Possible Mechanism of The Preventive Effect of Pentoxifylline on The Development of Diabetic Neuropathic Pain in Rats

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List of abbreviations

AGEs	Advanced Glycation End-products
AMP	Adenosine monophosphate
AR	Aldose reductase
Arg1	Arginase 1
ARIs	Aldose reductase inhibitors
ATP	Adenosine triphosphate
AUC	Area under the curve
B1R	kinin B1 receptor
BDNF	Brain-derived neurotrophic factor
DAG	Diacyl glycerol
DN	Diabetic neuropathy
DNP	Diabetic neuropathic pain
DRG	Dorsal root ganglia
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
GABA	Gamma Aminobutyric Acid
GFAP	Glial fibrillary acidic protein
H&E	Hematoxylin and Eosin
HPTX	Hydroxpentoxifylline
Iba 1	Ionized calcium-binding adapter molecule-1
IFN	Interferon

IGF1	Insulin-like growth factor-1
IL10	Interleukin 10
IL-1	Interleukin1
IL-6	Interleukin 6
INOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
Na^+, K^+-ATPase	Sodium-potassium adenosine triphosphatase
NCV	nerve conduction velocity
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-D-aspartate
PAI-1	Plasminogen activator inhibitor-1
PGs	Prostaglandins
PKC	Protein kinase c
PTX	Pentoxifylline
RAGE	Receptor for advanced glycation end products
RBCs	Red blood cells
ROS	Reactive oxygen species
STZ	Streptozotocin
TGF-ß	Transforming growth factor–B
TNF-α	Tumor necrosis factor-α
ICAM-1	Intercellular adhesion molecule-1

VEGF	Vascular endothelial growth factor

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Introduction and aim of the work

Diabetic neuropathy (DN) is the most common diabetic complication, which has a lifetime prevalence of about 50% of diabetic patients **(Singh et al., 2014a).** About 20 to 30% of patients with DN suffer from neuropathic pain (**Callaghan** impacting the ability **et al., 2012).**That can affect the quality of life of patients, to perform daily activities and having a negative influence on mood (**Tesfaye et al., 2013a).**

Many theories have been proposed to explain the pain related to the diabetic neuropathy, such as changes in the blood vessels that supply the peripheral nerves; metabolic and autoimmune disorders accompanied by glial cell activation **(Tesfaye et al., 2013b)**. Activation of spinal microglia has been demonstrated in STZ treated animals, the most commonly used model of diabetes **(Courteix et al., 1993).** That activated microglia might have the main role in sensitization of nociceptive neurons in the spinal cord **(Wang et al., 2014).**

The present study aimed to:

١. Assess the role of microglial and astrocytes activation in animal model of DN through studying Iba-1 and GFAP immunoreactivity in the thoracolumbar segments of the spinal cord**.**

٢. Assess the effect of early and late treatment of PTX on microglial activation through studying Iba-1 immunoreactivity in the thoracolumbar segments of the spinal cord.

٣. Assess the role of early and late treatment of PTX on diabetic neuropathic pain (DNP) development through using Von Frey filaments to report on mechanical allodynia and by estimation the area under the 60% mechanical threshold time curve

٤. Assess the role of early and late treatment of PTX on spinal cord inflammatory mediator's levels through measuring of NF-κB & TNF-α.

٥. Assess the role of early and late treatment of PTX on sciatic nerve inflammatory mediator's level through measuring TNF-α.

٦. Assess the role of early and late treatment of PTX on sciatic nerve Na^+/K^+ ATPase activity and the foot pad epidermal thickness.

In order to investigate that, male Wister rats were divided into 8 groups naïve, control, early PTX treated (50, 100 and 200 mg/kg/day, in drinking water starting one week after STZ injection and for 7 weeks) and late PTX treated (50, 100 and 200 mg/kg/day, in drinking water starting 6 week after STZ injection and for 2 weeks).

Review of literature

Diabetic neuropathy and neuropathic pain

Diabetic neuropathies are the most prevalent chronic complications of diabetes. This heterogeneous group of conditions affects various parts of the nervous system and presents with distinct clinical manifestations **(Pop-Busui et al., 2017).** Symptoms of peripheral nerve dysfunction could be either sensory symptoms or motor involvement. Sensory symptoms could be a decrease in sensation or positive neuropathic symptoms (**Bansal et al., 2006).**

Diabetic neuropathy has a lifetime prevalence of about 50% in diabetic patients **(Singh et al., 2014a).** It considered as a leading cause for disability owing to foot ulceration and amputation, gait disturbance, and fall-related injury (**Callaghan et al., 2012; Singh et al., 2014a).** It is significantly lowers quality of life and to a large extent increases health costs associated with diabetes **(Argoff et al., 2006)**.

Intensive glycemic control is effective for the primary and secondary preventions of neuropathy in people with type 1 diabetes **(Ang et al., 2014).** Duration of diabetes, cardiovascular disease independently doubled the risk of neuropathy, elevated triglyceride levels, smoking, obesity, albuminuria and retinopathy was also associated with an increased incidence of DN (**Edwards et al., 2008).**

Diabetic neuropathic pain (DNP) in diabetes, as proposed by the International Association for the Study of Pain is defined as "pain arising as a direct consequence of abnormalities in the peripheral somatosensory system in people with diabetes" **(Treede et al., 2008).** About 20 to 30% of patients with DN suffer from neuropathic pain (**Callaghan et al., 2012).** It is one of the main reasons that force the patients to seek medical care **(Tesfaye et al., 2005).** DNP

is characterized by tingling, burning, shooting, sharp, and lancinating or even as electric shock sensations (**Tesfaye et al., 2013a).** It is usually described as moderate to severe pain and often worse at night, causing sleeping disturbance. The pain can be constant and associated with cutaneous allodynia (increased sensitivity to innocuous stimuli), hyperalgesia (exaggerated response to noxious stimuli), and/or spontaneous pain which can substantially affect the quality of life of patients, impacting the ability to perform daily activities and having a negative influence on mood. The pain may also be a cause of withdrawal of recreational and social activities and may be associated with depression (**Tesfaye et al., 2013a).**

Symptoms are not a predictable marker of the severity of axonal loss, those with the most severe painful symptoms have minimal or no sensory deficit on exam or electrodiagnostic studies (**Tesfaye and Selvarajah, 2012).** In practice, the diagnosis of DNP is clinically detected, which depend on the patient's description of pain (**Tesfaye et al., 2010)**. A number of simple numeric rating scales can be used to assess the frequency and severity of painful symptoms **(Cruccu et al., 2004)**. Visual analog scale (VAS; the oldest and best validated measure) can be used (**Tesfaye et al., 2010)**. VAS is a graphic tool with a 10 cm horizontal line, the left end marked as "no symptom" and the right end marked as "worst imaginable symptom". The patient is asked to draw a vertical line to indicate the intensity of the symptom.

١. **Pathogenesis:**

The pathogenesis of DN is complex and is characterized by both metabolic and vascular factors (**Cameron et al., 2001)**. Hyperglycemia is considered one of the many key metabolic events known to cause axonal and microvascular injury. Other key players include, activation of the polyol pathway,

accumulation of advanced glycation end products (AGEs), protein kinase c pathway (PKC) activation, oxidative stress, increasing of inflammatory markers and alteration in Na^+/K^+-ATPase activity (Fig.1; **Sima and Sugimoto, 1999; Callaghan and Feldman, 2013)**.

Although nerve fiber loss is accepted as the genesis of insensitivity in DN (**Tesfaye and Selvarajah, 2012)**, the pathogenesis of DNP is not well understood even there is not yet a reasonable hypothesis to explain why some patients develop the painful pattern of disease while others do not. Many theories have been proposed to explain the pain related to the DN, as changes in the blood vessels that supply the peripheral nerves; changes in sodium and calcium channels expression and central pain mechanisms, such as increased thalamic vascularity, imbalance of the facilitatory/inhibitory descending pathways and glial cell activation **(Tesfaye et al., 2013a).**

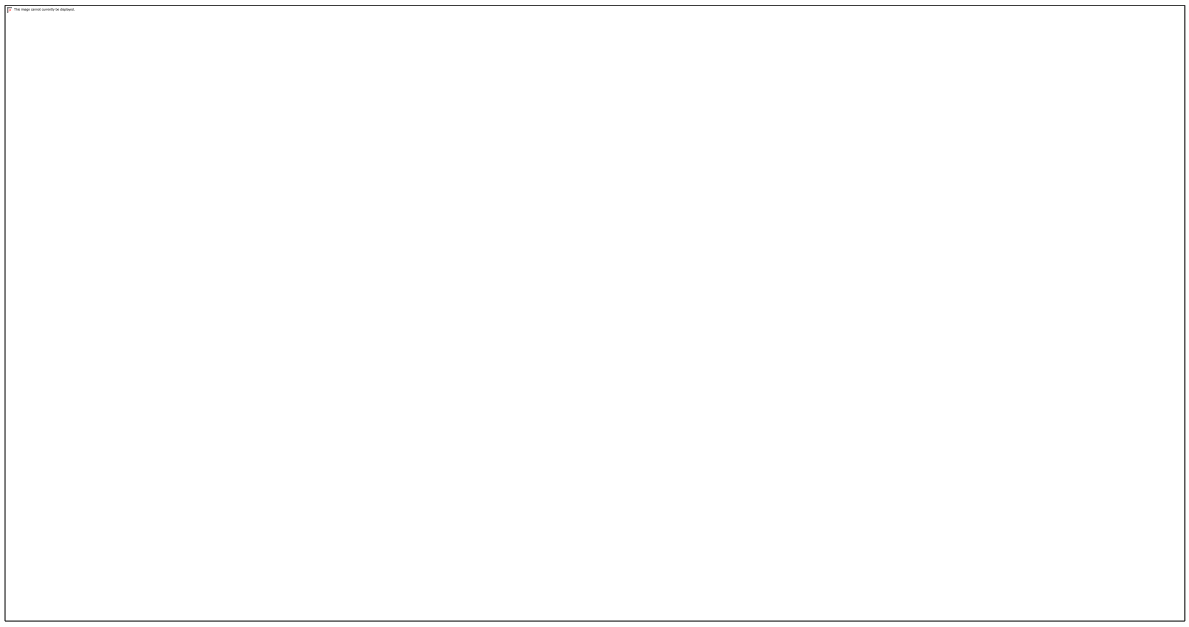

Figure (1): Different metabolic pathways involved in pathogenesis of diabetic neuropathy. AGEs, advanced glycation end products; TNF-α, tumor necrosis factor α; PKC, protein kinase c pathway(modified from **Satoh et al., 2003).**