Stem Cell Oncology

Editor
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Increased expression of TGF-β in the cochlear fibroblast of diabetic model rats

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ABSTRACT: The cochlea, as a microvascular organ, is very susceptible to conditions that result in a disruption of vascularisation. Transforming Growth Factor-β (TGF-β) plays a role in diabetic complication process, especially in microangiopathy, due to its role in thickening the basal membrane of the vessel. This study aims to see the TGF-β expression in cochlear fibroblasts diabetic model rats. Eight male Rattus norvegicus were divided into two groups> Group 1: control, rats were injected only with a single dose of sodium citrate intraperitoneally and then terminated on day 5. Group 2: rats were injected with intraperitoneal single-dose Streptozotocin (STZ) and terminated on day 5. Samples were terminated and necropsied for the removal of their cochlear tissue and their TGF-β expression determined by immunohistochemical examination. There was a significant difference (p <0.05) in TGF-β expression in group 1 compared to group 2.

Keywords: Cochlea, Diabetes, Transforming Growth Factor-β

1 INTRODUCTION

The hyperglycaemia that happens in this disease can induce the occurrence of inflammation in the cells of the body (Romero, Sadidi & Feldman, 2008). This plays a role in the emergence of diabetic microvascular complications (Navarro and Mora, 2004), wherein inflammation that occurs can be through the Protein Kinase C (PKC) pathway along with Transforming Growth Factor-β (TGF-β) (Romero et al., 2008).

2 METHODS

Male Rattus norvegicus Wistar strain (150–250g), treated at controlled temperature (25 ± 2°C) with a 12-hour light/12-hour dark cycle with free access to water and food and handled following the standard guide for the care and use of laboratory animals.

Streptozotocin (STZ) at 60 mg/kg BW dissolved in sodium citrate (22.5 mg STZ/ml) was injected in a single dose intraperitoneally. Blood sugar levels were examined in peripheral blood taken from the rats' tails daily using a glucometer. Forty-eight hours after the STZ injection, hyperglycaemia was positive if rats had blood sugar levels greater than 200 mg/dl (Patterson et al., 2015).

In this study, there were two groups with each consisting of four rats. Group 1 was the control group where they were injected only with a single dose of sodium citrate intraperitoneally and then terminated on day 5. In group 2, the rats were injected with an intraperitoneal single dose of STZ and terminated on day 5. Tissue samples were taken and fixed with a 10% formalin buffer solution and decalcified with EDTA for four weeks and continued by cutting the tissue until it became a slide preparation.

Immunohistochemistry (IHC) staining used Polyclonal Anti-TGF-β1. Examined using a light microscope with 100x magnification, the TGF-β expression was identified by IHC
imaging showing cytoplasmic colour changes to brown. The TGF-β expression ratings were assessed by multiplication of intensity with extend of staining score (Tan and Putti, 2004), and the data collected was analysed with SPSS 21 (SPSS Inc., NY, USA).

3 RESULT

TGF-β expression, characterised by a brown colour on the cytoplasm, was higher in the diabetic group (Figure 1B) compared to the control group (Figure 1A).

The following graph shows the average TGF-β expression in each group. TGF-β is higher in group 2 (8.4).

The table above shows a significant result of TGF-β expression ($p < 0.05$) when comparing the control group (group 1) with the diabetic model group (group 2).

4 DISCUSSION

Hyperglycaemia is a characterised condition in diabetes as a group of chronic disease that can cause macrovascular (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) where
aldose reductase pathway and oxidative stress, and TGF-β may play an important role in the cellular injury that is caused by hyperglycaemia (Fowler, 2008).

As a multi-functional cytokine, TGF-β was also increased the synthesis of extracellular matrix, and the increase in TGF-β expression is also known to be in line with the severity of glomerulosclerosis in diabetics (Chang et al., 2016). In 2013, a study showed that TGF-β serum level in patients suffering from diabetes with diabetic retinopathy was significantly higher compared to diabetic patients without diabetic retinopathy (Zorena et al., 2013).

Haemodynamic changes and microcirculation disorders that are often found in diabetic conditions also happened in the cochlea. The studies showed that, especially in *stria vascularis*, vascular changes in the cochlea including thickening of the capillary walls (Xipeng et al., 2013). The increase in extracellular matrix accumulation through the stimulation of type IV collagen and the production of fibronectin (Russo et al., 2007), and the increase in Connective Tissue Growth Factor (CTGF), as well as Vascular Endothelial Growth Factor (VEGF) (Lee, 2013), all known to be induced by TGF-β. In 2013, a study showed that the TGF-β serum level in patients suffering from diabetes with diabetic retinopathy was significantly higher compared to diabetic patients without diabetic retinopathy (Zorena et al., 2013).

In this preliminary study, we wanted to compare the expression of TGF-β in the cochlear fibroblast in diabetic model rats to the non-diabetic control group and found that the TGF-β expression was significantly higher in the cochlear of the diabetic group (p < 0.05). This result is similar to the study which stated that TGF-β renal expression is increased after induction of STZ diabetes in rats (Hill et al., 2000).

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REFERENCES


