REVIEW ARTICLE

Diabetic Cardiac Autonomic Neuropathy: A Review of NLRP3 Inflammasome Complicity

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ABSTRACT

Debilitating and often misdiagnosed cardiac autonomic neuropathy affects people with diabetes. Damage to the biggest sympathetic trunk ganglion of autonomic nerve fibers that innervate the heart and blood vessels, the superior cervical ganglion (SCG), is the cause of aberrant heart rate and vascular dynamics in people with diabetic cardiac autonomic neuropathy (DCAN). Mediating host immunological responses to microbial infection and cellular damage, inflammasomes are a class of cytosolic protein complexes that include NLRP3. This review compiled the findings of experimental investigations on DCAN and discussed their connection to the NLRP3 inflammasome's involvement. SCG P2X7 receptor expression can be boosted by inflammation brought on by hyperglycemia, leading to nerve injury. Unfortunately, there is a lack of research on DCAN-related inflammation in animal models, especially in inflammasome. Currently, only IncRNAUC.360+ shRNA and Schisandrin B, prove to alleviate the pathogenesis. Therefore, more research into the causes and potential remedies for DCAN is necessary.
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INTRODUCTION

Prevalence of DCAN

Inflammasomes, of which nucleotide-binding oligomerisation domain (NOD)-like receptor pyrin domain 3 (NLRP3) is a part, are a class of cytosolic protein complexes that coordinate host immunological responses. The prevalence of diabetes in Malaysia is one of the highest in the world, with economic burden of almost $600 million [1]. From 2011 to 2019, the rate of diabetes incidence increased from 11.2% to 18.3% [2]. There were an estimated 3.6 million adults in Malaysia with diabetes in 2019, with 49% of those cases going undiagnosed [2].

With a diabetes incidence of 31.3%, 7 million Malaysian adults aged 18 and over are anticipated to have the disease by 2025, posing a serious risk to the public's health. According to published research, the prevalence of diabetes mellitus (DM) in Malaysia varies between 7.3% and 23.8% [2]. Numerous factors, such as population growth, population aging, urbanization, increasing weight gainand physical inactivity rates, and population expansion all contribute to the upward trend [3].

Patients with diabetes often suffer from cardiovascular autonomic neuropathy (CAN), a disorder that is both disabling and sometimes misdiagnosed. The primary prevention cohort of the Diabetes Control and Complications Trial [4] reports a prevalence anywhere of 2.5% to 90% among patients with type 1 diabetes.

Diabetic cardiac autonomic neuropathy (DCAN) affects anywhere from 2% to 91% of type 1 DM patients and 25% to 75% of type 2 DM patients. There is a 16% to 50% mortality rate within the first five years after a diagnosis of DCAN in patients with type 1 or type 2 DM; the majority of these deaths are attributable to sudden cardiac arrest [4]. Treatment of diabetes complications is very expensive for society as a whole, and the 5-year mortality rate of DCAN patients is five times higher than that of DCAN patients without the disease [5]. There is currently no effective systemic medication for the medical management of

DCAN. Since there is a dearth of research on animal models of diabetic cardiac autonomic neuropathy, we elaborated on potential explanations and therapies, with an emphasis on inflammation. We focused on rodents in view of limited literatures of DCAN-NLRP3 inflammasome related.

Pathogenesis DCAN

The autonomic nervous system's greatest sympathetic trunk ganglion, the superior cervical ganglion (SCG), plays a role in the development of DCAN [3]. Prolonged hyperglycemia causes glycoxotic stress, which in turn increases reactive oxygen species (ROS) production. Excessive ROS also inhibits synaptic transmission in autonomic ganglia, which can lead to deadly cardiac arrhythmias and abrupt cardiac death due to myocardial infarction. Mitochondrial failure is associated with bioenergetic dysfunction, autophagy and apoptosis, inflammatory conditions, neurovascular dysfunction, and neurological degeneration of nerves, to name just a few processes.

Patients with DCAN may have a postganglionic sympathetic nerve that is particularly attuned to the nociceptive signals of acute myocardial ischemia and hypoxia, making the heart more excitable than usual [4, 6]. Increases in heart rate, blood pressure, and the excitability of the cardiac sympathetic nerve can cause significant damage in patients with myocardial ischemia.

SCG dysfunction in DCAN patients may also be linked to abnormalities in a subset of receptors involved in cardiovascular control, including P2X and P2Y receptor [7]. As a result, altering the levels of these receptors' expression could be a viable therapy option for DCAN [8].

NLRP3 Inflammasome

When a cell is damaged or infected with bacteria, a protein complex called an inflammasome is assembled in the cytosol [9]. Active caspase-1 is generated from inactive procaspase-1 upon inflammasome assembly [10, 11], which in turn transforms the pro-IL-1 and pro-IL-18 cytokine precursors into mature and physiologically active IL-1 and IL-18, respectively. Overactivation of the NLRP3 inflammasome, on the other hand, causes pathological states marked by excessive inflammation and collateral damage to the host [12].

The NLRP3 inflammasome, which also includes an adaptor protein, an apoptosis-related spot-like protein with a caspase activation recruitment domain (CARD) (ASC), and an effector protein, caspase-1, is formed and activated when the NLRP3 intracellular receptor detects signals from self- and foreign pathogens (Figure 1). The function of the inflammasome, which controls inflammation and the immunological response, is

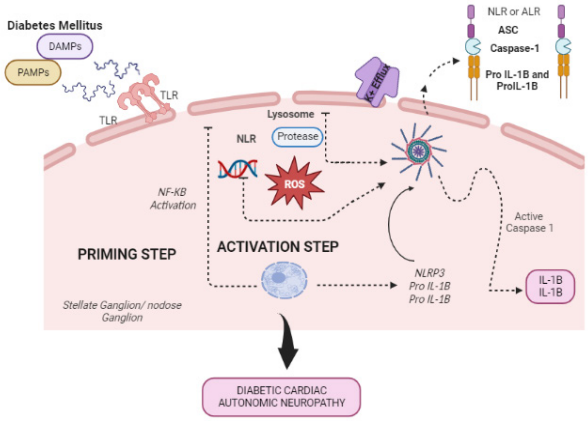

Figure 1 : A visual representation illustrating the activation of the NLRP3 inflammasome in the stellate/nodose ganglion due to increased sugar-induced oxidative stress is displayed in the schematic diagram. The process involves two main stages: priming and activation. During the priming step, the production of NF-kB is encouraged, which in turn triggers the activation steps. These activation steps lead to the generation of active IL-1β and IL-18 through the activation of caspase-1, serving as the final products of the NLRP3 inflammasome. Consequently, this process contributes to the development of diabetic cardiac autonomic neuropathy.

rigorously regulated by interactions between these three proteins [13].

There are many upstream signals that can lead to the activation of the NLRP3 inflammasome [14], such as K^+ efflux, Cl^- efflux, Ca^{2+} flow, lysosomal damage, mitochondrial dysfunction, and ROS generation.

DCAN and NLRP3 Inflammasome

SCG P2X7 receptor expression can be boosted by inflammation brought on by hyperglycemia, leading to nerve injury. Inhibiting P2X7 receptor expression in the superior cervical ganglion has been shown to reduce the dysregulation of nociceptive signalling caused by DCAN [15]. There is currently no known medicine that could downregulation of P2X7 receptor expression.

Traditional Chinese medicine, Schisandrin B, has been shown to have therapeutic effects in the treatment of DCAN [15] (Table I). Most research has linked NF-kB and Nrf2 to anti-inflammatory molecular pathways [16], but the P2X7 receptor has received less attention. Schisandrin B may reduce P2X7 expression levels via NLRP3 and diminish the chronic inflammation imposed by diabetes, as shown by the reversal of previously elevated levels of the P2X7 receptor, NLRP3 inflammasomes, and IL-1 following treatment. Schisandrin B has never been utilised in the study of DCAN before, therefore its potential utility in the treatment of DCAN as a supplementary regimen may need further validation [15].

Table I : Experimental DCAN studies related to NLRP3 inflammasome

No (Ref)	Title	Rodent	Type of Diabetes	NLRP3 Inflammasome	Treatment	Result
1.(17)	Schisandrin B Alleviates Diabetic Cardiac Autonomic Neuropathy Induced by P2X7 Receptor in Superior Cervical Ganglion via NLRP3.	Male Sprague Dawley (180-200 g)	Type 2 diabetes	IL-1β and NLRP3	Schisandrin B	1) Pathological blood pressure, heart rate, heart rate variability, and sympathetic nerve discharge were ameliorated after administration of Schisandrin B. 2) Upregulated protein level of P2X7 receptor, NLRP3, and interleukin-1β in diabetic rats were decreased.
2. (21)	Beneficial Effects of lncRNA-UC.360+ shRNA on Diabetic Cardiac Sympathetic Damage via NLRP3 Inflammasome-Induced Pyroptosis in Stellate Ganglion.	Male Sprague Dawley, 180g-220g	STZ induced Type 2 diabetes	NLRP3, ASC, Caspase-1	lncRNA-UC.360+ shRNA	1) lncRNA-UC.360+ shRNA modulates the NLRP3 inflammasome/inflammatory pathway in the SG, which alleviates diabetic heart sympathetic nerve damage. 3) 2) UC.360+ shRNA reduced the expression of nuclear factor kappa-B (NF-κB), NLRP3, ASC, caspase-1, interleukin-1β (IL-1β), and IL-18 in the SG of diabetic rats and inhibited the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK).

Upon hyperglycaemia, the body produces an abundance of ROS, which in turn activates MAPK signalling pathways, as well as several cytokines and transcription factors. Previous studies have shown that elevated levels of ROS activate p38 MAPK, which then modifies the expression of the pro-inflammatory transcription factor NF-B [17, 18]. Increased production of cell-injury molecules and pyroptosis are the results of activated NLRP3 inflammasomes caused by NF-B expression. Due to further cellular damage DCAN developed. In diabetics, this leads to sympathetic neuropathy over time. Self-oligomerization of the NLRP3 inflammasome adapter ASC generates a massive complex that triggers caspase-1 [19]. Amplification of local and systemic inflammatory responses [20], caused by cleavage of IL-1β and IL-18 precursors into active forms, which in turn promotes the production of other pro-inflammatory components.

In addition, Zhang et al. shown that the lncRNA UC.360+ reduced NLRP3 inflammasome-induced pyroptosis in the stellate ganglion, hence reducing the severity of DCAN [17] (Table I). ROS production was suppressed and stimulation of the ROS-NLRP3-IL-1 signaling pathway was prevented in a rat model of diabetes when the lncRNA UC.360+ was administered via short-hairpin RNA (shRNA). Therefore, pyroptosis does not harm the nerve cells of the cervical sympathetic ganglion [17].

Positive results were seen in the treatment of diabetic rodents with lncRNA-UC.360+ shRNA, with improvements in dyslipidemia and reduced cardiac sympathetic damage. In addition, lncRNA-UC.360+ shRNA treatment successfully decreased ROS overproduction, decreased phospho-p38 MAPK content in the stellate ganglion (SG), and counteracted the increment of NF-B protein levels in diabetic rats, indicating that lncRNA-UC.360+ shRNA could effectively inhibit ROS production and prevent activation of the p38 MAPK signaling pathway, thereby hindering the expression of NF-B protein.

The p38 MAPK-mediated instructing route is activated when cells produce too many ROS as a result of diabetes-induced hyperglycemia. This pathway then induces nuclear factor-kappa B (NF-B) and controls the activities of various elements of the NLRP3 inflammasome, ultimately leading to DCAN. However, it has been discovered that treatment with lncRNA-UC.360+ shRNA significantly reduces intracellular ROS generation and prevents activation of the ROS-NLRP3-IL-1 signaling pathway. This, in turn, improves diabetic cardiac sympathetic nerve injury [17] and decreases pyroptosis in cervical sympathetic ganglion nerve cells.

CONCLUSION

The pathogenesis and treatment in the experimental model of DCAN are still vague in view of mixed symptoms with other cardiac presentations (Fig. 1). Furthermore, the paucity of literature in the relationship between NLRP3 inflammasome leading to DCAN has yet to be explored further. Hence, more investigations are necessary to delineate their relationship, especially in prevention and managing the complications.

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