Int J Clin Exp Pathol 2020;13(7):1707-1711
www.ijcep.com /ISSN:1936-2625/IJCEP0108433

Original Article

Influence of dl-3-N-butylphthalide on infarction size in rats with acute myocardial infarction

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Received October 17, 2020; Accepted March 4, 2020; Epub July 1, 2020; Published July 15, 2020

Abstract: Objective: To study the influence of dl-3-N-butylphthalide (NBP) on infarction size in rats with acute myocardial infarction (AMI). Methods: AMI model was established by ligation of the left anterior descending artery. A total of 36 healthy male Sprague-Dawley rats (weight, 180 t, 180 ght, 180 ligation of left anterior descending artery. A total of 36 healthy males were assigned to model group, sham-operation (SO) group, and the NBP group (n=12 each). The rats in the NBP group were treated with intraperitoneal injection administration of 60 mg/kg/body weight NBP once a day. The rats in the other groups were given distilled water of the same volume. The MI area in each group was detected by TTC staining. The concentrations of CK-MB and LDH were detected. The concentrations of TNF-α, IL-6, MDA, and SOD were measured by ELISA. Results: Compared with the SO group, the myocardial infarct sizes in the model group and the NBP group were significantly increased (*P*<0.001), and the infarct size in the NBP group was lower than that in the model group (280.6±5.82% vs. 37.74±10.18%, P<0.05). The levels of TNF-α in the NBP group and model group were significantly increased compared with that in the SO group (*P*<0.001), while the level of TNF-α in the NBP group was significantly lower than that in the model group (29.01±0.81 pg/mg prot vs. 37.727 pg/mg prot, *P*<0.05). The level of IL-6 in the model group and NBP group was significantly higher than that in the SO group, and it was lower than that in the model group (24.13 group wamg prot vs. 27.53 pg/mg prot, *P*<0.05). The levels of MDA in the model group and the NBP group were significantly higher (<0.001), and the level of NBP group was lower than that of the model group (4.10 group wnmol/mg prot vs. 4.774.774. nmol/mg prot, *P*<0.05). The levels of SOD in the model group and NBP group were lower (*P*<0.001), and the SOD level in the NBP group was higher than that of the model group (53.490.001), and prot vs. 38.068.06he SOD prot (*P*<0.05). Conclusion: NBP can reduce the infarct size in SD rats with AMI.

Keywords: Acute myocardial infarction, Dl-3-N-butylphthalide, infarction size, SD rat

Introduction

Acute myocardial infarction (AMI) is one of the leading causes of morbidity and mortality worldwide with a rising trend in our country [1]. Although early reperfusion therapies have notably reduced the mortality in patients with AMI, the parallel increase of heart failure after AMI has emerged as a growing challenging health problem [2]. Previous studies [3] have indicated that AMI can lead to ventricular remodeling and heart failure [4]. However, there were few therapies found to limit the infarct size and prevent heart failure.

dl-3-N-butylphthalide (NBP), autonomously developed by Chinese scientists, is a chemical drug to treat acute cerebral infarction with the beneficial effects of anti-inflammatory, anti-apoptotic, antioxidant, pro-mitochondrial function, and pro-neurogenesis properties [5-7]. As a result, NBP was widely used in the treatment of acute cerebral infarction to limit the size of cerebral infarction. In this experiment, we established a rat model of AMI to study the influence of NBP on infarction size in AMI.

Materials and methods

Animals

A total of 36 healthy male Sprague-Dawley rats (weight, 180±20 g), were randomly assigned to the sham-operation group (SO group, n=12), the model group (n=12) and the NBP group (n=12). The animals were provided by the Experimental Animal Center of Wanleibio Co., Ltd (Shenyang, China) and bred in experimental

animal rooms of the Laboratory with good ventilation, natural light, and a normal circadian cycle. The indoor temperature was maintained at 22±1°C. The animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use guidelines.

Establishment of AMI model

AMI model was established by ligation of left anterior descending artery. Rats were first anesthetized with 10% chloral hydrate (300 mg/Kg body weight) intraperitoneal injection and fixed on an operating table. No rats exhibited any signs of peritonitis, pain or discomfort following administration of 10% chloral hydrate. After tracheal intubation, small animal ventilator was connected to assist respiration. The parameters of ventilator were adjusted as follows: tidal volume: 5 mL, respiratory rate: 80 beats/min, and respiratory ratio: 5:4. The chest hair was removed and the chest was cut open between the 3rd and 4th intercostal spaces to expose the heart. The left anterior descending artery was ligated at about 2 mm on the lower edge of left auricle using the 6-0 wire. Whitening of cardiac apex indicated the successful operation. After the chest was sutured and closed, the rats were treated with anti-infective therapy using penicillin. After resuscitation, the trachea cannula was removed and the rats were placed into the insulation cage.

The study protocol was approved by the Ethical Committee of the Second Hospital of Hebei Medical University. All participants knew the test very well and gave their informed consent.

Administration of NBP

The rats in the NBP group were treated with intraperitoneal injection administration of 60 mg/kg/body weight NBP (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) once a day. The rats in the other groups were given distilled water of the same volume.

Determination of MI area

All rats were sacrificed under thiopental sodium anesthesia and cervical vertebra dislocation after the 14-day treatment. The heart was taken and trimmed after 15-minute observation of vital signs. Meanwhile, the left ventricle was collected, weighed and sliced into sections. After incubation with pre-heated 1% TTC solution (Beijing Dingguo Biotechnology Co., Ltd., Beijing, China) at 37°C for 15 min in dark, the sections were fixed with 4% paraformaldehyde for 30 min and photographed. The staining area was measured using Image-Pro Plus image analysis software. MI area was calculated by the following formula: MI area (%) = (MI area per section/myocardial area in the section * myocardial weight in the section) $\sum$/left ventricular weight * 100%.

Detection of myocardial enzymes

After reperfusion for 120 min, blood was taken from the left common carotid artery and centrifuged at 3500 rpm for 10 min. The supernatant was collected and the concentrations of CK-MB and LDH were detected using the corresponding kits.

Enzyme-linked immunosorbent assay (ELISA) for the measurement of inflammatory factors

After homogenization of the heart tissue of each treatment group, the concentrations of TNF-α, IL-6, MDA, and SOD were measured by ELISA according to the manufacturer's instructions (LifeSpan BioSciences, Inc., Seattle, WA, USA).

Statistical analysis

All calculations were computed with the aid of SPSS 19.0 statistical software. Data are presented as mean ± SEM. The data were compared using one-way ANOVA, followed in case of significance, by a two-sided Tukey's test for multiple comparisons. $P<0.05$ was considered significant.

Results

A total of 36 healthy male Sprague-Dawley rats (weight, 323ts (weight, 323t, 323le Sprague-Dawley rats (weight, 323) using one-way ANOVA, were followed to determine significance.

AMI area

The heart was collected from rats in each group at 14 d after AMI. The MI area in each group was detected by TTC staining. It was found that the left ventricular MI area of NBP group was

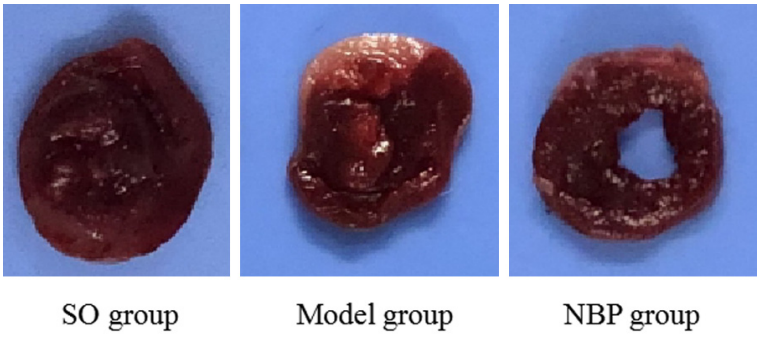

Figure 1. Comparisons of the infarct area among the three groups.

significantly larger than that of the SO group ($P<0.05$). However, MI area in NBP group was significantly smaller than that of the model group after NBP intervention ($P<0.05$) (**Figure 1**; **Table 1**).

The levels of biomarkers of infarction

The myocardial damage biomarkers (CK-MB and LDH) were significantly lower in the NBP group than those in the model group (**Table 1**).

Comparisons of the concentrations of inflammatory factors

The levels of concentrations of TNF-α inflammatory factor were scantly reduced in the NBP group compared to those in the model ($P<0.001$), while the level of TNF-α in the NBP group was significantly lower than that in the model group (29.01±0.81 pg/mg prot vs. 37.727.727.pg/mg prot, $P<0.05$). The level of IL-6 in the model group and NBP group was significantly higher than that in the SO group, and it was lower than that in the model group (24.13±0.74 pg/mg prot vs. 27.537.537.pg/mg prot, $P<0.05$). The levels of MDA in the model group and the NBP group were significantly higher ($P<0.001$), and the level in the NBP group was lower than that in the model group (4.10 high nmol/mg prot vs. 4.774.774. nmol/mg prot, $P<0.05$). The level of SOD in the model group and NBP group were lower ($P<0.001$), and the SOD level in the NBP group was higher than that of the model group (53.49 in the Sprot vs. 38.068.06 in the SOD prot, $P<0.05$) (**Table 2**).

Discussion

Acute myocardial infarction (AMI) is an ischemic heart disease, manifested as persistent ischemia accompanied by myocardial necrosis or apoptosis. It seriously threatens human health [8, 9]. A large amount of research has proven that the prognosis of AMI patients is closely related to the area of myocardial necrosis and the number of surviving myocardial cells. Moreover, the non-renewability of myocardial cells indicates that reversing myocardial remodeling and reducing the number of apoptotic myocardial cells are key steps to improve the prognosis of AMI [10, 11]. NBP is a small molecule drug used in ischemic stroke treatment in China [12]. It was first extracted from celery seeds and showed protective effects for ischemic stroke, attributing to its anti-inflammation, anti-apoptosis, and pro-angiogenesis properties [13, 14].

In this study, the SD rat model of AMI was established by ligation of left anterior descending artery. The MI area was measured. We found that NBP treatment could decrease the MI area.

Myocardial ischemia produces a large number of oxygen free radicals, which act on unsaturated fatty acids on the cell membrane and form the final product of oxygen radical lipid peroxidation, MDA [15]. As a result, the level of MDA reflects the extent of myocardial damage. SOD can scavenge oxygen free radicals and counteract the damage of MDA. It is an important antioxidant enzyme in the myocardium [16], with the effect of alleviating ischemia/reperfusion injury [17]. TNF-α and IL-6 are known to be closely related to myocardial ischemia-reperfusion injury [18]. The concentration of TNF-α in the serum increases with the prolongation of reperfusion time [19], and induces a neutrophil adhesion to cardiomyocytes and release of toxic substances to damage myocardial cells. IL-6 is located at the pivotal position of regulating inflammation and plays an important role in myocardial ischemia-reperfusion injury [20].

dl-3-N-butylphthalide (NBP), a small molecule drug used clinically in the acute phase of ischemic stroke, has been shown to improve functional recovery and promote angiogenesis and

Table 1. Comparisons of the infarct area among the three groups

	SO group (n=12)	Model group (n=12)	NBP group (n=12)	*P* value
Infarction area (%)	0	37.74#	20.06*,#	<0.001
CK-MB (U/L)	40.87	78.97#	63.16*,#	<0.001
LDH (U/g prot)	3992.25	8662.79#	7364.34*,#	<0.001

Note: #: compared with SO group, *P*<0.05. *: compared with model group, *P*<0.05.

Table 2. Comparisons of the inflammatory factors among the three groups

	SO group (n=12)	Model group (n=12)	NBP group (n=12)	*P* value
TNF-u (pg/mg prot)	18.68	37.72#	29.01*,#	<0.001
IL-6 (pg/mg prot)	11.48	27.53#	24.13*,#	<0.001
MDA (nmol/mg prot)	1.71	4.77#	4.10*,#	<0.001
SOD (U/mg prot)	104.57	38.06#	53.49*,#	<0.001

Note: #: compared with SO group, *P*<0.05. *: compared with model group, *P*<0.05.

collateral vessel circulation after experimental cerebral ischemia [21]. Previous studies found that NBP is useful in the treatment of acute cerebral infarction with the beneficial effects of anti-inflammatory, anti-apoptotic, antioxidant, pro-mitochondrial function, and pro-neurogenesis properties [22]. Our findings indicated that NBP can limit the infarct size in rats with AMI by anti-inflammatory effect. However, because of the small sample size, a further study should be carried out to investigate the mechanism of NBP on the infarct area in AMI rats.

In conclusion, NBP can reduce myocardial apoptosis in SD rats with AMI.

Acknowledgements

Supported by 2018 training fund of Department of Human Resources and Social Security of Hebei Province in China (A201803053). I am very grateful to my friend Fengtian Chi who has offered me useful literature to compose my thesis and discussed with me about my thesis.

Disclosure of conflict of interest

None.

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