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Regulation of the Ku70 and apoptosis-related proteins in experimental diabetic nephropathy

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ABSTRACT

Background. Apoptosis contributes nephropathy pathogenesis in diabetes. However, its mechanisms still remain unclear. We examined the extent to which the angiotensin-II type 1 receptor blocker (AT1RB) irbesartan and the angiotensin converting enzyme inhibitor (ACEI) perindopril affected the apoptosis-related proteins Bcl-2, Bax, caspase-3, cytochrome-c and Ku70 in streptozotocin (STZ)-diabetic rats.

Materials and methods. Animals were divided into five groups of eight each, four of which received STZ (60 mg/kg in a single dose, i.p.) to induce diabetes. The groups were performed as untreated diabetic; non-diabetic control; daily irbesartan (15 mg/kg/day) or perindopril (6 mg/kg/day) and also combined irbesartan and perindopril (respectively, 5 mg/kg/day, 3 mg/kg/day) were applied by gavage for 30 days to STZ-diabetic rats. The kidney tissue analysis was performed by using immunohistochemical staining with Bcl-2, Bax, caspase-3, cytochrome-c and Ku70 antibodies and by using Western blot analysis with caspase-3 and cytochrome-c antibodies.

Results. Immunoreactivity of Bax, caspase-3, cytochrome-c and Ku70 was increased in the tubuli and glomeruli of the untreated diabetic group, but decreased in all treated diabetic groups. In the irbesartan and perindopril treated diabetic groups Bcl-2 immunoreactivity was higher than that of the untreated diabetic group. Caspase-3 and cytoplasmic cytochrome-c protein levels increased in the untreated diabetic group.

Conclusions. We conclude that the increased expression of Bax and caspase-3, and the increased level of cytoplasmic cytochrome-c relate to renal tissue injury. This case is also seen in the early stages of diabetes as a result of the damage caused by local increased expression of renin angiotensin system (RAS) in the renal tissue, which is induced by hyperglycemia. The increase of the cytosolic cytochrome-c, caspase-3 and Ku70 expression in the tubuli is suggestive of apoptosis. Overall, our results show that treatments of irbesartan and perindopril are effective and efficient in preventing renal tissue injury and apoptosis by blocking the RAS in experimental diabetic nephropathy and reducing the expression of proteins associated with apoptosis.

Abbreviations: AII, angiotensin II; ARB, angiotensin receptor blocker; AT1RB, angiotensin-II type 1 receptor blocker; ACEI, angiotensin converting enzyme inhibitor; APAF-1, apoptotic protease activating factor-1; DSB, DNA double-strand breaks; nCLU, nuclear clusterin; ROS, reactive oxygen species; RAS, renin angiotensin system; sCLU, secreted clusterin; STZ, streptozotocin.

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1. Introduction

The pathogenesis of diabetic nephropathy is a chronic and complex process, in which the glomeruli are affected in the early stages. Thickening of glomerular basement membrane, hypertrophy and albuminuria are followed by glomerulosclerosis and then with the gradual decline of renal function, tubular atrophy and interstitial fibrosis [1]. Although glomerular damage is a hallmark of renal injury in diabetes, studies reported that tubular injury might be a better predictor of renal disease progression than glomerular pathology [2,3].

Hyperglycemia is one of the most important factors contributing to the pathogenesis of diabetic nephropathy [1]. In diabetic nephropathy a high concentration of glucose contributes and stimulates apoptosis but its mechanisms remain unclear [4]. Apoptosis, which is a common feature of both acute and chronic renal diseases, results in excessive renal cell loss [5,6]. Apoptosis plays a critical role in tubular atrophy in diabetic patients [7] as well as in the diabetic nephropathy animal models [6,8,9]. The mechanisms underlying the apoptosis of tubuli in the diabetic kidney are currently being studied, and appropriate approaches to minimize the damage are under development.

Metabolic changes induce apoptosis by regulating the expression of apoptosis controlling genes; this could be an important factor contributing to the pathogenesis of diabetic nephropathy. It has been shown that the increased glucose level causes a decrease in the expression of anti-apoptotic Bcl-2 gene and an increase in pro-apoptotic Bax in tubular epithelial cells [9]. Bcl-2 prevents apoptosis by binding to the external mitochondrial membrane and preventing the release of cytochrome-c from mitochondria [5]. The increase of Bax expression leads to release of cytochrome-c, formation of the apoptosome, and activation of caspase-9 [10]. On the other hand Ku70 as the DNA repair protein involves the regulation of cell death by suppressing Bax activity [11]. It has been reported that Ku70 has two localizations both in the nucleus and in cytoplasm, but still there is no clarity on the Ku70 functions in this cellular compartments [12,13].

The role of caspases in renal tubule and tubulointerstitial cell damage has been demonstrated by various experimental models [14–16]. Bamri-Ezzine et al. [17] reported increased caspase-3 activation in the kidneys of STZ-diabetic rats. Kang et al. [18] suggested that high glucose stimulates caspase-3 activation and DNA fragmentation in cultured human mesangial cells.

Blockade of the renin-angiotensin system (RAS) is a major therapeutic strategy in diabetic renal disease [19,20]. In addition to their beneficial effects on renal function, angiotensin type-1 receptor blockers (AT1RB) and inhibitors of angiotensin converting enzyme (ACEI) effectively prevent tubular damage in inflammatory, metabolic, hemodynamic and mechanically induced renal injury [21–23]. Intrarenal RAS activation and high glucose may act in concert to increase tubular apoptosis and pro-apoptotic gene expression in diabetes [24].

In this study, we researched the effects of irbesartan as an AT1RB and perindopril as an ACEI on the expression of apoptosis-regulator proteins including anti-apoptotic Bcl-2, pro-apoptotic Bax, caspase-3, cytochrome-c and Ku70 in renal tissue by immunohistochemical assays, and by Western blot analysis for detection of caspase-3 and cytochrome-c expression levels. The mechanism of increased apoptosis in the renal tubules in diabetes-induced nephropathy is still not clear. In this study, we aimed to investigate effects of RAS blockade on the expression of apoptotic genes and to reveal the role of Ku70.

2. Materials and Methods

2.1. Ethics Statement

The study was approved by the Animal Welfare and Ethics Committee of Istanbul University. The procedures involving experimentation on animal subjects were performed in accordance with both the guidelines of Istanbul University, and with the National Research Council's guidelines for the care and use of laboratory animals.

2.2. Animals

Wistar type albino male rats of weighing approximately 210–240 g were purchased from Istanbul University, Institute of Experimental Medicine Research (DETAE). The animals had free access to standard rat chow and drinking water.

2.3. Experimental Induction of Diabetes

Diabetes was experimentally induced with an intraperitoneal injection of streptozotocin (STZ; Sigma, St. Louis, MO) at a single dose of 60 mg/kg body weight freshly dissolved in 0.9% sodium chloride. To confirm the diabetic state, blood glucose concentration was measured three days after STZ injection. The rats with blood glucose 350 mg/dL were considered to be diabetic.

The diabetic animals were divided into four groups (n = 8 in each group): Group 1 received no treatment, group 2 received irbesartan (Sanofi, TURKEY) dissolved in 0.9% sodium chloride (15 mg/kg/day, gavage, 30 days), group 3 received perindopril (Servier, Turkey) dissolved in 0.9% sodium chloride (6 mg/kg/day, gavage, 30 days) and group 4 received both irbesartan (5 mg/kg/day, gavage, 30 days) and perindopril (3 mg/kg/day, gavage, 30 days). All diabetic animals (STZ-diabetic, ACEI and AT1RB treated diabetics) did not receive any insulin therapy. Eight animals served as non-diabetic controls (group 5).

After 31 days of study, animals were killed under anesthesia [60 mg/kg ketamine (Ketalar, Parke-Davis, Eczacıbası, Istanbul, Turkey) and 9 mg/kg xylazine (Rompun, Bayer, Istanbul, Turkey), i.m.]. Kidneys were removed for histological examination, immunohistochemistry and Western blot analysis.

2.4. Blood Glucose

On the 1st day, 15th day and experiment end blood glucose levels of all groups were measured using glucose test reagent strips (Accu-Check Active Glucose test strips, Roche, Germany) with a glucometer (Accu-Check Active, Roche, Germany) in samples obtained from the tail vein.