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Original research article

The comparative effects of perindopril and catechin on mesangial matrix and podocytes in the streptozotocin induced diabetic rats

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ABSTRACT

Background: Hyperglycemia and advanced glucose end substance (AGE) are responsible for excessive reactive oxygen species (ROS) production, which causes oxidative stress in diabetes mellitus. Oxidative stress and high blood pressure may cause injury and glomerulosclerosis in the kidney. End-stage kidney failure induced by glomerulosclerosis leads to microalbuminuria (Ma) in diabetic nephropathy. We investigated the effects of an angiotensin converting enzyme inhibitor (ACEI), perindopril, and an antioxidant, catechin, on podocytes and the glomerular mesangial matrix in experimental diabetic nephropathy using ultrastructural visualization and immunohistochemical staining.

Methods: We compared 5 groups of male adult Wistar albino rats: a control group, an untreated diabetic group, and diabetic groups treated with perindopril, catechin, or catechin + perindopril.

Results: Blood glucose values in all diabetic groups were significantly higher than in the control group ($p < 0.001$). The body weight in all diabetic groups was significantly lower than in the control group ($p < 0.001$, $p < 0.05$). The kidney weight in the catechin + perindopril-treated diabetic group was significantly lower than in the untreated diabetic group ($p < 0.001$). In all treated diabetic groups, Ma levels decreased significantly ($p < 0.001$). Mesangial matrix and podocyte damage increased in the untreated diabetic group, but the group treated with catechin + perindopril showed less damage. TGF-beta 1 immunostaining was significantly lower in the catechin-treated and perindopril-treated groups than in the untreated diabetic group ($p < 0.001$). Catechin was more effective than ACEI in preventing podocyte structure. Podocytes appeared to be the first cells affected in diabetes mellitus. When exposed to hyperglycemia, podocytes caused the mesangial matrix to expand.

Conclusions: Catechin and perindopril were more effective in preventing renal corpuscle damage when administered together.

Introduction

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus that causes end-stage renal disease. Hyperglycemia, high glomerular capillary pressure, various cytokines, and oxidative stress play important roles in the development of DN [4,34,44,60]. The characteristic properties of DN in the early stages of diabetes are increased glomerular volume and kidney size, which are detected by microalbuminuria (Ma), glomerular extracellular matrix accumulation, and glomerulosclerosis [46,51].

Podocytes, terminally differentiated and highly specialized cells, are the main components of the selective glomerular filtration barrier. They have primary cytoplasmic processes, which originate from the cell body, and secondary cytoplasmic processes, called pedicels, which originate from the primary processes.

Abbreviations: AII, angiotensin II; ACE, angiotensin converting enzyme; ACEI, angiotensin converting enzyme inhibitor; AGE, advanced glucose end substance; AII-RB, angiotensin II receptor blocker; BG, blood glucose; BW, body weight; DN, diabetic nephropathy; ECM, extracellular matrix; EM, electron microscopy; GBM, glomerular basement membrane; KW, kidney weight; Ma, microalbuminuria; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PAS, periodic acid-Schiff; RAAS, renin–angiotensin–aldosterone system; RAS, renin–angiotensin system; ROS, reactive oxygen species; STZ, streptozotocin; TGF-beta 1, transforming growth factor-beta 1.

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Pedicels are aligned under the glomerular basal membrane (GBM) [11,42]. GBM thickening, mesangial matrix expansion, and podocyte hypertrophy have been observed in many studies of diabetic nephropathy. According to these studies, the loss of podocytes and the abnormal function of slit pore membranes cause proteinuria [30,38,45].

High blood glucose levels and hyperglycemia are responsible for excessive ROS production, which causes oxidative stress. This is the major cellular event responsible for DN [22]. Angiotensin II (AII) causes ROS to accumulate in the vessel walls of hypertensive patients. The increase in ROS causes oxidative damage in the slit pore membrane, which can lead to the development of Ma [1,38].

In addition, hyperglycemia accelerates the formation of diacylglycerol and activates protein kinase C, leading to the overexpression of the gene encoding transforming growth factor beta (TGF-beta) and thereby to the expansion of matrix components in mesangial cells and glomeruli [23]. TGF-beta 1, a fibrogenic growth factor produced locally in the kidney, is involved in the pathogenesis of kidney damage [8,39]. Locally increased AII in diabetes, increases NADPH oxidase by stimulating the production of superoxide. Superoxide reacts with nitric oxide (NO), and oxidized NO induces podocyte hypertrophy and mesangial cell proliferation. By stimulating the synthesis of collagen and fibronectin, TGF-beta promotes the expansion of the mesangial matrix and the interstitial matrix [2]. Expression of TGF-beta 1 by podocytes stimulates sclerosis in the mesangial matrix, induces the apoptosis of glomerular cells, and causes hypertrophic and ultrastructural changes in podocytes [51,52].

Some studies have reported that traditional and new therapies administered separately or together can prevent or reverse the development of diabetic nephropathy. Podocytes were the target cells in these studies [30,37,45].

Green tea, prepared from the leaves of *Camellia sinensis* L., is popular worldwide [36]. Catechins in green tea bind ROS, free radicals, and peroxynitrites and thereby act as antioxidants. Their antioxidant effects are renoprotective [57]. Green tea polyphenols inhibit mesangial cell proliferation and prolong the survival of the kidney in experimental glomerulosclerosis.

It has been reported that ACEIs prevent mesangial proliferation in the kidney. According to some studies, perindopril, an ACEI, decreases the capillary blood pressure in the glomerulus, which reduces Ma [1]. Despite treatment of diabetic nephropathy patients with agents such as ACEIs, angiotensin II receptor blocker (AII-RB), and antihypertensives, large numbers of diabetic patients continue to suffer from nephropathic complications [3].

In this study, using ultrastructural, histological, and biochemical methods, we compared the renoprotective effects of catechin and perindopril, administered separately and together, on the mesangial matrix and podocyte damage.

Materials and methods

Animals and protocols

All experiments were approved by the ethics committee of Istanbul University Medical School of Cerrahpasa. Animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Male Wistar albino rats (180–200 g, 10–12 weeks old) were obtained from the Experimental Research Laboratory at Istanbul University, Cerrahpasa Faculty of Medicine. The blood glucose levels and body weights of all animals were measured at the beginning of the study. Forty rats were randomly assigned to 5 groups, each containing 8 rats. One group was used as the control group. All other groups received a single injection of streptozotocin (STZ; 60 mg/kg, *ip*; Sigma, St. Louis, MO, USA). The second day after the injection of STZ, the rats became diabetic. Of the 4 STZ-treated groups, one was used as an untreated diabetic group. A second was treated with perindopril (6 mg/kg/day, gavage; Servier, Istanbul, Turkey) for 30 days after STZ injection. A third was treated with catechin (20 mg/kg/day; C0567; Sigma, St. Louis, MO, USA) starting 7 days before the injection of STZ and continuing for 30 days after. The fourth was treated with catechin and perindopril as described for the individual treatments. All animals had free access to standard rat chow and drinking water. At the end of the experiment (day 31), the rats were anesthetized with ketamine (90 mg/kg, *ip*) and xylazine (10 mg/kg, *ip*) and sacrificed after both kidneys were excised.

Blood glucose (BG)

The glucose levels (mg/dl) in blood taken from the rat tail were measured using the Freestyle MediSense Optium Blood Glucose Monitoring System (Abbott Laboratories, Illinois, USA) at 3 different times: before the experiment, 48 h after STZ injection, and at the end of the experiment.

Microalbuminuria (Ma)

Rats were placed in metabolic cages and housed for 24 h. Urine samples were collected after 24 h on days 1, 15, and 30. Ma levels were measured by the Central Biochemistry Laboratory of the Cerrahpasa Faculty of Medicine.

Kidney weight (KW)

At the end of the experiment, rats were anesthetized and sacrificed. The kidneys were excised and washed with physiologic saline solution. The kidney weights were measured using a sensitive scale and recorded.

Body weight (BW)

On the first day of the experiment and before anesthetization on the last day of the experiment, the BW of each rat was measured using a scale and recorded.

Immunohistochemistry

The right kidney of each rat was excised, placed into 10% neutral formalin for fixation, embedded in paraffin, and cut into sections 5-μm thick. The sections were mounted on slides coated with poly-L-lysine (PLL; Sigma, St. Louis, MO, USA), deparaffinized with toluene (Merck, Germany), and rehydrated with a graded alcohol series (Merck, Darmstadt, Germany).

Immunoperoxidase staining was performed using the Histostain-*Plus* Bulk Kit (Zymed LAB-SA Detection System, 85-9043) and a rabbit polyclonal TGF-beta 1 antibody (1:200; sc-146; Santa Cruz Biotechnology) according to the streptavidin–biotin-peroxidase method. Immunostaining procedures were carried out following the guidelines of the manufacturer. The sections were heated for 15 min in 10 mM citric acid buffer, pH 6 (DAKO, Glostrup, Denmark) in a microwave oven. Afterwards, to inactivate the endogenous peroxidase activity, the sections were incubated with 0.3% H_2O_2 in methanol for 10 min. The sections were then washed with PBS and incubated with normal blocking serum (goat serum). Sections were incubated overnight with TGF-beta 1 antibodies at 4 °C, washed with PBS, incubated with biotinylated secondary antibody (goat-anti mouse), and washed again with PBS. Sections were incubated with a substrate-chromogen solution (AEC; Zymed, San Francisco CA, USA) for 5–6 min and then incubated with a horseradish peroxidase–streptavidin complex. Sections were counterstained with hematoxylin.