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TGF-b and Diabetes Mellitus

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Abstract: Diabetes mellitus is categorized as a major metabolic disease. Hyperglycemia is the populest mediator. The oxygen radicals' production or the peroxide production in the mitochondria is preceded as a possible mechanism. TGF-beta is characterized as the main protagonist. TGF-b is a molecular mediator with adherent and hypertrophic properties in diabetic nephropathy. 20 Balb/c male and female mice were used. They were separated in two age groups, one of 4 weeks old (childhood, n= 10) and one of 6 weeks old (puberty, n=10). An immunochemical study for TGF-beta was undertaken. The basal petal of the external membrane of Bowmen is thickened. In significant percentage the basal membrane was thickened and the peduncles merge together. Mature sperm cells were not normal. The two age groups did not present significant differences in morphology of the exocrine pancreatic cells. The augmented levels of TGF-beta expression promote the increased proliferation of the pancreatic glandular cells, the derangement of differentiation and the apoptosis rhythm. The renal function is diminished sooner than the clinical manifestations. The testicles are less influenced concerning principally the spermiogenesis.

Key words: TGF-b, diabetes mellitus, kidney, pancreas, testicles.

1. Introduction

Diabetes mellitus is categorized as a metabolic disease. It is characterized by the highest morbidity and mortality in the past few years. The phenotype of hyperglycemia is observed. There are two major subgroups: type 1 and type 2. Other types of diabetes are also observed.

One of the major complications of diabetes is diabetic nephropathy. Almost one third of diabetic patients suffer from this kind of disease. 44% of patients with end stage renal failure in United States have been diagnosed with diabetes. Four theories attempt to explain the mechanism of developing diabetic nephropathy [1, 2].

At first, glucose promotes the production of enzymatic mediated glucozilated protein, intracellular and extracellular. These products accelerate the glomerular dysfunction, the disposal of extracellular matrix and the derangement of the structures architecture. There is a linear relation between the quantity of products and the GFR reduce.

In the second theory, the percentage of metabolized glucose in the sorbitol pathway is examined. This process is mediated by reductase of aldolase. Sorbitol augments the redox dynamic, the cytoplasmic osmolality, the production of active oxygen radicals, and finally the cytoplasmic dysfunction. The results in nephropathy therapy in humans were not the ones expected when the reductase inhibitors were used [3].

The third hypothesis approaches the dialcyglycerol production by glucose. The first one activates protein kinas C. This kinase transform the expression of genes encoding collagen type IV, extracellular matrix, fibronenctin, contraction proteins in the endothelium cells [4-6].

In the fourth theory glucose follows the exozamine and the phosphate glucose. The last one consists the underlying for the oxygen depended glycosylation and the glycozamine production. The exozamine production possibly alternates the endothelium synthetase of NO. The TGF-b or the inhibitor of the plasminogen activator expression is also influenced [7, 8].

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In all four cases the hyperglycemia is the common mediator. A possible common mechanism in all theories could be the oxygen radicals' production or the peroxide production in the mitochondria. Definitely the hyperglycaemia is the first and most important cause. The specific mechanism affecting certain organs remains unknown [9].

Despite the fact that the clinical marks of the nephropathy are noticed 15-20 years after the disease onset, the thickening of the basal membrane and the increase of the extracellular matrix are obvious in the first decade. TGF-beta is characterized as the protagonist in all cases.

Additionally it is proved in cell cultures, animal experimental models and clinical studies that hyperclycaemia has a definitive role in diabetic nephropathy. Different growth factors and cytokines have been discovered. The most dominate seems to be TGF-beta. It is a cytokine with fibrogenetic and hypertrophic role. The hyperexpression of TGF-beta has been noticed in the tubules and the mesangium. As a result the receptor and the cataract of Smad pathway are activated.

All the molecular mediators and the endoplasmic pathways of signaling that are part of the development of diabetic nephropathy, promote the action of TGF-beta as an intermediate mediator. The high glucose concertation , non- enzymatic glycolization proteins, the oxidetive stress , the peroxide hyperproduction by the mitochondrial chain of electron transportation, the cyclic process of contraction and relaxation of the mesangial cells in cell cultures (the process resembles to the intraglomerular hypertension), the de novo diacylglycerol production and the activation of proteinic kinase by mitogenetic factors, the hyperproduction of glycozamines and the high levels of vasoactive substances such as angiotensin II, thromboxanes, and endothelin enforce the action of TGF-b [10, 11].

TGF-b is a molecular mediator with adherent and hypertrophic properties in diabetic nephropathy. More precisely this molecule promotes the production and the deposition of extracellular matrix components such as collagen type I and IV, fibronectin and laminin. In the same time TGF- beta prevents the decomposition of the extracellular matrix and inhibits the ptrotease action. The inhibition of proteases such as the inhibitor of the plasminogen activator is also noticed. Additionally TGF-beta promotes the interaction of the cell with the matrix. This is achieved by intergrin production and the surface receptors of cells [12].

Treg cells are suppressed by TGF-beta. Treg cells are responsible for diabetes influencing the pancreas function. Treg cells suppress the macrophage function that takes place in pancreas and repress the pancreatic cell proliferation. The mechanism is autoimmune. The procedures are not unambiguous [13-15].

The hyperglycaemia influences all the systems, including the genetic. The bibliography is not sufficient. Experimental data prove the sperm's production derangement [4, 16].

The discovery of factors that promote the degradation and the rebuilding of the matrix in diabetic nephropathy, are necessary for the design of therapies targeting the glomerural sclerosis and the tubular fibrosis. These therapies reduce the proteinuria and the developing renal failure [6, 8, 17].

2. Materials and Methods

20 Balb/c male and female mice were used. They were separated in two age groups, one of 4 weeks old (childhood, n = 10) and one of 6 weeks old (puberty, n = 10).

Each age group was afterwards separated in two subgroups. In the first, saline water was administered subcutaneously. The second group received alloxane (200 mg/kg of body weight). Diabetes was developed. After 20 hours fasting, alloxane was administered. After two days the blood glucose was measured with the help of a saccharimeter. As diabetic mice were characterized those with glucose levels above the level of 250 mg/dl. The animals were provided a full free

access to food, water and an environment free of certain germs. Every age group was observed for 5 weeks during which fasting glucose was measured every second day.

In diabetic mice, every two days 0,2 U of slow release insulin were administered. The target was the maintenance the glucose levels between 250-300 mg/dl and the avoidance of ketosis. After 5 weeks the mice were sacrificed. Pancreas, kidneys, testicles were taken. These organs were studied with electron microscope. An immunochemical study for TGF-beta was undertaken. The Rabbit anti-Human TGF Beta 1 Polyclonal Antibody was used.

3. Results and Discussion

The main abnormalities are noticed in the kidney in renal corpsules. The distal urinary cavity is distended. The basal petal of the external membrane of Bowmen is thickened. In significant percentage the basal membrane was thickened and the peduncles merge together. The majority of proximal and distal convoluted tubules were normal. In some big vacuoles were noticed in the cells and the epithelium was multilayer. Focally the connective tissue was increased with collagen fibre bundles.

Nothing has been referred in bibliography for the testicles. Normal spermatogonial cells, sperm cells type I and II and spermatids have been noticed. Nevertheless mature sperm cells were not normal except some heads of them. In tails, there is a lack of microtubules and mitochondria of the media sheath. Leydig cells were mainly isolated and some of them degenerated.

The two age groups did not present significant differences in morphology of the exocrine pancreatic cells. The number and the size of Langerhans islets were remarkably diminished in mice of the second age group (6 weeks old). From the immunochemical aspect TGF-beta was located in the connective tissue, vessels and the cytoplasm of Langerhans islets. The expression of the factor was more boosted in the second age group.

4. Conclusions

The augmented levels of TGF-beta expression promote the increased proliferation of the pancreatic glandular cells, the derangement of differentiation and the apoptosis rhythm. The farther result was the disvantageous insulin secretion due to the devastation of Langerhans islets [6, 17].

The renal function is diminished sooner than the clinical manifestations. The testicles are less influenced concerning principally the spermiogenesis [18].

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