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Research report

Preventive and therapeutic effect of brozopine on stroke in Dahl Salt-sensitive hypertensive rats

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ABSTRACT

Our aim was to explore the preventive and therapeutic effects of sodium (±)-5-bromo-2-(α-hydroxypentyl) benzoate (brand name: brozopine, BZP) on stroke in Dahl Salt-sensitive (Dahl-SS) hypertensive rats. Dahl-SS rats were fed a high-salt diet to observe the effect of BZP on blood pressure, and brain, heart, and kidney tissues. Additionally, the incidence of stroke was recorded according to the neurological score. The relative mechanisms investigated included anti-oxidative effects and anti-platelet aggregation. BZP reduced the incidence of stroke, neuronal necrosis in the brain, and cell swelling and inflammatory infiltration in the kidney. Its mechanisms were related to the increased activities of gluthatione peroxidase and catalase and the decreased level of plasma nitric oxide. BZP inhibited arachidonic acid (AA) – induced platelet aggregation (IC_{50}: 12 μM) rather than that of adenosine diphosphate (ADP) – and/or thrombin-induced platelet aggregation *in vitro*. Interestingly, BZP inhibited ADP-, thrombin-, or AA-induced platelet aggregation and elevated the level of AMP-activated protein kinase, cyclic guanosine monophosphate, and vasodilator-stimulated-phosphoprotein, and attenuated ATP contents and mitogen-activated protein kinase levels in platelet and inhibited thrombus formation in a carotid artery thrombosis model, dose-dependently, in Dahl-SS hypertensive-induced stroke rats. In conclusion, BZP can have therapeutic and preventive effects on stroke in Dahl-SS hypertensive rats, the mechanisms of which may be related to anti-oxidant, anti-platelet aggregation and anti-thrombus formation.

1. Introduction

Hypertension has become one of the most common cardiovascular diseases and has been an independent risk factor of stroke in developing and developed countries. Stroke has a high incidence in China and exhibits a rising trend annually. Salt has been considered one of the most important environmental factors of hypertension, and high salt intake is closely related to blood pressure. Platelet activation was thought to play a vital role in the initiation and maintenance of atherosclerosis (Garcia Carrascal et al., 2017) and was implicated in the pathogenesis of vascular diseases. Moreover, these changes were exaggerated during sodium loading, especially in salt-sensitive hypertension (Somova and Mufunda, 1993). Patients with hypertension revealed that platelets were more sensitive to platelet aggregation inducer (Mugellini et al., 2005) and showed an elevation in their intracellular free calcium (Rendu et al., 1993). This later potentiated platelet activity and increased the risk of thromboembolic diseases such as myocardial infarction and peripheral artery disease (Dogne et al., 2002). Platelet activation was abnormal in cerebral ischemia, but usually returned to normal with anti-platelet therapy. This activation of platelets might contribute to the clinical manifestations of occlusive vascular diseases. Platelet activation, induced by platelet agonists such as adenosine diphosphate (ADP), thrombin, and arachidonic acid (AA) at the site of the injury, was a vital process for platelet adhesion and aggregation (Kazianka et al., 2017). Currently, anti-platelet therapy is considered one of the valid approached to ameliorate cerebral ischemia injury (Furie and Furie, 2005; Huo and Ley, 2004; Li et al., 2007).

To date, several anti-thrombotic drugs have been developed and widely used in clinical practice. Generally, the acetylsalicylic acid (ASA), which is a cyclooxygenase inhibitor, preventing thromboxane A_2 synthesis, has been used as an anti-platelet agent in the prevention of ischemic stroke. However, its use became greatly limited due to severe adverse effects and drug resistance in many patients (Fitzgerald and Pirmohamed, 2011; Fong et al., 2011). Sodium (±)-5-bromo-2-(α-hydroxypentyl) benzoate (BZP) is derived from 1-3-*n*-butylphthalide (NBP) and its chemical

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structure is similar to that of ASA. Based on our previous findings, BZP has the ability to protect from focal cerebral ischemia-reperfusion injury in rats, through mechanisms including anti-apoptosis, anti-inflammation, and promoting synaptic plasticity (unpublished data). Currently, BZP is used in phase I clinical trials with encouraging efficacy results. The preventive and therapeutic effect of BZP on stroke in Dahl-SS hypertensive rats have not been studied yet. Accordingly, we assessed the modulatory effects of BZP on Dahl-SS hypertensive rats at 28 days after stroke.

2. Results

2.1. No difference was observed in BP in Dahl-SS hypertensive-induced stroke rats treated with BZP

The mean arterial pressure was significantly higher in the Dahl-SS group as compared with the SS-13^{BN} group (135.66 ± 6.18 mmHg *vs* 99.33 ± 3.96 mmHg, respectively) after 2 weeks of high salt diet. The systolic (SBP) and dystolic blood pressure (DBP) were significantly increased in the Dahl-SS group compared with the controls (SBP: 155.18 ± 4.58 mmHg *vs* 115.19 ± 3.39 mmHg, respectively; DBP: 125.88 ± 7.94 mmHg *vs* 91.40 ± 5.23 mmHg, respectively). After establishing the hypertensive model, the drug was administrated by caudal vein once daily for the next 28 days. The results indicated no statistical difference between the BZP (0.75, 3, 12 mg/kg) and Model group ($P > 0.05$), indicating that BZP is not an anti-hypertensive drug. (Data was shown in Supplementary Fig. 1).

2.2. Behavioral testing

The prehensile traction, beam-walking test, and neurological deficit (Bederson) score were examined before administration. Subsequently, all rats were tested subjected to behavioral tests at least every 7 days. Compared with the Control group that exhibited an intact performance, the Dahl-SS hypertensive-induced stroke rats displayed behavioral deficits following cerebral lesions on the beam-walk test as evidenced by a reduced time on the beam. In contrast, rats in the BZP groups remained longer time on the beam compared with the Model group. Moreover, the Model group demonstrated a decrease in the time spent on the rope in the prehensile traction test, whereas an increase was observed in rats treated with different doses of BZP. There were significant differences in the time spent on the rope between the BZP and Model groups. Additionally, the BZP (12 mg/kg) group exhibited an improvement in their motor function that was more prevalent than in the NBP-K (9.6 mg/kg) and Br-NBP (10.5 mg/kg) groups. A significant group effect in the grip strength was observed at days 7, 14, and 28, whereby rats treated with BZP (0.75, 3, and 12 mg/kg) achieved significantly lower scores than the other experimental groups (Fig. 1).

2.3. BZP reduced the incidence of stroke and increased the survival rate in Dahl-SS hypertensive rats

Stroke was defined by comprehensive scores equal or greater than three points according to the motor changes. Following the intake of high salt diet, the incidence of stroke increased gradually in the Model group, which tended to be higher than in the other groups (Fig. 2A). The incidence of stroke was 83.3% in the Model group with apathetic, lethargy, and motor dysfunction (such as circling behavior) after 4 weeks of high salt diet. In contrast, the incidence of stroke was obviously lower in the BZP-treated rats (0.75, 3, and 12 mg/kg). This observation was dose -dependent manner and rats displayed a good physiological state and no circling behavior. The highest stroke incidence was merely 8.3% and was

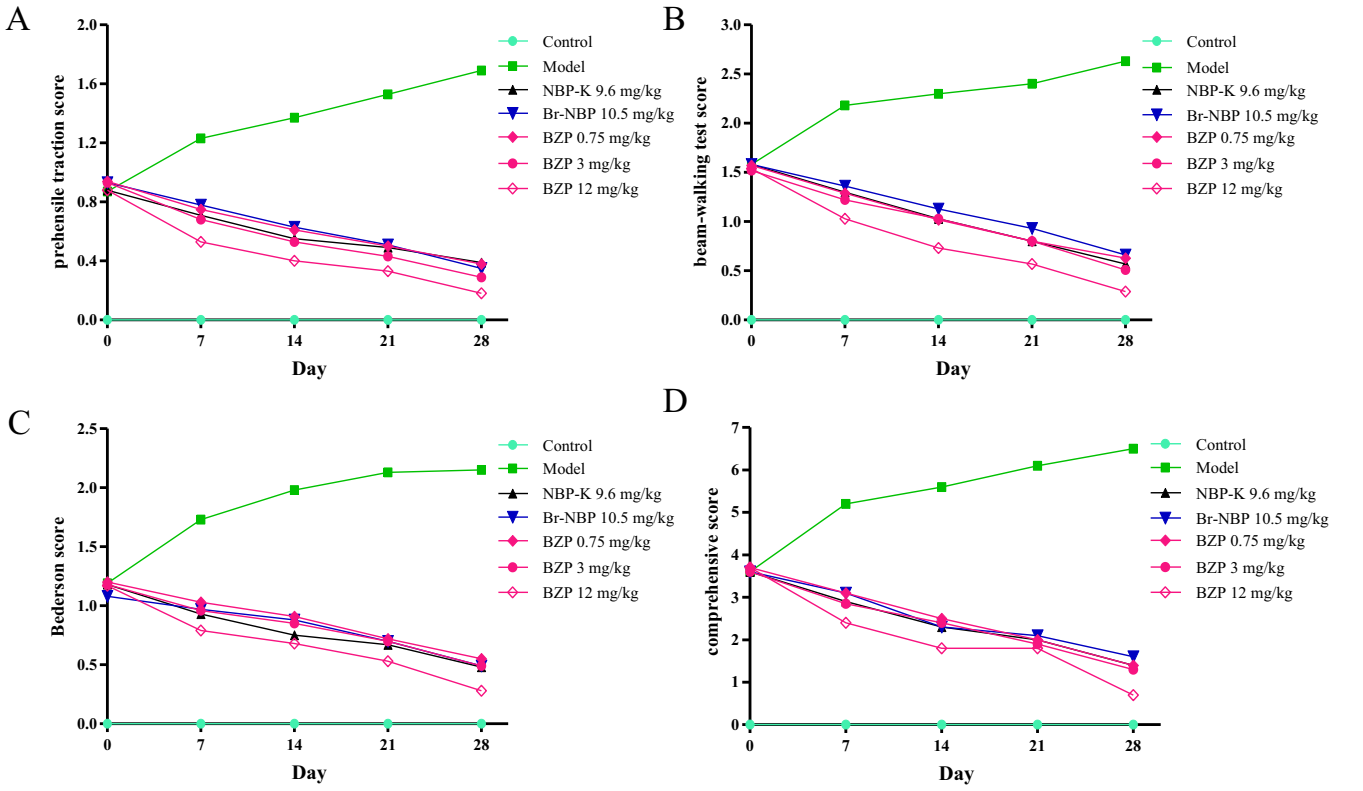

Fig. 1. Stroke administration of BZP improved motor dysfunction and decreased neurological scores in Dahl-SS hypertensive-induced stroke rats. (A) Prehensile traction test. (B) Beam-walking test. (C) Neurological defect (Bederson) scoring. (D) Comprehensive scoring. X -bar represents the day for administration, "0" means the day before administration, and "7" means 7 days after administration.