Effects of Cobalt Nitrate on Histopathological and Morphometric Changes in Rat Femoral Artery Vasospasm Model

Saygın Ucar¹, Yasar Dagistan² and Ali Guler³

ABSTRACT

Objective: To determine the effects of cobalt nitrate on the arterial wall in a rat femoral artery vasospasm model.

Study Design: Experimental study.

Place and Duration of Study: Faculty of Medicine, Istanbul University, between November 2009 and September 2010.

Methodology: Twenty-four rats were separated into three groups named group 1 (n=8, sham-control); group 2 (n=8, vasospasm), and group 3 (n=8, vasospasm + cobalt nitrate treated). Group 3 rats were subjected to intraperitoneal administration of 0.1 mg/kg/day cobalt nitrate for 7 days, followed by histological and morphometric analyses. ANOVA and post hoc analyses were carried out.

Results: The mean vascular wall thickness of the group 1, 2 and 3 rats was found to be 133 ±13, 210 ±15 and 160 ±11 micron, respectively. The mean vascular lumen diameter of the group 1, 2 and 3 rats was 698 ±20, 240 ±15 and 540 ±22 micron, respectively. Arteries in the group 3 had thin smooth endothelium, thin mildly folded internal elastic lamina, and concentric smooth muscle cells. The mean vascular lumen diameter of the group 3 rats showed a significant decrease compared to the group 2 rats.

Conclusion: Cobalt nitrate may potentially serve as an agent in preventing cerebral vasospasm after a hemorrhagic episode.

Key Words: Cobalt nitrate. Subarachnoid hemorrhage. Femoral artery vasospasm. Rat.

INTRODUCTION

In patients suffering from subarachnoid hemorrhage (SAH), vasospasm of cerebral arteries is the most common cause of delayed neurological defect.¹⁻³ Vasospasms after subarachnoid hemorrhage depend on a wide variety of causes such as blood, blood products, eicosanoid, endothelin, free radicals, and inflammation. Although various pharmacological drugs have been developed to treat patients with SAH-induced delayed cerebral vasospasm, but in the majority of patients the medications are not satisfactory.⁴⁻⁶

Cobalt nitrate is the inorganic cobalt (II) salt of nitric acid, often with various amounts of water. Cobalt nitrate is generally found as a hexahydrate, a red-brown deliquescent salt that is soluble in water and other polar solvents.⁷ Cobalt (II) complexes have antimicrobial, anticancer, antiproliferative, and antioxidant activity.⁸⁻¹⁰

The aim of this study was to determine the effects of cobalt nitrate on morphometric and histopathological changes in the rat femoral artery wall in an experimental vasospasm model.

METHODOLOGY

This experimental study was conducted with the approval of the Ethics Committee Institute for Experimental Medical Research (DETAE), Istanbul Faculty of Medicine, Istanbul University between November 2009 and September 2010 at the Research Institute, University of Istanbul, Istanbul, Turkey. Twenty-four female Sprague-Dawley rats weighing 180 - 220 g were used. The animals were fed ad libitum, and a 12:12-hour light/dark cycle was maintained.

Rats were separated into three groups: group 1 (n=8, sham-control); group 2 (n=8, vasospasm), and group 3 (n=8, vasospasm + cobalt nitrate treated). The group 1 rats were delivered 1 cc 0.9% saline. In the group 2 and 3 rats, 0.1 cc percutaneous intracardiac blood was collected and injected into the silastic sheath to generate a peripheral vasospasm model. In addition, the group 3 rats were subjected to 0.1 mg/kg/day intraperitoneal cobalt nitrate for 7 days.

For the femoral artery vasospasm model, Okada et al. model was preferred.¹¹ Rats were sedated with intraperitoneal 2 mg/kg ketamine HCl (Ketalar vial 50 mg/ml, Pfizer). The femoral vascular bundle was exposed with a longitudinal 2 cm skin incision under a surgical microscope. The femoral artery was dissected from the femoral vein and nerve without being traumatised.

¹ Department of Neurosurgery, Gaziosmanpasa Taksim Training and Research Hospital, Istanbul, Turkey.
² Department of Neurosurgery, Abant Izzet Baysal University, Medical School, Bolu, Turkey.
³ Department of Neurosurgery, Derince Training and Research Hospital, Kocaeli, Turkey.

Correspondence: Dr. Yasar Dagistan, Assistant Professor, Department of Neurosurgery, Abant Izzet Baysal University, Medical School, Bolu, Turkey.
E-mail: dagistanyasar@hotmail.com

Received: June 23, 2014; Accepted: May 31, 2016.
A 1-1.5 cm silastic sheath was wrapped around the femoral artery and sutured. Autologous cardiac blood of the subjects was used as whole blood.

Rats were fed a mouse diet and kept at room temperature in separate cages for 7 days. No subject required exclusion due to mortality or disease. At the seventh day, the silastic sheath wrapped around the femoral artery was accessed by opening the earlier surgical incisions, and the femoral artery was exposed from the silastic sheath. In all rats, the femoral artery was observed to be within the silastic sheath. In all groups, the right femoral artery was incised 1 - 1.5 cm and removed for histopathological morphometric analysis.

The extracted femoral artery specimens were immediately placed in 10% formalin solution. The tissue specimens were placed in paraffin blocks, sectioned at 5 µm, and stained with hematoxyline and eosine. The preparations were examined under 100x, 200x, and 400x magnification with an Olympus (Olympus BX7, Japan) microscope, and photographs were taken for morphometric analysis. The vascular wall thickness and lumen area were measured as unit values with the Image J 1.34 program. Measurements were carried out on the photographs taken under 40x magnification. The specimens were compared in terms of the vascular lumen area and wall thickness in the morphometric analysis.

SPSS 11.0 software (SPSS Inc., USA) for Windows was used for the statistical analyses. Data are expressed as mean ±S.D. and analysed for statistical significance by one-way analysis of variance (ANOVA) followed by post-hoc for multiple. Differences between mean values were considered significant when p < 0.05.

**RESULTS**

Histopathologically, the sham-control group rats had essentially normal femoral artery morphology (Figure 1A). In the group 2 rats, the arteries had marked narrowing in the lumen width as well as thickening in the vascular wall, disruption of endothelial integrity, folding in the internal elastic lamina, and vacuolizations in the muscle layer (Figure 1B). Arteries in the group 3 rats had thin smooth endothelium, thin and mildly folded internal elastic lamina, and concentric smooth muscle cells (Figure 1C).

After the femoral artery wall thickness and lumen area of all rats were calculated, the groups were compared. The mean vascular wall thickness of the group 1, 2 and 3 rats was found to be 133 ±13, 210 ±15 and 160 ±11 micron,
respectively (Figure 2a). In the group 2 rats, the vascular wall thickness had increased and the lumen diameter had decreased compared to those of the control group and the cobalt nitrate treated group (p < 0.003 and p < 0.001, respectively). The mean vascular lumen diameter of the group 1, 2 and 3 rats was 698 ±20, 240 ±15 and 540 ±22 micron, respectively (Figure 2b). The mean vascular lumen diameter of the group 3 rats had increased compared to the group 2 rats (p < 0.001).

**DISCUSSION**

Spontaneous SAH from the rupture of a cerebral aneurysm is an important clinical problem. After aneurysmal SAH, cerebral vasospasms are still one of the most important contributors to morbidity and mortality. It is a common and potentially devastating condition that is not completely understood and for which the underlying pathological mechanism remains obscure, despite much research. Additionally, despite the advances in pharmacological and surgical treatment of SAH, there is still no exact therapy for cerebral vasospasm. Recent studies have determined that inflammation and free oxygen radicals play a key role in the pathophysiology of cerebral vasospasms. In the present study, the authors demonstrated that cobalt nitrate reduced the development of peripheral vasospasms in a rat model.

Cobalt is an element of biological interest due to its presence in the active center of cobalamin. Cobalt indirectly regulates the synthesis of DNA, and is involved in the co-enzyme of vitamin B12. Numerous cobalt complexes showing antitumor, antiproliferative, antimicrobial, antifungal, antiviral, and antioxidant activity have been reported in the literature. They demonstrated that tolfenamic acid and its cobalt (II) complexes exhibit good binding propensity to human or bovine serum albumin protein, with high binding constant values. In a similar study, Dimiza et al. suggested that mfenamic acid and its cobalt (II) complexes exhibit good binding propensity to human or bovine serum albumin protein, with high binding constant values. Additionally, the antioxidant activity of the compounds has been evaluated indicating their high scavenging activity against hydroxyl-free radicals and superoxide radicals. Wu et al. investigated the coordinating ability of various benzimidazole ligands. In their study, the synthesis, structure, DNA-binding properties, and antioxidant activity of copper (II) and cobalt (II) complexes with bis (N allylbenzimidazol-2-ylmethyl) benzylamine are presented. Both complexes exhibited potential antioxidant properties in in vitro studies.

In this study, the arteries in the group 2 rats narrowed significantly in the lumen width and thickness in the vascular wall, disruption of endothelial integrity, folding in the internal elastic lamina, and vacuolization in the muscle layer. Whereas, arteries in the group 3 rats had thin smooth endothelium, thin mildly folded internal elastic lamina, and concentric smooth muscle cells. In the group 2 rats, the wall thickness increased significantly and the lumen diameter decreased compared to the cobalt nitrate treated group.

**CONCLUSION**

Cobalt nitrate histopathologically and morphometrically prevented the development of peripheral vasospasm in rats. Thus, these findings suggest that cobalt nitrate is a promising therapeutic agent for preventing cerebral vasospasm.

**REFERENCES**

7. Tikhomirov GA, Znamenkov KO, Morozov IV, Kemnitz E, Troyanov SI. Anhydrous nitrates and nitrosonium nitratometallates of manganese and cobalt, (NO)2[Mn(NO3)2], (NO)2[Co(NO3)2]3, and (NO)2[Co(NO3)2]. Synthesis and crystal structure. Z Anorg Allg Chem 2002; 628:269-73.
10. Emirdag-Ozturk S, Babahan I, Ozmen A. Synthesis,


12. Suarez JI. Diagnosis and management of subarachnoid hemorrhage. *Continuum (Minneap Minn)*. *Neurocrit Care* 2015; **21**:1263-87.


