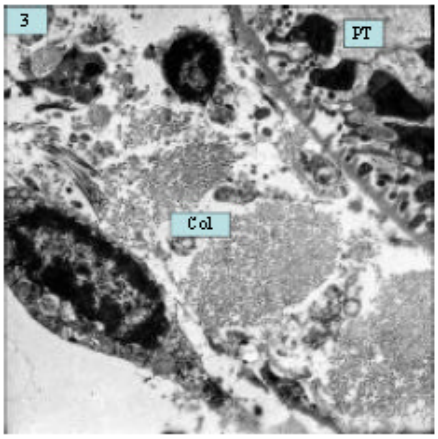

Fig. 3: STZ-induced diabetic rat kidney. Irregular basal invaginations in some of the Proximal Tubules (PT) and peritubular heavy clusters of collagen fibers (Col) can be observed

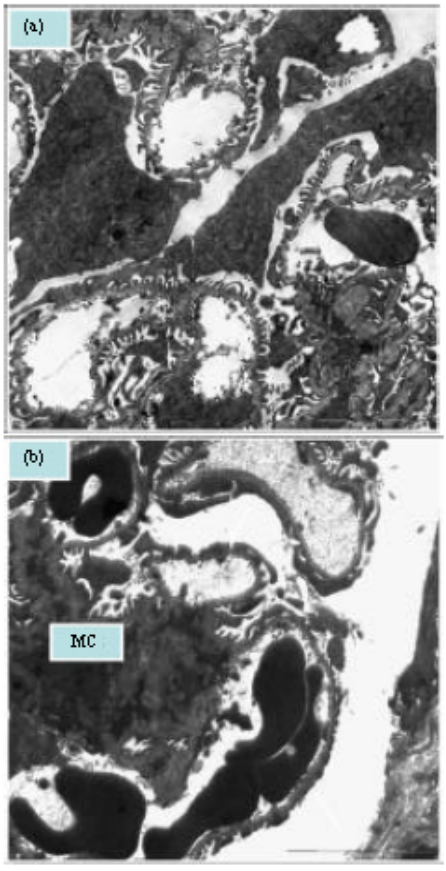

Fig. 4: Comparison of podocyte extensions and infiltration slits. a) Control group, b) Diabetic rat kidney. Shortened podocyte extensions and obscure infiltration slits (arrow) are marked

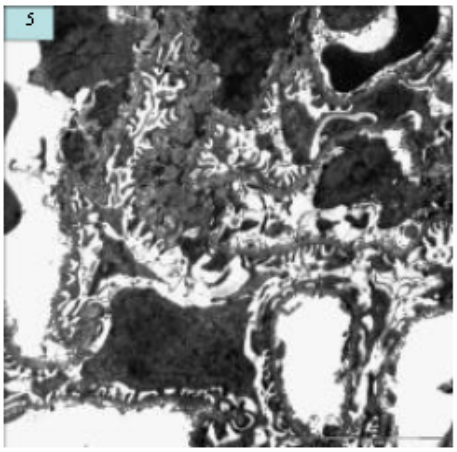

Fig. 5: Rats treated with a combination of vitamins C and E had more intact glomeruli in comparison to the diabetic group and increase in mesengial cell was not noted

than the diabetes group. Increase in mesangial cells was not observed. Podocyte processes and filtration slits generally appeared normal (Fig. 5).

DISCUSSION

Diabetic nephropathy is the most common cause of chronic renal disease and the foremost indication for dialysis and renal transplantation (Estacio and Schrier, 2001). Diffuse or nodular glomerulosclerosis, arteriosclerosis, tubulo-interstitial fibrosis and atrophy occur, proteinuria gradually increases and so does the blood pressure (Alsaad and Herzyenberg, 2007). Glomerulosclerosis develops as a result of injury to the podocytes, which play the key role in glomerular filtration (Lee *et al*., 2007). Podocyte injury can present itself with two forms: metabolic (biochemical) and hemodynamic (associated with hyperfiltration and hyperperfusion) (Hostetter, 2003; Wolf *et al*., 2003). Glomerular hyperfiltration and hyperperfusion are essential in mesangilisation and changes in the glomerular basal membrane (Wolf *et al*., 2003; Menini *et al*., 2007). In diabetic nephropathy, Prostaglandin E_2 (PGE_2) synthesis in the glomeruli is significantly increased. This is a result of an increase in mesangial cells (Iino *et al*., 2005). γ-tochopherole inhibits PGE_2 (Peerapatdit *et al*., 2006; Wu *et al*., 2007b). Oxidative stress and free oxygen radicals, which develop during nephropathy, trigger apoptosis of the tubular epithelial cells and podocytes of the glomeruli (Blauwkamp *et al*., 2008; Jung *et al*., 2008; Ruster *et al*., 2008; Susztak *et al*., 2006). Various agents used to inhibit apoptosis have been tried in the treatment of nephropathy (Isermann *et al*., 2007). In present study, we observed an increase in mesangial cell in the glomeruli of diabetic kidneys and mesangial accumulation but not apoptosis of podocytes and tubular epithelial cells.

Antioxidants are frequently used for diabetes and its complications. Plasma Vitamin C and vitamin E concentrations are reduced in diabetes (Murugan and Pari, 2006; Ramesh *et al*., 2006; Peerapatdit *et al*., 2006; Wu *et al*., 2007a, b; Lee *et al*., 2007). A positive relation has been demonstrated between high plasma vitamin C level and reduction in complications of diabetes (Harding *et al*., 2008). Vitamin C plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells (Sadi *et al*., 2008; Afkhami-Ardekani and Shojaodding-Ardekani, 2007; Al-Shamsi *et al*., 2006). Furthermore, vitamin C regenerates the oxidized vitamin E (Chen *et al*., 2005). Vitamin E, on the other hand, acts as a non-enzymatic antioxidant and reduces lipid peroxidation and glutathione (Punithavathi *et al*., 2008; Minamiyama *et al*., 2008; Lee *et al*., 2007). Vitamin E is very effective in glycemic control, lowering the HbA_{1c} levels (Ihara *et al*., 2000) and preventing the hypertrophic effects of hyperglycemia (Nascimento *et al*., 2005). However, this is in contrast to the results of some studies, which showed that vitamin E was not beneficial in glycemic control and lipid metabolism in Type II diabetes (Ble-Castillo *et al*., 2005). In another study, the researchers demonstrated that a combination of vitamins C and E improved the glomerular functions but did not have any effect on the tubular functions (Farvid *et al*., 2006). When exercise is given to rats with STZ-induced diabetes in addition to Vitamins C and E, it was observed that lipid peroxidation was significantly reduced, Glutathione Peroxidase (GSH-Px) was increased and reduced Glutathione (GSH) level was decreased (Kutlu *et al*., 2005).

The earliest structural changes in diabetic nephropathy are the increase in mesangial cells and mesangial dilatation. Diffuse thickening of the glomerular basement membrane depends on the severity of the disease. Vitamins C and E reduce the thickness of the basement membrane (Kedziara-Kornatowski *et al*., 2003; Davila-Esqueda *et al*., 2005). It has been argued that glomerular changes can occur in diabetic nephropathy without arterial or tubulo-interstitial changes (Fioretto and Mauer, 2007). Vacuolization within the podocytes, myelin figures and blebs were noted (Farvid *et al*., 2006). In the results, thickening of the glomerular basement membrane was not diffuse but they were in certain regions. Occasional irregularities of the podocyte processes, shortened podocyte processes and obscureness of the infiltration slits were observed.

CONCLUSION

Alterations were not observed in the renal tubules of rats with STZ induced-diabetes while increased mesangial cells, increased capillary permeability and obscureness of the filtration slits were noted in the glomeruli. Neither apoptosis of the podocytes nor thickening of the basement membrane was observed. These alterations were less pronounced in the diabetic rats treated with VCE. VCE helped alleviation of the renal degeneration by protecting the glomerular structures from oxidative injury. Concomitant administration of Vitamins C and E would be more effective in preventing the complications of diabetes.

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