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Effect of coenzyme Q10 in combat with mesangial expansion in diabetic rats (stereological study)

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Abstract

Introduction: Mesangial expansion is the main factor that leads to renal failure in diabetic patients. Oxidative stress is the most important factor in induction of diabetic mesangial expansion.
Objectives: The purpose of this research was to evaluate the effect of Coenzyme Q10 (Co Q10) as an antioxidant against diabetic mesangial expansion through the stereological methods.
Materials and Methods: Thirty male rats (180–200 g) were divided into three groups: control (group 1), diabetic without treatment (group 2) and diabetic treatment with Co Q10 (group 3). The animals received Co Q10 (15 mg/kg/d i.p.) during the study. After eight weeks of treatment, the left kidneys of animals were fixed in 10% formal saline. Kidney slices (1 mm thickness) processed and then paraffin sections (5 µ thickness) were prepared and stained through periodic acid Schiff (PAS) method. Glomerular volume, mesangium volume, glomerular capillary volume and mesangial cells were estimated stereologically.
Results: Diabetes increased significantly mesangium volume, mesangial cells, and glomerular volume and decreased lumen of glomerular capillary when compared with the control group. Co Q10 therapy ameliorated these variables in comparison with group 2 ($P<0.05$) but could not save these variables at the same level as that of the control group.
Conclusion: Coenzyme Q10 ameliorated significantly mesangial expansion in diabetic rats via inhibition of mesangial matrix production or mesangial cell number proliferation through inhibition of oxidative stress.

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Introduction

Diabetic nephropathy, the main cause of end-stage renal disease (ESRD) is consequence of renal microvascular complications of diabetes mellitus. Oxidative stress plays a pivotal role in pathogenesis of diabetic nephropathy. Hyperglycemia increase reactive oxygen species (ROS) via induction of NADPH oxidase overproduction in mesangial cells. Reactive oxygen species induce increase of cytokines such as protein kinase C (PKC), mitogen-activated protein kinase, nuclear factor-kappa B (NF-κB) and transforming growth factor beta (TGF-β) in mesangial cells (1-4). Also ROS induce renal injuries through cell membrane peroxidation, protein oxidation, renal vasoconstriction, DNA destruction, matrix overproduction and advanced glycation end-products (AGEs) formation (3-5). Besides there are many agents that induce oxidative stress in mesangial cells that include angiotensin II (AgII), AGEs, TGF-β, aldosterone, oxidized LDL, serotonin and amino acids (6,7).

Core tip
One of the most important goals in prevention of renal failure in diabetic patients is inhibition of mesangial expansion. Oxidative stress induced by hyperglycemia is the main cause of mesangial expansion. Coenzyme Q10 as an antioxidant was used to inhibition of oxidative stress. Stereological rules were used to estimating quantitative variables such as mesangial volume and mesangial cell numbers.

Oxidative stress increases mesangium due to increase of mesangial matrix production, mesangial cells proliferation and decreased degradation of matrix by inhibiting metalloproteinases (5,6).

In addition to the above mechanisms, deficiency or inactivation of innate renal tissue antioxidants may contribute to oxidative stress in mesangial cells (6).

Mesangium has intracapillary location and then mesangial expansion leads to narrowing and occlusion of glomerular capillaries lumen and subsequently reducing

glomerular filtration rate and finally renal failure.

Coenzyme Q10 an ubiquinone take part in ATP production and showed antioxidant and scavenging free radicals effects (8 -10).

In this research Co Q10 as an antioxidant was used to inhibit diabetic mesangial expansion and was studied through stereological methods.

Objectives

Because of antioxidant property of CoQ10 we decided to assess outcome of CoQ10 therapy in inhibition of mesangial expansion in diabetic rats.

Materials and Methods

Thirty male mature Sprague–Dawley rats (180–200 g) were divided into three groups: control (group 1), diabetic without treatment (group 2) and diabetic treatment with Co Q10 (group 3). Diabetes was induced after overnight fasting in groups 1 and 2 through subcutaneous injection of alloxan monohydrate (120 mg/kg) (11). The rats received 10% sucrose solution in the first two days instead of tap water to prevent hypoglycemia. Blood glucose was measured via glucometer five days after diabetes induction and the rats with blood glucose level of ≥300 mg/dL considered as diabetic (12). Two or three rats per group died throughout the first five days after diabetes induction. Animals maintained at 12 h dark/light cycle at 21±3°C temperature. All animals received food and water ad libitum for eight weeks. The third group was injected with coenzyme Q10 (15 mg/kg/d) intraperitoneally (13). After eight weeks left kidneys of rats sampled under anesthesia (Thiopental 50 mg/kg i.p). The samples were weighted and immersed in 10% formal saline fixative. Forty eight hours later 1 mm thickness slices were prepared by a special instrument. Paraffin sections (5 µ thickness) were prepared from all slices. Two serial sections (section pairs) were selected from each slice. The Sections (slides) were stained through periodic acid Schiff (PAS) technique.

Estimation of Stereological parameters

Estimation of renal cortex volume

The cortex volume fraction or volume density of cortex per kidney [V_v (cortex/kid)] was estimated through point counting technique. 8-11 sections (One section per slice) were studied from each kidney. A point grid (pluses with 6 mm apart) was superimposed on kidney section image (linear magnification 14x) and the points that hitted cortex or whole kidney section were counted respectively (Figure 1). If the plus fall on the border of cortex and medulla the plus (point) consider inside the cortex that part of up and right of plus fall on the cortex.

The volume density of cortex per kidney was estimated by bellow equation (14,15).

$$V_v\ (cortex/kid) = \Sigma P_p/\Sigma P_t$$

ΣP_p = points falling on cortex

$\Sigma P_{t=}$ points falling on cortex and medulla.

Estimation of glomerular volume

Glomerular volume fraction (glomerular volume density) was estimated by point counting technique. In this manner, the kidney sections (one section from each slice) were studied. The image of kidney section on microscopic field was transferred to the monitor via Leica DFC camera. A transparent sheet frame (10 cm × 10 cm with 100 +) was superimposed on microscopic image on monitor (Figure 2). At final magnification of 240, the points (plus) that touched or hitted glomerular lobules were counted. The glomeruli were studied that fall inside of the frame or touch right and upper lines of frame. Finally 100–70 glomeruli were studied from all slices (14). Glomerular volume density (V_v) was estimated by bellow formula (15).

$$V_v\ (glom/cortex) = \Sigma P_p/\Sigma P_t$$

ΣP_p = points touch glomeruli

ΣP_t = points in frame (100).

Total glomerular volume was estimated by bellow formula

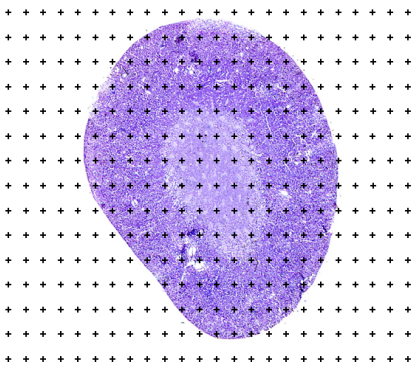

Figure 1. A grid point (6 mm apart) superimposed on kidney image and the plus hitted cortex and whole section were counted. Final linear magnification of 14X.

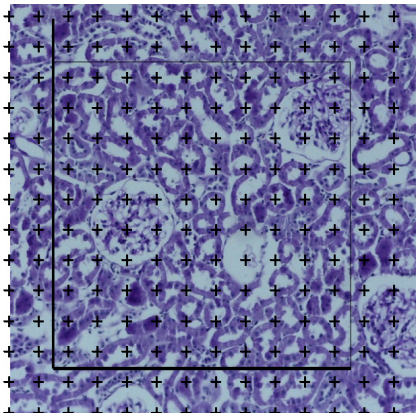

Figure 2. A frame (10cm × 10 cm with 100 plus) superimposed on microscopical kidney image and point hitted glomeruli were counted. PAS staining, 240X.

(14).

V_{total} *(glom/kid)* = V_v *(glom/cortex)* [V_{kid} . V_v *(cortex/kid)]*

Kidney weight was put instead of kidney volume (1 g ≈1 cm^3) (16).

Estimation of total mesangium volume

Volume density (volume fraction) of mesangium per glomerule estimated by point counting method. From each kidney 8-11 sections (1 section per slice) were used. Microscopical image moved to a monitor via camera-equipped microscope (Leica DFC camera). At the magnification of 490X a coarse grid (points 6 mm apart) superimposed on microscopical image (Figure 3) and points counted that fall on glomerule. Then a fine grid (points 3 mm apart) superimposed on this image and points counted that fall on mesangium (Figure 4).

At least 100 glomeruli per animal were assessed. Volume density of mesangium per glomerule (V_v mes/glom) was estimated via bellow formula (17).

V_v *(mes/glom) = FPM / CPG. 4*

FPM =point hitting mesangium

CPG =points hitting glomeruli

4 = coarse point area/fine point area

Total mesangium volume per kidney was estimated by the following formula:

V_{total} *(mes/kid)* =V_v *(mes/glom).*V_{total} *glom/kid*

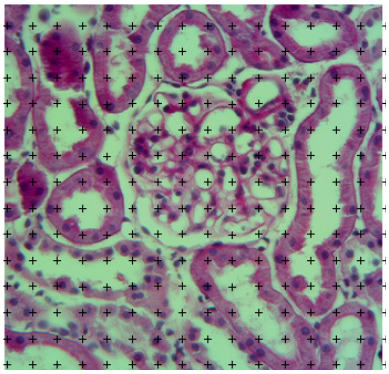

Figure 3. The coarse point grid (6 mm apart) superimposed on micrograph and point fall on glomerule were counted. PAS staining, 490X.

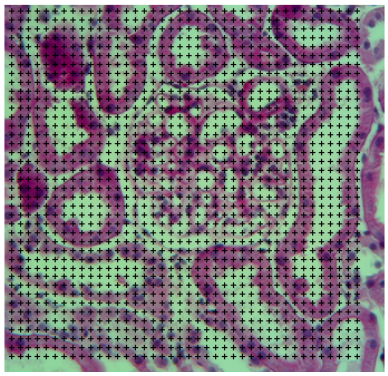

Figure 4. The fine point grid (3 mm apart) superimposed on micrograph and point that fall on mesangium or capillary lumen were counted. PAS staining, 490X.

When the fine grid superimposed on glomerular image, points that fall on capillaries lumen counted and then volume density of capillary per glomeruli estimated through the following formula:

V_v *(capill/glom) = FPC / CPG. 4*

FPC =point hitting capillary

CPG =points hitting glomeruli

Finally the total volume of glomerular capillary per kidney was estimated by bellow formula (17).

V_{total} *(glom cap/kid)* = V_v *(capill/glom).* V_{total} *glom/kid*

Estimation of mesangial cells number per kidney via physical disector

Numerical density of mesangial cells per glomerule estimated through physical disector. Sections from all kidney slices (two sections per slice) were assessed. Section pairs 5 micron apart (the first and second section) from each kidney slice were used. The first glomerule image((reference section) was projected on disector frame 1(square 5cm×5cm on paper) and image of the same glomerule from second section (look up) was projected on disector frame 2 (square 5 cm × 5 cm on paper).Two projecting system were used simultaneously in dark room. The mesangial cell were counted if observed in frame 1 but did not observe on frame 2. The cells counted that fall on the probe or touch upper and right lines of probe and did not touch with forbidden lines of probe (lower and left lines of frame 1). Finally about 100 mesangial cells were counted in each kidney sections. The final linear magnification was calculated 1200 times. The numerical density of mesangial cells per glomerule [N_v (mes/glom)] estimated via bellow formula (18).

N_v *(mes/glom)* = $\Sigma Q.M^2/\Sigma P.a.d$

ΣQ = counted mesangial cells

ΣP = sum of studied frames (reference sections)

a = disector area (2500 mm^2)

d = disector height (5 μ= 0.005 mm)

M= final linear magnification (1200)

Total mesangial cells number per kidney was estimated by the bellow equation (18).

N_{total} *(mes/kid)* = N_v *(mes/glom).* V_{total} *glom/kid*

Ethical issues

Ethical Committee of Lorestan University of Medical Sciences approved this study. The guidelines are in accord with the ethical principles of the International Committees for the Protection of Animal Rights Laboratory and Medical Research Council guidelines.

Statistical analysis

Statistical analysis was performed via Mann Whitney U-

test by SPSS 12 software. All values were showed as Mean ± SEM. A P value of < 0.05 was considered significant.

Results

Diabetes increased volume of renal cortex, glomeruli and mesangium when compared with the control group significantly ($P<0.05$). Treatment with CoQ10 improved these variables in comparison to diabetic – without treatment – group but the treatment could not save these variables at the same levels as that of the control group ($P<0.05$, Table 1).

The glomerular capillary volume (lumen of capillaries that blood circulate) decreased by diabetes in group 2 in contrast to group 1 ($P<0.05$). Although CoQ10 therapy improved it but could not save this variable at the same level as that of group 1 (Table 2).

The mesangial cells number increased in group 2 in contrast to group 1 ($P<0.05$). Coenzyme Q10 therapy in group 3 improved mesangial cells number in comparison to group 2 but could not save it at the same level similar to group 1($P<0.05$, Table 2).

Discussion

Mesangial cells are pericyte like cells that originate from metanephrogenic blastema in developing metanephros. These cells are located intracapillary (no intercapillary) in glomerular capillary tuft. Mesangial cells function includes maintaining organization of glomerular capillary, making and turnover of glomerular basement membrane (GBM) and mesangial matrix, regulating of filtration surface area and filtration pressure, cytokines production, cleaning of GBM and matrix of the mesangium (19,20).

In diabetes mesangial cells divide and also make extraordinary amount of mesangial matrix. The increased mesangium spreads into the capillary lumen and makes stenosis and finally occlusion of the capillary lumen (19,21).

High glucose condition induces generation of ROS in mesangial cells via enzymatic reactions through NADPH oxidase, cyclooxygenase, lipoxygenase, xanthine oxidase, and myeloperoxidase. High glucose increases activity of polyol pathway that leads to increased intracellular uric acid synthesis and finally uric acid stimulates NADPH oxidase enzyme (2). Reactive oxygen species also create through nonenzymatic reaction such as mitochondrial electron transport chain defects, advanced glycation end products (AGEs) generation, glucose autoxidation and fenton reactions (17,22). Between the ROS generating pathways, NADPH oxidase, AGEs and uncoupled nitric oxide synthase (NOS) are the most important molecules involved in the pathogenesis of diabetic nephropathy (1,2,19,23).

In diabetic condition ROS induce production of many cytokines that promote mesangial cells to division or over production of matrix (see introduction).

Coenzyme Q10 or ubiquinone is a lipid electron transport molecule found in all cell membranes, which participates in many redox reactions, involved in bioenergetics, nucleotide biosynthesis and antioxidant mechanisms (24,25). Also Co Q10 is a cofactor of other dehydrogenases, a modulator of the permeability transition pore and an essential antioxidant. The ability of this peculiar molecule to sustain continuous oxidation–reduction cycles makes it an excellent membrane antioxidant (10). Besides Co Q10 showed anti-apoptotic activities in the research study (26).

There are many studies that showed amelioration of mesangial expansion and glomerulosclerosis by different antioxidants, but most of these studies used semiquantitative or morphometric methods (27-29) that measured variables such as mesangial area, glomerulosclerosis or mesangial expansion semiquantitatively by scoring rule (30). In this study and our recent study (31), mesangial volume (mm^3) and

Table 1. Volume changes of cortex, glomeruli and mesangium through Co Q10 therapy in diabetic rats

Groups	Cortex volume (mm^3)	Glomerular volume per kidney (mm^3)	Mesangium volume per kidney (mm^3)
1: Control	616.18 ± 19.75	23.67 ± 0.82	3.248 ± 0.3
2: Diabetic without treatment	986.26 ± 28.41*	28.17 ± 1.019*	9.112 ± 0.51*
3: Diabetic treated with Co Q10 (15 mg/kg i.p.)	729.74 ± 23.36*#	25.84 ± 1.04*#	4.968 ±.0.29*#

Data were expressed as mean as Mean±SEM. * shows significant difference in contrast to group 1 at $P < 0.05$. # shows significant difference in contrast to group 2 at $P < 0.05$.

Table 2. The effect of Co Q10 therapy on glomerular capillary volume and mesangial cells number in diabetic rats

Groups	Glomerular capillary volume (mm)	Mesangial cells number per kidney
1: Control	21.21 ± 0.47	1412615.2 ± 102473.1
2: Diabetic without treatment	17.38 ± 0.32*	243525 6.6 ± 148524.4*
3: Diabetic treated with Co Q10 (15 mg/kg i.p.)	19.25 ± 0.36*#	2116417 ± 15998.15*#

Data were expressed as mean as Mean±SEM. * shows significant difference in contrast to group 1 at $P < 0.05$. # shows significant difference in contrast to group 2 at $P < 0.05$.

mesangial cells number were estimated by unbiased designed based stereological methods. Design-based stereology offers a powerful collection of methods for quantitative analysis of 3-dimensional tissue structure from histological 2-dimensional sections and these sensitive and efficient methods provide precise estimates with the guarantee of accuracy that give unbiased estimates (15).

Our result showed that diabetes increased mesangial volume. Increased mesangium ameliorated by using of Co Q10 similar to our recent study by using of *Satureja khuzestanica* essential oil (31). There is no other stereological study for comparing to our study by using of other antioxidant or other antidiabetic agents. Mesangial expansion is mainly the consequence of ROS effects on mesangial cells. Amelioration of mesangial expansion by Co Q10 showed that Co Q10 inhibited matrix overproduction or improvement of metalloproteinase activities probability via ROS inhibition or unknown pathways.

The glomerular capillary volume per kidney (lumen of capillary that blood circulate) decreased in diabetic animals and improved by CoQ10 treatment similar to use of *Satureja khuzestanica* essential oil (31). Although mesangium volume and glomerular capillary volume ameliorated by CoQ10 compared to diabetic rats but the treatment could not save mesangium and capillary volume at the same level as that of normal animals. There is no basal lamina between plasma membrane of mesangial cell and capillary endothelium and endothelial cell adjacent to mesangium is lack of GBM. The location of mesangium is intracapillary (no intercapillary) and increase of mesangium occupies the capilllary lumen and leads to stenosis and finally capillary occlusion. Stenosis and occlusion of capillaries decreases filteration area and finally reach ESRD.

Our results showed that diabetes raised mesangial cells number per kidney and use of CoQ10 inhibits it but could not save the number of mesangial cells as the same level as control group significantly. In our recent study *Satureja khuzestanica* essential oil maintains mesangial cells number at the same level of normal group that showed SKEO is the more effective than Co Q10 in inhibition of mesangial cell proliferation in diabetic condition. May be said that inhibition of mesangial cells proliferation related to ROS inhibition via free radical scavenging property of Co Q10 or probability by other unknown molecular mechanisms. Although our results showed that Co Q10 ameliorated mesangial expansion but this study did not show molecular mechanisms pathways involved in inhibition of mesangial matrix production or inhibition of mesangial cell proliferation by CoQ10 and is needed to future molecular investigations.

Conclusion

Coenzyme Q10 ameliorates significantly mesangial expansion in diabetic rats via inhibition of mesangial matrix production or mesangial cell number proliferation that is mediated through inhibition of oxidative stress.

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Authors' contribution

MT and HA designed the research. MT, HA and AT performed experimental works. MT analyzed the data and prepared the final draft of the article.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Conflicts of interest

The authors declare that they have no conflict of interest.

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