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The effects of clenbuterol on cerebral vasospasm in an experimental rat model of subarachnoid hemorrhage

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Abstract

Objective: To investigate the possible therapeutic effects of clenbuterol on cerebral vasospasm after subarachnoid hemorrhage (SAH) in rats.

Methods: Eighteen male albino Wistar rats, each weighing 200-250 g, were randomized into three groups; Group 1 (Control group) (n = 6) having no SAH and no treatment; Group 2 (Sham group) (n = 6) having only SAH and Group 3 (Experimental group) (n = 6) having SAH treated with clenbuterol. Group 2 has been accepted as sham group to the experimental group. Experimental SAH was induced using a modified rat double hemorrhage model. Clenbuterol was administered twice daily in 12-hour intervals for three days at a dose of 0,1 mg/kg/day. The luminal diameter of the basilar artery was measured on each section with an optic micrometer by an experienced pathologist blinded to the groups.

Results: Mean basilar artery diameters were found to be different between the three groups ($p < 0.001$). Mean value of Group 2 was significantly lower than that of Group 1 ($p < 0.001$). While mean value of Group 3 was significantly greater than that of Group 2 ($p = 0.001$), Groups 1 and 3 were found to be similar ($p = 0.242$).

Conclusion: Clenbuterol has favorable effects in the treatment of rat cerebral vasospasm (CVS). Further investigations are needed to evaluate both molecular effects and to find out effective treatment dose of clenbuterol on CVS.

Key words: Subarachnoid hemorrhage; cerebral vasospasm; clenbuterol; experimental vasospasm.

Introduction

Cerebral vasospasm (CVS) is described as delayed-onset luminal narrowing of the cerebral arteries in response to blood clots left in the subarachnoid space after spontaneous aneurysmal subarachnoid hemorrhage (SAH) (4, 16, 22, 27). It is the most serious complication affecting both morbidity and mortality (3, 11, 15). In spite of new therapeutic approaches (7, 11, 13, 17), successful treatment of CVS after subarachnoid hemorrhage remains a dilemma (9, 25).

An imbalance between endothelin-mediated vasoconstriction and nitric oxide (NO) mediated vasodilation has been implicated in the pathogenesis of cerebral vasospasm (21, 27). Inflammation has also been proposed to play a role in the mechanism of CVS (2, 18, 27). In this regard, clenbuterol, a β2-adrenoceptor agonist that has antiinflammatory (12, 28), neuroprotective (20) and NO releasing effects (14), may be considered as a new alternative for the treatment of CVS. To our best knowledge, it has not been studied in CVS (14). In this study, we aimed to investigate the possible therapeutic effects of clenbuterol on CVS after SAH in rats.

Materials and methods

The experiment was performed in accordance with Institutional Guidelines for the care and use of laboratory animals and all protocols were approved by Pamukkale University Medical Faculty Ethics Committee. Eighteen male albino Wistar rats, each weighing 200-250 g, were randomized into three groups; Group 1 (Control group) (n = 6): having no SAH and no treatment, Group 2 (sham to the experiment group) (n = 6): having only SAH and Group 3 (experiment group) (n = 6): having SAH treated with clenbuterol. All animals were kept at the same environmental temperature (22°C) and humidity. Throughout the experiment, the rats were let free access to food and water.

Experimental SAH model and treatment protocol

The rats were initially anesthesized with intramuscular injections of ketamine hydrochloride (40 mg/kg) and xylazine (10 mg/kg) and were allowed to breathe spontaneously throughout the procedures.

Experimental SAH was induced using a modified rat double hemorrhage model (25). Under sterile conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna and 0.1 ml cerebrospinal fluid (CSF) was withdrawn, thereafter the same amount of fresh nonheparinized autologous blood obtained from the tail artery was slowly injected into the subarachnoid space to induce the first SAH. The same procedure was repeated after 48 hours in identical manner for induction of the second SAH.

Clenbuterol tablets were pulverized manually and dissolved in saline solution by mechanical mixing. No precipitation was observed in the prepared solution. Clenbuterol was administered twice daily in 12-hour intervals for three days at a dose of 0,1 mg/kg/day. The drug was given directly into the stomach through a gastric tube.

The animals were sacrificed under general anesthesia after 72 hours following SAH and were transcardially perfused with 50 ml of phosphate-buffered saline and then 100 ml of 4% paraformaldehyde to fix the basilar arteries at their insitu diameter. After perfusion and fixation, the brains were removed totally. Widespread subarachnoid hemorrhage was macroscopically observed on the basal side of the brain stem around the vertebro-basillary system in groups 2 and 3 rats.

Morphometric analysis

All tissue specimens were kept in 10% formaldehyde solution for 24 hours. They were embedded in paraffin and cut into 5-μm slices using steel knife rotator microtome. Then they were stained with hematoxylin and eosin. Three sections from the top, middle, and bottom of the basilar artery were used for measurements. The slices were evaluated with a light microscope and each section was digitally photographed. The luminal diameter of the basilar artery was measured on each section with an optic micrometer by an experienced pathologist blinded to the groups. The mean value was calculated for each basilar artery.

Statistical analysis

Shapiro Wilk's test was used to assess the distribution of the data. One-way ANOVA was used to compare the mean values of the groups followed by Tukey's multiple comparisons. SPSS 13.0 was used for the statistical analyses and p values less than 0.05 were considered significant.

Results

The groups were similar in terms of body weight and mean arterial blood pressure. Photomicrographs of representative slices showed typical features of vasospasm.

The distribution of the basilar artery diameters are shown in Figure 1. Mean basilar artery diameters were found to be different between the three groups ($p < 0.001$). Mean value of Group 2 was significantly lower than that of Group 1 ($p < 0.001$). While mean value of Group 3 was significantly greater than that of Group 2 ($p = 0.001$), Groups 1 and 3 were found to be similar ($p = 0.242$).

Discussion

In this study, we have investigated the impact of clenbuterol on CVS in a rat model and our results have shown that it had favorable effects.

Cerebral vasospasm is an important cause of cerebral ischemia after SAH (2, 22). Several cytokines or neuropeptides have been proposed to play role in the pathogenesis of vasospasm (13, 22, 27). Accordingly, many treatment alternatives have been tried and those include calcium antagonists, magnesium, antiinflammatory drugs, NO-related vasodilators, endothelin antagonists, statins, antioxidants and free radical scavengers, serine protease inhibitors,

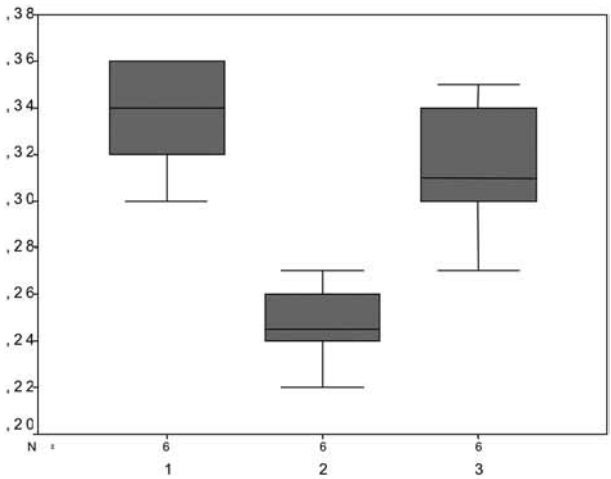

Fig. 1. — Box-plot graphics demonstrating the distribution of the basilar artery diameters in each group (x axis = group, y axis = basilar artery diameter).

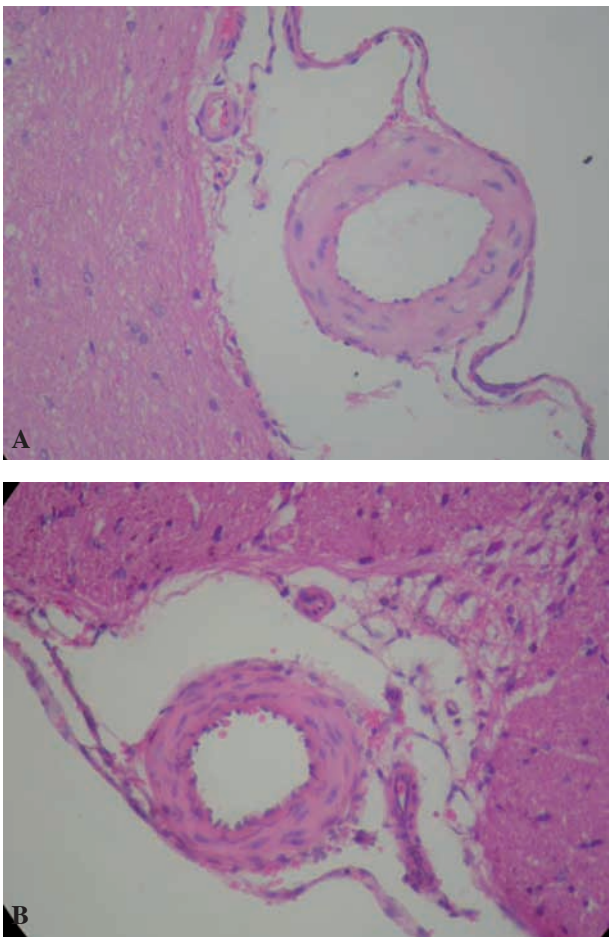

FIG. 2. — Photomicrographs of the basilar artery from a normal rat (A) after SAH (B) and after treatment (C). Corrugation of the internal elastic lamina, thickening and vacuolization of the arterial wall, and narrowing of the cross-sectional diameter is seen (B).

raloxifene etc. (1, 5, 6, 8, 11, 13, 17, 19, 22, 23, 26). However, despite accumulating experimental and clinical data, its mechanism is yet unclear.

The release of NO from the endothelium for autoregulation of cerebral vascular tone has an important role in the pathogenesis of CVS after SAH (17, 24, 27). Clenbuterol has been shown to reduce contraction in the endothelium-denued mesenteric arteries of the rat via NO release, through the activitation of B2-adrenoceptors probably present in nitrergic nerves (14). Inflammation has also a significant role in the pathophysiology of cerebral vasospasm (18, 27).

Thus, anti-inflammatory agents that modulate the early extravasation of leukocytes into the subarachnoid space have been proposed to be adjuncts in the treatment of CVS (2, 17). Beta agonists, by increasing the intracellular cAMP levels, inhibit the production of proinflammatory cytokines (28). Due to its diffusion through the blood-brain barrier, direct effects on the central nervous system, anti-inflammatory and neuroprotective features and capability in reducing the contractile responses of the arteries can make clenbuterol a potential therapeutic agent in the treatment of CVS (12, 18, 20).

We may conclude that clenbuterol has favorable effects in the treatment of rat CVS. The effective treatment dose of clenbuterol and its mechanism (its molecular effects) on CVS need further investigations.

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