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Effects of raloxifene on cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits

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Abstract

Background: The aim of this study was to investigate the ability of a SERM, RLX, to prevent vasospasm in a rabbit model of SAH.

Methods: Thirty-four New Zealand white rabbits were allocated into 3 groups randomly. Subarachnoid hemorrhage was induced by injecting autologous blood into the cisterna magna. The treatment groups were as follows: (1) sham operated (no SAH [n = 12]), (2) SAH only (n = 12), and (3) SAH plus RLX (n = 10). Basilar artery lumen areas and arterial wall thickness were measured to assess vasospams in all groups.

Results: There was a statistically significant difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements of the control and SAH-only groups ($P < .05$). The difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements in the RLX-treated group was statistically significant ($P < .05$). The difference between the SAH group and the SAH + RLX group was also statistically significant ($P < .05$).

Conclusions: These findings demonstrate that RLX has marked vasodilatatory effect in an experimental model of SAH in rabbits. This observation may have clinical implications suggesting that this SERM drug could be used as possible anti-vasospastic agent in patients without major adverse effects.

Keywords: Raloxifene; Subarachnoid hemorrhage; Vasospasm; Rabbit

1. Introduction

Delayed vasospasm after SAH is a major component of brain damage after SAH. It is characterized by the prolonged and reversible contraction of the cerebral arteries that adds to the ischemic process [14].

Raloxifene is a nonsteroidal benzothiophene that has been classified as a SERM [15]. It binds to ER subtypes α and β [13]. Approximately 60% of orally administered RLX is absorbed from the gastrointestinal tract, and the reported bioavailibity is 2%. The drug has been shown to cross the blood-brain barrier in sufficient quantities to alter ER expression [25]. Its use has been approved for the treatment

Abbreviations: cGMP, cyclic guanosine monophosphate; ER, estrogen receptor; MLC, myosin light chain; NO, endothelin-derived relaxing factor, nitrous oxide; RLX, raloxifene; SAH, subarachnoid hemorrhage; SERM, selective estrogen receptor modulator; VDCC, voltage-dependent Ca^{2+} channels; $[Ca^{2+}]_i$, intracellular Ca^{2+}.

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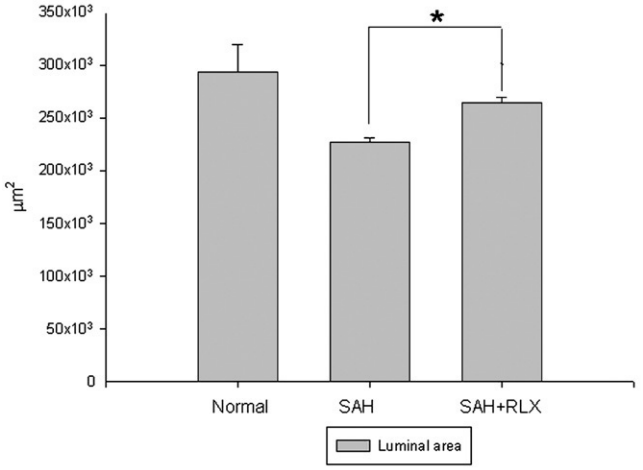

Fig. 1. Graph showing arterial luminal area measurements in 3 different groups.

of osteoporosis. It has estrogen-agonistic effects on bone and lipid metabolism, and estrogen-antagonistic effects on uterine endometrium and breast tissue [6,7,24]. Effect of RLX on endothelial function in the coronary arteries or in the peripheral arteries is widely studied with contradictory results [2,4]. It has been shown to induce endothelium-dependent vasodilation and directly activate NO synthesis in endothelial cells [9,20]. Leung et al [12] reported a vasodilatatory effect of RLX on rat intrarenal arteries by inhibiting Ca^{2+} influx into vascular smooth muscle cells. They did not find any sex-specific differences for the vasorelaxing effect of RLX. On the basis of these findings, we aimed to investigate the effect of RLX on cerebral arteries after SAH-induced vasospasm in a rabbits.

2. Materials and methods

All protocols were approved by Ministry of Health Ankara Diskapi Research and Education Hospital Ethics Committee. The animals were initially anesthetized with ketamine-xylazine (35/10 mg/kg) intramuscularly, and all animals breathed spontaneously throughout the procedures. Arterial blood samples (P_{O_2} and P_{CO_2}) were taken from each animal from the catheterized ear arteries for blood gas analysis during the procedures, and only those animals with P_{O_2} more than 70 mm Hg and P_{CO_2} less than 40 mm Hg were included in the study (n = 12 in the control group, n = 12 in the SAH group, and n = 10 in the SAH + RLX group). Heart rate and systemic blood pressure were measured with the use of an ear artery catheter and mean blood pressures before and after SAH were recorded. Core body temperature was monitored rectally and maintained at 37°C ± 0.5°C with a heater.

2.1. Cerebral vasospasm model

Under sterile conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna, as described before [19]. In the control group, 1.0 mL of CSF was withdrawn and 2.0 mL of saline (%0.9 NaCl) was injected into the subarachnoid space in 2 minutes. In the SAH group, after withdrawal of 1.0 mL of CSF, 2.0 mL of nonheparinized fresh autologous blood, drawn from central ear artery, was injected into the subarachnoid space in 2 minutes. The animals were then placed in a head-down position for 30 minutes so that the blood would reach the basal cisterns. After recovering from anesthesia, the rabbits were observed for possible neurologic deficits and then returned to the vivarium.

2.2. Drug treatments and groups

Thirty-four male New Zealand white rabbits weighting 2400 to 3600 g were allocated to 3 groups. Group 1 (sham group, n = 12) and group 2 (SAH only, n = 12) did not receive any treatment. Group 3 (SAH + treatment, n = 10) received RLX at a loading dose of 1 mg/kg through a gastric tube 6 hours before the formation of SAH. Raloxifene was given directly into the stomach through a gastric tube. The maintenance dose was 1 mg/kg per day. The animals tolerated RLX well without any gastrointestinal intolerance during the procedures. The rabbits were killed under general anesthesia after 72 hours.

2.3. Morphometric analysis of the basilar artery

Rabbits were pressure perfused 72 hours after the insult. The perfusion was begun initially with 300 mL of physiologic saline and then 250 mL of 4% paraformaldehyde under a pressure of 120 cm H_2O. The entire basilar artery was sectioned at 5 segments 2 mm in length. Tissues are embedded in paraffin, and hematoxylin and eosin stain is performed on the 0.5-μm cross sections of the basilar artery. Measurements of the basilar artery lumen area (cross sectional area) and arterial wall thickness were made in a single-blind fashion by one pathologist. Morphometric measurements on all 5 cross sections from basilar artery were performed by using the Image Analysis System (Spot Software: 4.1, Diagnostic Instruments Inc, USA). The luminal area was calculated from the perimeter of the luminal border, and the area contained within the boundaries of the internal elastic lamina is neglected. The luminal area for each basilar artery was obtained by averaging these measurements. The wall thickness between lumen and external border of muscle layer was measured at 4 quadrants

Table 1
Physiologic parameters of the animals during surgery

Group	Control (n = 12)	SAH (n = 12)	SAH + RLX (n = 10)
HR	170.7 ± 4.4	173.6 ± 6.7	172.8 ± 5.2
MABP	81.5 ± 2.6	84.4 ± 3.5	83.4 ± 3.0
CBT	36.8°C ± 0.4°C	37.4°C ± 0.3°C	37.2°C ± 0.4°C

All values are expressed as mean ± standard deviation. No significant differences were seen among groups ($P > .05$). HR indicates heart rate; MABP indicates mean arterial blood pressure (mm Hg); CBT, core body temperature.