EXTENDED ABSTRACT

Preclinical Evaluation of Therapeutic Potential and Mechanism of Tetramethylpyrazine in the Amelioration of Type-2 Diabetes and its Nephropathic Complication

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Abstract:

Diabetes mellitus (DM) is a metabolic disorder characterised by fasting and postprandial hyperglycaemia, and hyperlipidaemia, which results from an impairment in protein, fat and carbohydrate metabolism. It is identified as the world-wide disorder persistently affecting people of all age groups. Diabetic nephropathy (DN) is a major complication of diabetes mellitus, also recognised as diabetic kidney disease being the most predominant element of end-stage renal failure. However, there is a lack of constraining DN treatments and the mechanism that is potentially able to ameliorate renal injury is still unclear. Therefore, the present study was designed to investigate the therapeutic response of tetramethylpyrazine (TMP), a major active constituent of *Ligusticum chuanxiong*, a traditional medicinal plant, in high-fat diet (HFD)-streptozotocin (STZ)-induced type-2 diabetic (T2D) rats and to identify its possible mechanism of action. We also explored the protective actions of TMP on DN in STZ - nicotinamide (NCT) – induced T2D rats and further investigated the underlying mechanism.

Dose-dependent effect of oral treatment of TMP (100, 150 and 200 mg/kg/day) for 28 days was evaluated by calculating the alteration in body weight, level of fasting blood glucose (FBG), fasting serum insulin (FSI) level, homeostasis model assessment (HOMA), serum lipids, oral glucose and intraperitoneal insulin tolerance and glycosylated haemoglobin in HFD-STZ-induced T2D rats. The effect of TMP treatment on the levels of pro-inflammatory cytokines C-reactive protein (CRP) and interleukin-6 (IL-6) was also investigated and the underlying molecular mechanisms of TMP was

studied through western blot analysis and real-time polymerase chain reaction (RT-PCR). The different doses of TMP (100 mg/kg, 150 mg/kg and 200 mg/kg) were orally given each day for 8 weeks in STZ - NCT – induced T2D rats also and the metabolic parameters of diabetes such as body weight, FBG, FSI, oral glucose tolerance, glycosylated haemoglobin and serum lipid profile were evaluated. The oxidative stress markers like superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) and, the renal functional parameters being blood urea nitrogen (BUN), serum creatinine (SCR) and urinary protein content were also assessed. Microstructural changes in kidney were observed through histopathological analysis, and the expression of Akt signalling pathway proteins was measured by western blotting.

TMP treatment predominantly reduced the level of FBG, glycosylated haemoglobin and restored body weight gain, level of serum insulin and serum lipid profile dose-dependently in diabetic rats. TMP treatment significantly improved insulin resistance, as identified through oral glucose tolerance and insulin tolerance tests. The results of HOMA-insulin resistance (IR) were indicating the prominent reduction of insulin resistance and the values of HOMA-β-cell function (B%) were showing the significant increase in the functioning of β-cell after TMP treatment. Moreover, dose-dependent reduction in the level of pro-inflammatory cytokines, IL-6 and CRP was observed and their levels were found to be significantly reduced in highest dose TMP (200 mg/kg) treated diabetic rats, pointing towards TMP mediated recovery of insulin signalling and a decrease in insulin resistance. The expressions of p-PI3K-p85/p-Akt/GLUT-4 were also significantly up-regulated by TMP (200 mg/kg) as observed through western blotting and RT-PCR studies, suggesting the connection of the PI3K/Akt signal pathway in the anti-hyperglycemic action of TMP. TMP administration in STZ-NCT

induced T2D rats also improved diabetic condition, as demonstrated by significant increase of body weight, reduction of FBG and glycosylated haemoglobin level, and regulation of lipid profile, FSI and oral glucose tolerance in a dose-dependent manner. TMP treatment decreased oxidative stress through an increase in SOD and GSH-Px activity and reduction of MDA level and also reduced BUN, SCR and urinary protein content. TMP treatment significantly alleviated renal morphological defects such as glomerular mesangial matrix expansion and tubulointerstitial injury. TMP activated the Akt signalling pathway, increased the levels of p-Akt and Bcl-2, and diminished the expressions of p-GSK-3β, Bax and cleaved caspase-3 as identified through western blot analysis.

Therefore, it can be stated that TMP produces antidiabetic activity in T2D through PI3K/Akt/GLUT-4 signalling and suppression of inflammation-induced facilitation of insulin resistance. TMP also ameliorates diabetic nephropathy in T2D rats by initiating the Akt signalling, improving the metabolic markers of diabetes and suppressing oxidative stress. In conclusion, tetramethylpyrazine may be considered to have a potential therapeutic value in the treatment of type-2 diabetes and its nephropathic complication.