·Basic Research·

Expression of transforming growth factor-β type 1 receptor and transforming growth factor-β type 2 receptor in diabetic rat retina

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Foundation items: National Natural Science Foundation of China (No.30271391); Bureau of Heath Project, Shanghai (No.034124); Training Plan for Hundred Excellent Subject Bell Wether Going Beyond the Century in Shanghai Public Heath System (No.057)
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Received: 2008-02-20 Accepted: 2008-10-21

Abstract

• AIM: To quantitatively detect gene expression level of transforming growth factor-β type 1 receptor (TβR 1) and transforming growth factor-β type 2 receptor (TβR 2) in different stage of diabetic rats' retina and to observe and analyze the effect of transforming growth factor-β receptors on the retina of rat diabetic animal model.

• METHODS: Twenty-eight healthy adult Sprague-Dawley rats were chosen and randomly divided into two groups of normal control (CON) and diabetes mellitus (DM). Diabetes was induced by streptozotocin (STZ) intraperitoneal injection. Gene expression was detected quantitatively with real-time fluorescence quantitative reverse transcription polymerase chain reaction (QRT-PCR).

• RESULTS: The mRNA level of TβR 1 and TβR 2 was 0.000493±0.000133 and 0.000166±0.000057 at 4 weeks. The mRNA level of TβR 1 and TβR 2 was 0.000608±0.000232 and 0.000113 ±0.000049 at 12 weeks. TβR 1 expression was gradually elevated during the progression of diabetic retinopathy. TβR 2 expression was up-regulated at 4 weeks, but down-regulated at 12 weeks.

• CONCLUSION: TGF-β and its receptors (TβR 1 and TβR 2) may play important role in the pathogenesis of diabetic retinopathy.

• KEYWORDS: diabetic retinopathy; transforming growth factor-β; transforming growth factor-β receptor; streptozotocin; real-time quantitative PCR

Cao D, Liu L, Shen W. Expression of transforming growth factor-β type 1 receptor and transforming growth factor-β type 2 receptor in diabetic rat retina. *Int J Ophthalmol* 2008;1(4):297-300

INTRODUCTION

Diabetic retinopathy (DR) is the main complication of diabetes mellitus (DM), and is one of the most leading causes of blindness world-wide. DR is a progressive microangiopathy characterized by small vessel damage and occlusion. The earliest pathologic changes are thickening of the capillary endothelial basement membrane and reduction of pericytes. With the progressive microvascular damage, proliferative diabetic retinopathy (PDR) develops. Neovascularization is the main pathologic changes of PDR. But the pathogenesis of DR is still not fully understood. Recent studies show that cytokines are involved in the pathogenesis of DR in general.

Transforming growth factor-β (TGF-β) has been shown to be a multifunctional cytokine that modulates biological events as diverse as cell-cycle control, the regulation of early development, differentiation, proliferation, migration, extracellular matrix formation, hematopoesis, angiogenesis, chemotaxis, immune functions, and the induction of apoptosis[1]. Since its initial discovery, a myriad of biological effects, both paracrine and autocrine, have been attributed to TGF-β, qualifying it as a fundamental regulatory cytokine. All these functions are transduced through the TGF-β signaling pathways. TGF-β transduces signals through the mediation of its specific receptors [2,3]. There are two major types of receptors, transforming growth factor-β type 1 receptor (TβR 1) and transforming growth factor-β type 2 receptor (TβR 2), transmembrane serine/ threonine kinase receptors, which are widely expressed and are believed to be the signaling molecules. In the earlier study [4], it determined that TGF-β acts as mediator in development of PDR. There is, however, little information available on the process of TGF-β receptors induction in retina of Diabetic rats.

In this study, we investigated the expression of TβR 1 and TβR 2 in different stage of diabetic rats' retina with real-time fluorescence quantitative reverse transcription polymerase chain reaction (QRT-PCR), and discussed the

possible functions of TβR 1 and TβR 2 in the pathogenesis of diabetic retinopathy.

MATERIALS AND METHODS

Experimental Animals Twenty-eight adult male Sprague-Dawley rats (180-200g) were provided by Shanghai Laboratory Animal Center. Adaptability feeding for 2 weeks in a well-ventilated environment with an ammonia concentration of ≤20ppm, a relative humidity of 40%-70%, 24 hours natural light illumination, and a room temperature of 18 to 22℃. Feed with ordinary lump-like rat feedstuff, with free intake of water and food.

Diabetic Rat Preparation Rats were randomly divided into two groups, 12 in the normal control (CON) and 16 in the diabetes mellitus (DM). With 0.1mol/L, pH4.2 citrate buffer, streptozotocin (STZ) was dissolved for the preparation of 10g/L STZ solution. Diabetic rats were induced by streptozotocin (STZ) intraperitoneal injection with a dosage of 55mg/kg body weight. At 24 to 48 hours after the injection, the tail blood was collected to determine the level of blood glucose. If the blood glucose level > 16.65mmol/L, the rat then is considered as a diabetic rat. One rat in the DM group died. The blood glucose level and body weight were determined regularly after that. In experiment period, 15 rats were successful induced DM, 1 rat died. So 7 in DM were killed at 4 weeks, 8 in DM were killed at 12 weeks.

Total RNA in Retina and the First Strand cDNA Preparation The eye globes of the experimental animals were enucleated after the determination of their body weight and fasting blood glucose levels at 4 weeks and 12 weeks. The anterior of the globe and lens were removed and the retina were peeled off and immediately placed into liquid nitrogen for preservation. With Trizol reagent (Invitrogen. USA), total RNA was extracted. Then its purity and content were determined and its integrity was determined by 10g/L formaldehyde agarose gel electrophoresis. 5μg sample of total RNA was reverse transcripted into the first strand cDNA by 1μL of SuperScript 2-RTase.

Primer Design On the basis of the principle of standard QRT-PCR primer design, 6-carboxyfluorescein (FAM) was selected as the fluorescent reporter group for the TaqMan fluorescent probe, and 6-carboxytetramethy-lrhodamine (TAMRA) was selected as the quenching group. Based on the PCR primer sequence of gene TβR 1, TβR 2 and 18s of rats, as well as the TaqMan probe sequence, the sequence of the primer synthesis is as follows: T (RI-F: 5'>ACC TTC TGA TCC ATC CGT T<3'; T (RI-R: 5'>CGC AAA GCT GTC AGC CTA G<3'; T (RI-TM: FAM 5'>CAG AGC TGT GAG GCC TTG AGA GTG<3' TAMRA; T (RII-F: 5'>CCC TAC TCT GTC TGT GGA TGA<3'; T(RII-R: 5'>GAC GTC ATT TCC CAG AGT AC<3'; T(RII-TM: FAM 5'>CAG GTG GGA ACA GCG AGA TAC ATG G<3' TAMRA; 18s-F: 5'> GTA ACC CGT TGA ACC CCA TT <3'; 18s-R: 5'> CCA TCC AAT CGG TAG TAG CG <3'; 18s-TM: FAM 5'> ATG GGG ATC GGG GAT TGC AAT <3' TAMRA.

Table 1 The levels of body weight of normal rats and diabetic rats in different stage ($\bar{x}\pm s$)

Group	4wk	12wk
Normal	292.00±29.50	375.83±13.57
Diabetic	177.86±14.39	167.50±14.64
t	8.970	27.160
P	<0.01	<0.01

Table 2 The blood sugar levels of normal rats and diabetic rats in different stage ($\bar{x}\pm s$)

Group	4wk	12wk
Normal	4.94±0.97	4.42±0.27
Diabetic	21.09±4.09	25.20±3.24
t	8.540	15.511
P	<0.01	<0.01

Quantitative PCR Reaction and Analysis The quantitative PCR determination and analysis were performed in accordance with the methods introduced in the reference [5]. With the fluorescent curve and CT values, calculate the ratio of TβR 1/18s and TβR 2/18s respectively in the same tissue at different time points. Results were expressed in the form mean ±SD of and two-sample t-test was used for the comparison of the values between the groups.

RESULTS

Observation of the Animal General Conditions The blood glucose levels of all the diabetic rats were higher than 16.65mmol/L, with the symptoms of polydipsia, polyphagia, polyuria, gradual loss of body weight. Compared with the rats in control group, the diabetic rats had the characteristics of bradykinesia, acedia, non-aggressive and susceptible to infections.

Changes in Blood Glucose and Body Weight of Rats in Both Groups at Different Stage At each time point, the statistic differences in body weight were highly significant between diabetic rats and normal control rats (P<0.01, Table 1). In the normal control group, the blood glucose level was steady and no significant differences among different time points (P>0.05, Table 2). However, the blood glucose level of the diabetic rats maintained a high level throughout the experiment, and the statistic differences were highly significant when compared with that of the normal control group (P<0.01, Table 2).

Expressions of TβR 1/2 mRNA in Different Stage The RNA of rat retina was integrative enough to be used to further ORT-PCR analysis. Compared with control group, the mRNA level of TβR 1 and TβR 2 was 0.000493±0.000133 and 0.000166±0.000057 at 4 weeks. The mRNA level of

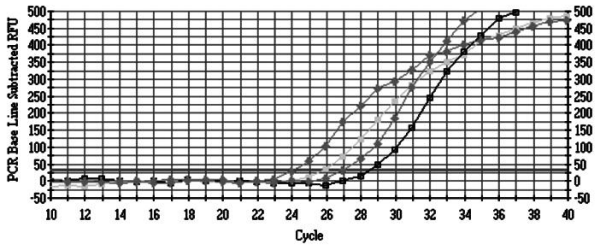

Figure 1 QRT-PCR curve for TβR 1/2 in retina of normal rats and diabetic rats. From left to right is TβR 1 in retina of diabetic rats, TβR 1 in retina of normal rats, TβR 2 in retina of diabetic rats and TβR 2 in retina of normal rats

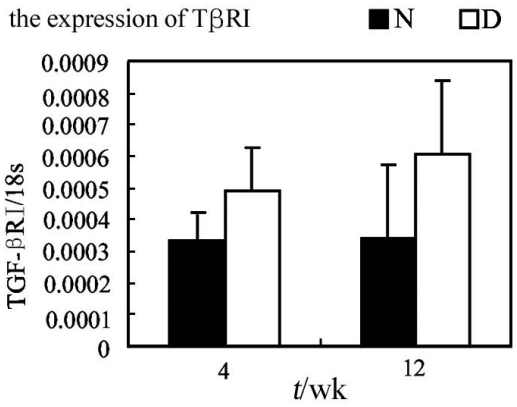

Figure 2 The mRNA levels of TβR 1/18s is 0.000493±0.000133 at 4 weeks (P<0.05), the mRNA levels of TβR 1/18s is 0.000608±0.000232 at 12 weeks (P<0.05)

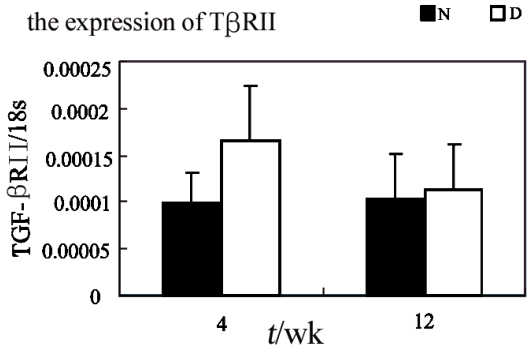

Figure 3 The mRNA levels of TβR 2/18s is 0.000166±0.000057 at 4 weeks (P<0.05), the mRNA levels of TβR 2/18s is 0.000113±0.000049 at 12 weeks

TβR 1 and TβR 2 was 0.000608±0.000232 and 0.000113±0.000049 at 12 weeks. The expressions of TβR 1 mRNA in diabetic rats' retina were up-regulated obviously at 4 weeks and 12 weeks, the difference being statistically insignificant (P<0.05). TβR 2 expression was up-regulated at 4 weeks, the difference being insignificant (P<0.05), but down-regulated to normal level approximately at 12 weeks (Figures 1-3).

DISCUSSION

Diabetic retinopathy (DR) is one of the most important complications of diabetic microangiopathy. Proliferative diabetic retinopathy (PDR) is featured as neovascularization and fibrosis. Along with the technological development in molecular biology, it has been discovered that the occurrence and development of DR is related to the abnormal regulation of cell proliferation, and several cytokines and growth factors are closely related to the growth and proliferation of several retinal cell types, among which TGF-β is an important factor. Three isoforms are present in mammals, *i.e.*, TGF-β1, TGF-β2, TGF-β3, and mainly expresses TGF-β2 in retina. Previous studies indicated that the concentration of TGF-β2 in the vitreous of PDR patients is significantly higher than that in those without PDR[4]. Under the functions of TGF-β and other cytokines, retinal pigment epithelial cells and retinal glial cells are relocated under the vitreous and retina, and the macrophages and fibroblasts are stimulated to produce collagen, fibronectins (FN) which might make the capillary basement membrane thickened and promote the formation of capillary lumen[6].

After ligand binding and activation of TGF specific cell membrane receptors and signals are transduced from the membrane to the nucleus via intracellular effectors, termed Smads, which then produce biological effects [2]. We think that the higher gene expression level of TGF-β receptors should be observed in the disease process. TGF-β receptors is a kind of surface membrane receptor, including three distinct forms, *i.e.* TβR 1, TβR 2 and TβR 3, among which TβR 1 and TβR 2 are the two important receptors in the TGF-β signals. Currently, it is considered that Tβ R 1 and Tβ R 2 are essential to the TGF-β signals [7]. TGF-β signals through a heteromeric complex of TβR 1 and TβR 2 transmembrane serine/threonine kinase receptors. In addition, it was found that, by the methods of immunohistochemistry and *in situ* hybridization, the expression of both TβR 1 and TβR 2 could be detected on the retinal pigment epithelial cells and retinal glial cells of both normal rats and the rats with retinal neovascular disease [8]. Both of the immunohistochemistry and in situ hybridization are the methods for the locating and semi-quantitative determination of gene expression. However, real time fluorescence quantitative reverse transcription PCR (QRT-PCR) could specifically and accurately detect gene expression level in rat retina. In our study, we detected the gene expression level of TβR 1/2 in different stage of diabetic rats' retina using fluorescence Quantitative reverse transcription PCR. The expression of TβR 1/2 gene mRNA were demonstrated quantitatively by QRT-PCR.

The current study showed that the expressions of TβR 1 mRNA in diabetic rats' retina were up-regulated obviously at 4 weeks and 12 weeks, the expressions of TβR 2 mRNA up-regulated at 4 weeks, but down-regulated to normal level approximately at 12 weeks. Our finding demonstrated that

TβR 1 expression was gradually elevated during the progression of diabetic retinopathy, whereas elevated expression of TβR 2 mRNA was a short term phenomenon. These results suggest that TβR 1 and TβR 2 plays an important role in the occurrence and development of early diabetic retinopathy, it may relate to the distinct structural characteristics of TβR 1 and TβR 2, as well as their distinct functions in TGF-β signaling pathways. Due to its structural characteristics, TβR 2 itself has kinase activities and needs no activation, whereas TβR 1 doesn't have such natural activities, but after the formation of heteromeric complex of type 1 and type 2 receptors, the kinase domains of TβR 1 and TβR 2 become similar, TβR 1 could be activated by TβR 2, then to activate Smads to transduced signals to nucleus, hence regulate the transcription of target genes. Experimental studies have confirmed that TβR 1 is involved in the formation of extracellular matrix and TβR 2 is involved in the cell proliferation[9]. Our experiment indicated that with the expression level of TβR 1 and TβR 2 changing, the content ratio of TβR 1 and TβR 2 changes accordingly, which may affect TGF-β signaling pathways and alter the regulation on target cells, hence promote the formation of diabetic retinopathy. Our data suggest that inhibiting the combining between TGF-β and its receptor type 1 may slowdown the formation of diabetic retinopathy.

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