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Antinociceptive effects of fisetin against diabetic neuropathic pain in mice: Engagement of antioxidant mechanisms and spinal $GABA_A$ receptors

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ABSTRACT

Peripheral painful neuropathy is one of the most common complications in diabetes and necessitates improved treatment. Fisetin, a naturally occurring flavonoid, has been reported to exert antidepressant-like effect in previous studies. As antidepressant drugs are employed clinically to treat neuropathic pain, this work aimed to investigate whether fisetin posses beneficial effect on diabetic neuropathic pain and explore the mechanism(s). We subjected mice to diabetes by a single intraperitoneal (i.p.) injection of streptozotocin (200 mg/kg), and von Frey test or Hargreaves test was used to assess mechanical allodynia or thermal hyperalgesia, respectively. Chronic treatment of diabetic mice with fisetin not only ameliorated the established symptoms of thermal hyperalgesia and mechanical allodynia, but also arrested the development of neuropathic pain when given at low doses. Although chronic fisetin administration did not impact on the symptom of hyperglycemia in diabetic mice, it reduced exacerbated oxidative stress in tissues of spinal cord, dorsal root ganglion (DRG) and sciatic verve. Furthermore, the analgesic actions of fisetin were abolished by repetitive co-treatment with the reactive oxygen species (ROS) donor *tert*-butyl hydroperoxide (*t*-BOOH), but potentiated by the ROS scavenger phenyl-*N*-*tert*-butylnitrone (PBN). Finally, acute blockade of spinal $GABA_A$ receptors by bicuculline totally counteracted such fisetin analgesia. These findings indicate that chronic fisetin treatment can delay or correct neuropathic hyperalgesia and allodynia in mice with type 1 diabetes. Mechanistically, the present fisetin analgesia may be associated with its antioxidant activity, and spinal $GABA_A$ receptors are likely rendered as downstream targets.

1. Introduction

Diabetic neuropathies are highly prevalent complications in diabetes and cause serious problems afflicting patients, such as chronic neuropathic pain. This painful condition may occur spontaneously or as a result of exposure to only mildly painful stimuli (hyperalgesia) or to stimuli not normally perceived as noxious (allodynia). Mounting evidence suggests that the management of hyperglycaemia alone may be insufficient to prevent or arrest this progressively diabetic complication [1,2] and its early identification and treatment are of utmost importance for both clinicians and diabetic patients [3].

Management of diabetic neuropathic pain represents an emerging therapeutic challenge in clinical practice, since it was reported that as many as 39% of patients suffering such type of chronic pain may be untreated [4]. Fundamentally different from acute pain treatment that relies heavily on over-the-counter drugs and prototypical analgesics such as morphine and non-steroidal anti-inflammatory drugs, recommendatory pharmacological treatment against chronic neuropathic pain is however based on drugs initially developed to treat other CNS diseases such as antidepressants and anticonvulsants [5]. Although the two categories of agents (antidepressants and anticonvulsants) have been ranked as first-line drugs to treat neuropathic pain, some disadvantages (including modest efficacy, extensive limitations, noticeable adverse effects and poor patient compliance) limit their clinical utility to quench

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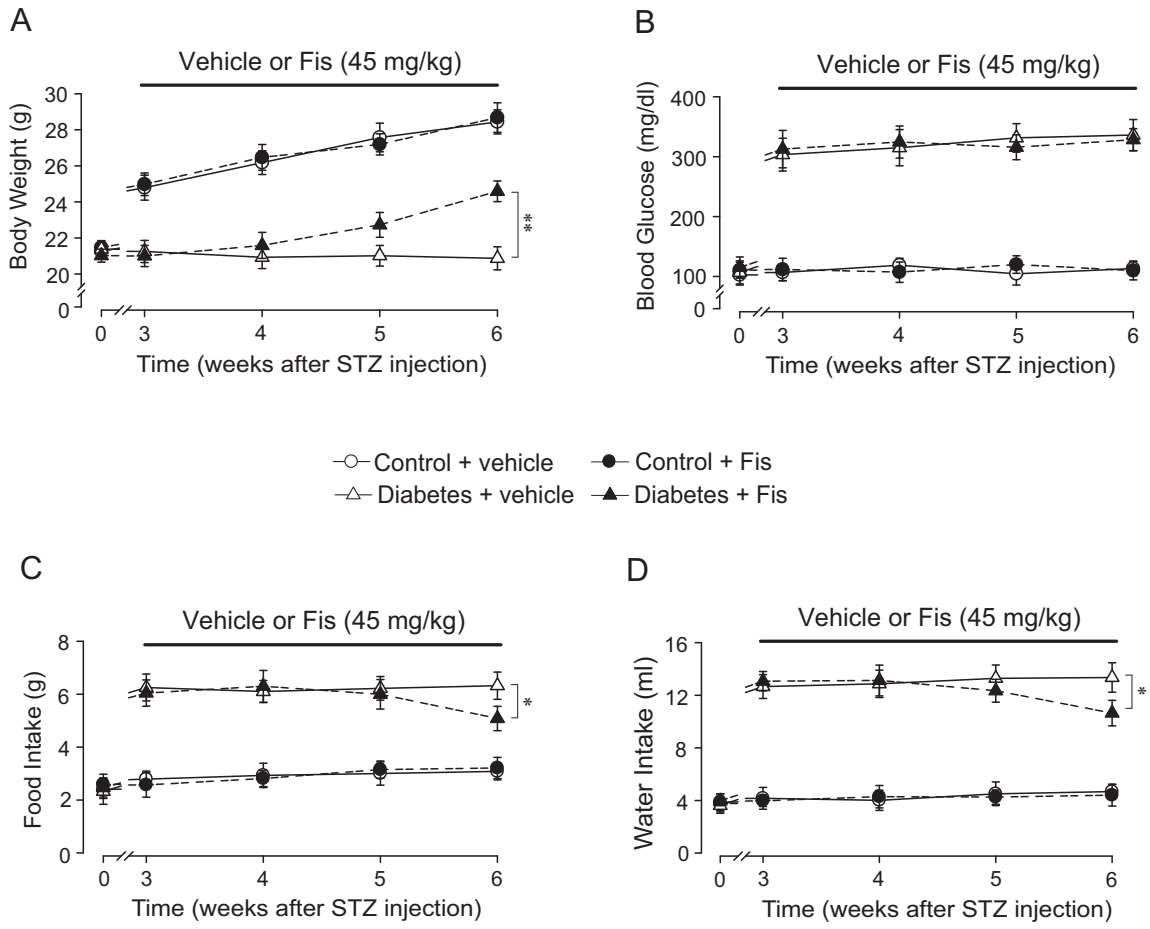

Fig. 1. Time courses for the effect of fisetin (Fis) on metabolic parameters in control and diabetic mice. During the period of fisetin treatment (i.e., from the 3rd week to 6th week following STZ injection), the mice were assayed weekly. (A) Effect of fisetin on body weight in control and diabetic mice. (B) Effect of fisetin on blood glucose in control and diabetic mice. (C) Effect of fisetin on food intake in control and diabetic mice. (D) Effect of fisetin on water intake in control and diabetic mice. Data are expressed as mean ± SEM ($n = 12$ for control mice, $n = 11$ for vehicle or fisetin treated diabetic mice), assessed by multifactor ANOVA followed by Duncan test.

pain [5,6]. Thus, the development of novel pharmacotherapy to relieve this painful symptom in diabetic patients is in great need.

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a flavonoid found in vegetables and fruits (particularly strawberries) [7]. It has a wide variety of pharmacological activities including antioxidant [8], anti-allergic [9] and cancer chemo-preventive activities [10]. Due to fisetin being a hydrophobic compound, Krasieva et al. observed, using in vivo label-free two-photon imaging, that fisetin (i.p. or p.o.) rapidly distributed to the blood vessels of brain followed by its dispersion into brain parenchyma [11], supporting for the transportation of fisetin across blood–brain barrier and its effects in central nervous system (CNS) such as neuro-protection [12]. Moreover, fisetin has been reported to exert modulatory effects on hyperglycemia in streptozotocin-induced diabetic rats [13], but it is not known whether it posses beneficial activities against painful diabetic neuropathy. Recently, fisetin was shown to possess anti-depressant properties in experimental animals [14]. This finding provides a rationale that fisetin may also have therapeutic potential in treating diabetic neuropathic pain, since antidepressant drugs such as tricyclic antidepressants (TCAs) are used clinically to treat persistent and neuropathic pain. Therefore, the primary aim of this study is to probe the possible analgesic capacity of fisetin in a mouse model of painful diabetic neuropathy produced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ, 200 mg/kg). Furthermore, after determining fisetin antinociception in diabetic mice, we explored its potential action mechanism(s) with focus on its antioxidant potentials [8], since oxidative stress is generally believed as a key pathological process involved in diabetic neuropathy [15,16].

2. Materials and methods

2.1. Animals

All animal experiments were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ningbo University Committee on Animal Care and Use. We further attest that all efforts were made to minimize the number of animals used and their suffering. Male C57BL/6J mice (6–7 weeks old upon arrival and obtained from the Laboratory Animal Center of Chinese Academy of Sciences) were used throughout the experiments. They were housed in groups (4–6 per cage) with food and water available ad libitum and kept in controlled laboratory conditions with the temperature maintained at 22 ± 0.5 °C and a relative humidity of 60 ± 2% in 12 h light cycles (on at 07:00 AM). Animals were randomly assigned to experimental groups at the start of study. Experimental behavioral tests were performed in a soundproof and air-regulated room and were done in blind respect to drug treatment. Following streptozotocin (STZ) injection, some animals were excluded from the study for measuring pain-related behaviors, due to not developing diabetes or die of diabetes. These dropouts were not replaced.