Dexmedetomidine promotes the functional recovery of mice after acute ischemic stroke via activation of the α2-adrenoceptor

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Abstract

Ischemic stroke (IS) is a well-known acute cerebrovascular disease characterized by high disability, morbidity, and recurrence rates with no effective treatments. Dexmedetomidine (DEX), a selective α2-adrenoceptor agonist used in anaesthesiology and pain management, has been found to exhibit neuroprotective effects in various diseases. However, its role in IS and the underlying mechanisms remains to be determined. Hence, the aim of the present study was to investigate the neuroprotective role of DEX in the recovery of mice following middle cerebral artery occlusion (MCAO). Mice were used to establish the animal model, and then DEX was injected. Behavioural tests (neurological function assessments, grip test, and rotarod test), brain water content measurement, ELISA, and measurement of oxidative stress were performed. DEX activated α2-adrenoceptor and resulted in reduced brain injury, as indicated by the decreased brain water content, S100 Calcium Binding Protein B (S100B) content, and neuron-specific enolase (NSE) content, whilst also inhibiting oxidative stress, as indicated by the increased total antioxidant capacity, catalase, glutathione, and superoxide dismutase levels, and decreased malondialdehyde and glutathione oxidized levels. Neuroinflammation was also reduced as indicated by the decrease in IFN-γ, IL-1α, IL-1β, IL-6, TNF-α, and MMP levels, improved the recovery of neurological function, as indicated by the decreased neurological function score and mNSS, and increased grip strength and rotarod performance in MCAO mice. These combined results suggest that DEX may be a novel strategy for the treatment of IS.

Key words: ischemic stroke, dexmedetomidine, α2-adrenoceptor, oxidative stress, neuroinflammation.

Introduction

Stroke is the second leading cause of mortality and morbidity worldwide, with ischemic stroke (IS) accounting for ~70% of cases of stroke [12,32], and the proportion increasing progressively. Ischemic stroke is primarily the cerebral ischemia and reperfusion injury [9,34]. To date, the therapeutic means for acute ischemic stroke (AIS) have primarily been focused on the restoration of cerebral blood flow (CBF) and the survival of the ischemic penumbra by using thrombolytic drugs [14,31,37]. However, given the strict limitations regarding the time window for intravenous thrombolysis, a significant proportion of stroke patients do not benefit from these clinical interventions, suffering from various degrees of disabilities, which affect a patient’s quality of life [26,29]. As treatment options for IS remain limited, there is an urgent need to explore novel therapeutic approaches.

Cerebral ischemia-reperfusion injury (CIRI) involves several complicated pathophysiological mechanisms, including inflammation, oxidative stress, and apoptosis [35,38]. Accumulating evidence has reported that inflammation and oxidative stress exerts essential roles in the pathogenesis of IS [23,40] as these factors can...
indirectly or directly result in the apoptosis of neurons in the brain, and further lead to neurological dysfunction. Hence, neuroprotective agents with anti-oxidative and anti-inflammatory properties may serve as treatments for IS.

The α-adrenoceptor (AR) is a member of the superfamily of G protein-coupled adrenoceptors [25]. Based on the pharmacological and molecular properties, ARs can be further divided into 3 subtypes: α1-AR, α2-AR, and β-AR [4]. Adrenoceptors include α- and β-adrenoceptor that mediates reactions to endogenous catecholamines in multiple target cells and modulates a range of intracellular processes, including DNA synthesis by activating MAPKs [25]. Targeting α2-adrenoceptor function, either using genetic tolls or drug interventions, may translate to clinically important effects [6].

Dexmedetomidine (DEX), a well-established anaesthetic adjuvant, sedative, and opioid-sparing medication [15], is a strongly selective α2-adrenoceptor agonist owing to its cardioprotective activity and exhibits multiple other biological functions including neuroprotective, renal protective, and lung protective effects against I/R injury [20], and translates well from animal models to clinical settings [3,18,30]. An increasing number of studies have confirmed its potential use as an neuroprotective agent, several neuroprotective mechanisms have been found in animal models of brain disease due to its anti-inflammatory effects through inhibition of the TLR4/NF-κB pathway, and inhibition of apoptosis and autophagy [3,5,13]. Pretreatment of DEX improves postoperative cognitive impairment by suppressing hippocampal neuroinflammation [27]. DEX can reduce cognitive dysfunction following MCI in rats by inhibiting the release of inflammatory cytokines [41]. DEX can attenuate neuropathic pain by reducing the release of inflammatory factors and inhibiting oxidative stress [22].

Given the key neuroprotective roles of DEX in the nervous system, the functional roles of DEX in the treatment of IS and the related underlying mechanisms were investigated in the present study. It was shown that DEX could inhibit inflammation and oxidative stress to promote the functional recovery of mice following acute ischemic stroke via activation of the α2-adrenoceptor.

Material and methods

Animals

In the present study, 8-week-old male C57BL/6J mice, purchased from the Hunan Medical Laboratory Animal Center, were housed at 22°C with a 12-h light/dark cycle and provided ad libitum access to food and water. All experimental protocols involving animals were approved by the Laboratory Animal Ethics Com-

mittee of The First Affiliated Hospital of the University of South China (approval no. 20211012004).

MCAO and treatment

The MCAO model using C57BL/6J mice was established as previously described [36]. Generally, mice were anesthetized with 5% isoflurane in O2 using a facemask, following the ligation of the middle cerebral artery on the left side with a 6-0 monofilament. After occlusion of this artery for 1 h, the monofilament was removed to allow reperfusion. A homeothermic heating pad was used to monitor and maintain the body temperature at 37 ±0.5°C.

To determine the neuroprotective role of DEX on IS, mice suffering from MCAO were randomly divided into 3 groups: 1) MCAO, 2) MCAO + DEX, or 3) MCAO + DEX + SKF86466. The groups were intraperitoneally injected with 100 μl PBS or DEX (100 μg/kg) with or without SKF86466 twice daily. For the Sham group, the mice underwent the same procedure without monofilament ligation.

Behavioural tests

All behavioural tests were performed post-MCAO for 24 h by 2 researchers who were blinded to animal allocation. Following the behavioural tests, animals were anesthetized with 5% isoflurane and then sacrificed, and tissues were collected for further analysis.

Neurological function assessment

The neurological function tests were performed using the Longa scoring method [19] and modified neurological severity score (mNSS). For Longa scoring, the function was scored as follows: 0 – no neurological deficits, 1 – inability to fully extend the contralateral forepaw, 2 – circling to the contralateral side, 3 – falling to the contralateral side, and 4 – inability to walk spontaneously and with a depressed level of consciousness. For mNSS, several aspects are listed as follows: 1) motor tests (muscle status-hemiplegia, normal = 0 – maximum = 6); 2) sensory tests (normal = 0 – maximum = 2); 3) beam balance tests (normal = 0 – maximum = 6); and 4) reflexes absent and abnormal movements (normal = 0 – maximum = 4). The total score of mNSS ranged from 0 to 18.

Grip test

The muscular co-ordination of mice following IS was evaluated by the grip strength (GSM Grip Strength Meter 47200). The forelimbs were placed on the test grid, and the mice were gently pulled after grasping...
Dexmedetomidine promotes the functional recovery of mice after acute ischemic stroke via activation of the α2-adrenoceptor

them. The highest value in grams (g) was selected for the grasping strength of each mouse.

Rotarod test

The motor coordination of the mice following IS was evaluated using an accelerating rotarod (SD Instruments, Inc.) [39]. Prior to formal testing, the mice were pre-trained at an acceleration mode for 4-40 rpm for 5 min once a day for 3 days. After MCAO surgery had been performed, the rotarod test was performed as before by a blinded observer 24 h post MCAO.

Brain water content

The two hemispheres were evaluated on an electronic balance for wet weight and subsequently dried in an oven for 24 h at 100°C for determining the dry weight. Brain water = (wet weight – dry weight)/wet weight × 100%.

Enzyme-linked immunosorobent assay

The levels of S100 calcium-binding protein B (S100B; cat. no. H258, Nanjing Jiancheng Bioengineering Institute), neuron-specific enolase (NSE; cat. no. H240, Nanjing Jiancheng Bioengineering Institute), interferon γ (IFN-γ; cat. no. H025, Nanjing Jiancheng Bioengineering Institute), interleukin (IL)-1α (cat. no. PIS65, Beyotime Institute of Biotechnology), IL-1β (cat. no. EK0412, Wuhan Boster Biological Technology, Ltd.), IL-6 (cat. no. EK0526, Wuhan Boster Biological Technology, Ltd), tumour necrosis factor α (TNF-α; cat. no. EK0393, Wuhan Boster Biological Technology, Ltd), and matrix metalloproteinase (MMP)-9 (cat. no. H146-4, Nanjing Jiancheng Bioengineering Institute).
Measurement of oxidative stress

The levels of total antioxidative capacity (T-AOC; cat. no. A015-1-2, Nanjing Jiancheng Bioengineering Institute), CAT (cat. no. A007-2-1, Nanjing Jiancheng Bioengineering Institute), glutathione (GSH; cat. no. A061-1-2, Nanjing Jiancheng Bioengineering Institute), superoxide dismutase (SOD; cat. no. A001-3-2, Nanjing Jiancheng Bioengineering Institute), malondialdehyde (MDA; cat. no. A003-1-2, Nanjing Jiancheng Bioengineering Institute), and oxidized glutathione (GSSG; cat. no. A061-1-2, Nanjing Jiancheng Bioengineering Institute) in brain tissues were measured using the respective kits according to the manufacturer’s protocol.

Statistical analysis

All analyses were performed using GraphPad Prism 6 (GraphPad Software Inc.). Data are presented as the mean ± SD. A one-way ANOVA followed by a post-hoc Bonferroni test was used to compare the differences between groups. P < 0.05 was considered to indicate a statistically significant difference.

Results

DEX promotes the functional recovery of mice following ischemic stroke via activation of the α2-adrenoceptor

To evaluate the effect of DEX on the functional recovery of mice following IS, Longa scoring, mNSS, grip tests, and rotarod tests 24 h post MCAO were assessed. In Longa scoring, compared with the sham group without any neurological dysfunction, neurological function score increased in the MCAO group, whereas DEX treatment improved (as determined by a decrease in) the neurological function score in mice following MCAO; moreover, DEX did not improve the neurological function score in mice following MCAO when co-treated with SKF86466 to inhibit α2-adrenoceptor (Fig. 1A). A similar pattern was observed regarding the mNSS (Fig. 1B).

In the grip test, compared with the sham group, the grip strength of the mice following MCAO was...
Dexmedetomidine promotes the functional recovery of mice after acute ischemic stroke via activation of the α2-adrenoceptor

significantly decreased. DEX treatment can increase the grip strength compared with the MCAO group; moreover, DEX did not improve the grip strength after MCAO when co-treated with SKF86466 to inhibit α2-adrenoceptor (Fig. 1C). A similar pattern was observed for the rotarod performance in the rotarod test (Fig. 1B).

DEX reduces brain injury in mice following ischemic stroke via activation of the α2-adrenoceptor

To determine the influence of DEX on brain injury in the MCAO mice, the brain water content, S100B content, and NSE content were calculated 24 h post MCAO.

**Fig. 3.** Determination of the effect of dexmedetomidine (DEX) on the oxidative stress in the brain of mice suffering from ischemic stroke (IS). DEX activates α2-adrenoceptor to inhibit oxidative stress in the brain of mice following middle cerebral artery occlusion (MCAO), as indicated by the changes in A) total antioxidative capacity (T-AOC), B) catalase (CAT), C) glutathione (GSH), D) superoxide dismutase (SOD), E) malondialdehyde (MDA), and F) oxidized glutathione (GSSG) levels; n = 6. *p < 0.05, **p < 0.01, ***p < 0.001.
Compared with the sham group, the brain water content was increased after MCAO, but decreased in mice treated with DEX; moreover, it was increased when mice were co-treated with SKF86466 to inhibit α2-adrenoceptor (Fig. 2A). Similar patterns were seen for S100B (Fig. 2B) and NSE content (Fig. 2C).

**DEX suppresses oxidative stress in mice following ischemic stroke via activation of α2-adrenoceptor**

To evaluate the effect of DEX on oxidative stress in MCAO mice, T-AOC, CAT, GSH, SOD, MDA, and GSSG levels were measured 24 h post MCAO in mice.
Compared with the sham group, the T-AOC levels were decreased following MCAO, but increased following DEX treatment; moreover, it was decreased when mice were co-treated with SKF86466 to inhibit α2-adrenoceptor (Fig. 3A). Similar patterns were observed regarding CAT (Fig. 3B), GSH (Fig. 3C), and SOD levels (Fig. 3D), and the reverse was seen for MDA (Fig. 3E) and GSSG levels (Fig. 3F).

**DEX inhibits neuroinflammation in mice following ischemic stroke via activation of the α2-adrenoceptor**

To evaluate the effect of DEX on neuroinflammation in MCAO mice, the levels of IFN-γ, IL-1α, IL-1β, IL-6, TNF-α, and MMP were detected 24 h post MCAO in mice using ELISA.

Compared with the sham group, IFN-γ levels were increased following MCAO but decreased in response to the treatment with DEX; moreover, it was increased when mice were co-treated with SKF86466 to inhibit α2-adrenoceptor (Fig. 4A). Similar patterns were observed regarding IL-1α (Fig. 4B), IL-1β (Fig. 4C), IL-6 (Fig. 4D), TNF-α (Fig. 4E), and MMP levels (Fig. 4F).

**Discussion**

In previous studies, the neuroprotective roles of DEX have been reported, and in the present study, it was shown that DEX activated α2-adrenoceptor to reduce brain injury, inhibit oxidative stress and neuroinflammation, and improve neurological function recovery in MCAO mice.

Neurological function evaluation is commonly performed to determine the degree of injury and further assess the therapeutic effects of treatments. In the present study, it was shown that DEX promoted the functional recovery of mice following IS via activation of the α2-adrenoceptor.

In the early acute phase of IS injury, the pathophysiological response of the brain is primarily reflected by the degree of brain oedema; in the middle stage, inflammation and neuronal apoptosis are observed; and this is followed by angiogenesis and neurogenesis [1]. The levels of S-100B and NSE are used as a measure to assess the severity of brain injury after IS [24]. In the present study, it was shown that DEX alleviated brain injury in mice following IS via activation of the α2-adrenoceptor.

The pathogenesis of IS involves multiple mechanisms, among which, oxidative stress and apoptosis are considered critical events [17]. After IS, the generation of large quantities of reactive oxygen species (ROS) is observed in brain tissues. The imbalance between the production of ROS and the antioxidant systems to remove ROS leads to excess accumulation of ROS to aggravate oxidative stress lesions. In the present study, it was shown that DEX inhibited oxidative stress in mice following IS via activation of the α2-adrenoceptor.

ROS interact with DNA, lipids, RNA, and proteins, leading to oxidative stress, inflammatory responses, and ultimately, neuronal cell death [10,33]. Neurological impairment following stroke is modulated by pro-inflammatory cytokines and inflammatory reactions [7,11]. IS injury results in extensive infiltration of immunocytes that activate glial cells to excrete proinflammatory cytokines, which participate in the development and induction of brain oedema [8,16]. IS injury results in the release of inflammatory cytokines, pro-inflammatory mediators, and anti-inflammatory mediators in ischemic brain tissues [2]. Recent studies reported that DEX can alleviate inflammation in animals and patients [21,28]. In the present study, it was revealed that DEX could inhibit neuroinflammation in mice following IS via activation of the α2-adrenoceptor.

Although the effect of DEX on MCAO is promising, further studies for no doubt are needed to be performed to find more about the effect of DEX, for example, whether DEX can decrease the incidence of ischemic stroke in the patients who were treated with DEX when performing the surgery. In conclusion, DEX may serve as a novel strategy for the treatment of IS.

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**Disclosure**

The authors report no conflict of interest.

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