Inverse Association Between Basilar Artery Volume and Neuron Density in the Stellate Ganglion Following Bilateral Common Carotid Artery Ligation: An Experimental Study

Ilhan Yilmaz[1], Metehan Eseoglu[2], Mehmet Resid Onen[3], Osman Tanrıverdi[1], Mustafa Kilic[1], Adem Yilmaz[1], Ahmet Murat Musluman[1], Mehmet Dumlu Aydin[4], Cemal Gündogdu[5]

■ OBJECTIVE: **This study examined the relationship between neuron density in the stellate ganglion and the severity of basilar artery (BA) enlargement after bilateral common carotid artery ligation.**

■ METHODS: **Rabbits ($n = 24$) were randomly divided into 3 groups: unoperated control group ($n = 4$), experimental group subjected to bilateral common carotid artery ligation ($n = 15$), and sham-operated control group ($n = 5$). Histologic examination of the BAs and stellate ganglia was performed 2 months later. Permanent bilateral common carotid artery ligation was induced by ligation of common carotid arteries at prebifurcation levels as a model for steno-occlusive carotid artery disease.**

■ RESULTS: **Mean BA volume and neuron density in stellate ganglia for all animals were 4200 $\mu m^3 \pm 240$ and 8325 $\mu m^3 \pm 210$. In sham-operated animals, the mean values were 4360 $\mu m^3 \pm 340$ and 8250 $mm^3 \pm 250$. For the experimental group, mean volume and density in animals with slight dilatation of the BA ($n = 6$) were 4948 $\mu m^3 \pm 680$ and 10,321 $mm^3 \pm 120$, whereas in animals with severe dilatation ($n = 9$), the values were 6728 $\mu m^3 \pm 440$ and 6300 $mm^3 \pm 730$. An inverse association was observed between degree of BA enlargement and stellate ganglia neuronal density.**

■ CONCLUSIONS: **High neuron density in stellate ganglia may protect against steno-occlusive carotid artery disease by preventing BA dilatation and aneurysm formation in the posterior circulatory arteries.**

INTRODUCTION

Bilateral common carotid artery ligation (BCCAL) results in a major redistribution of blood to the head, with increased intraluminal pressure and retrograde blood flow through the posterior vertebral artery and basilar artery (BA), leading to morphologic and histopathologic alterations, including vascular remodeling and trophic changes in craniocervical vessels.[1-6] Retrograde blood flow can protect carotid bodies from ischemic insults, allowing them to restore a normal circulation.[7] Cerebral vascular innervation is maintained by various autonomic nerve fibers and humoral and chemical factors. Parasympathetic cranial nerves provide vasodilatory outflow,[8] whereas sympathetic outflow of stellate ganglia has vasospastic effects on cerebral arteries.[9] Additionally, trigeminal nerve endings provide dense, vasodilatory innervation to cerebral vessels.[8,10] Innervation of the BA serves a regulatory function by altering lumen diameter, permeability, and sensory and secretory functions; thus, it is predicted that these nerves play important roles in determining BA characteristics after BCCAL. In this study, a rabbit model of steno-occlusive carotid artery disease was developed to examine the relationship between stellate ganglia neuron density and volumetric changes in the BAs after BCCAL.

MATERIALS AND METHODS

Experiments were performed on 24 anesthetized adult male albino New Zealand rabbits (3.7 kg ± 0.4). All animal protocols were approved by the Ethics Committee of the Medical Faculty of Atatürk University, and animal care and experiments were performed according to the committee's guidelines. Animals were randomly assigned to 1 of 3 groups: unoperated control group (n = 4); sham-operated control group (n = 5); and experimental (BCCAL) group (n = 15). After inducing anesthesia with isoflurane administered

Key words
- Basilar artery
- Bilateral common carotid artery ligation
- Neuron density in stellate ganglion

Abbreviations and Acronyms
BA: Basilar artery
BCCAL: Bilateral common carotid artery ligation

From the [1]Department of Neurosurgery, Sisli Etfal Education and Research Hospital, Istanbul; [2]Department of Neurosurgery, Medipol University, Istanbul; [3]Department of Neurosurgery, Umraniye Education and Research Hospital, Istanbul; and Departments of [4]Neurosurgery and [5]Pathology, Medical Faculty of Atatürk University, Erzurum, Turkey

To whom correspondence should be addressed: Mehmet Resid Onen, M.D. [E-mail: mresid@gmail.com]

Citation: World Neurosurg. (2017) 100:138-143.
http://dx.doi.org/10.1016/j.wneu.2016.12.034

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

through a face mask, 0.2 mL/kg of an anesthetic combination (ketamine 150 mg/1.5 mL, xylazine 30 mg/1.5 mL, and distilled water 1 mL) was subcutaneously injected before surgery; during the operation, booster doses of 0.1 mL/kg were given as required. All animals were placed in the supine position and secured to the operating table. BCCAL was performed on the anterior cervical region. After making a midcervical medial incision 3 cm long, the common carotid artery—vagal nerve—jugular vein—sympathetic chain was located on each side. Common carotid arteries were dissected, and BCCAL was applied to 15 animals in the experimental group but not to sham animals. The animals were allowed to recover and were sacrificed 2 months later. Brains and stellate ganglia were removed from each animal and placed in 10% formalin solution for 7 days for later histologic examination.

All BAs were assumed to be cylindrical, and simple geometric formulas were used to estimate their volumes. The use of BA volume was preferred over the lumen radius because volume estimation can be readily performed. The volume of BAs was determined by a cylinder volume estimation method in which micrographs of the same field of view were taken from 20 parallel and adjacent thin sections separated by a distance of 1 mm (e.g., Ar_{1-20}, Br_{1-20}, and Cr_{1-20} in Figure 1). These were fused and considered as a 20-unit cylinder with height hBA, and sections A, B, and C were defined as the postfusion level of the vertebral arteries, mid-BA level, and prebifurcation level of the posterior cerebral artery. The volume was calculated with the following formula: $V = \Pi r^2_{ABC}{}^{BC}$.

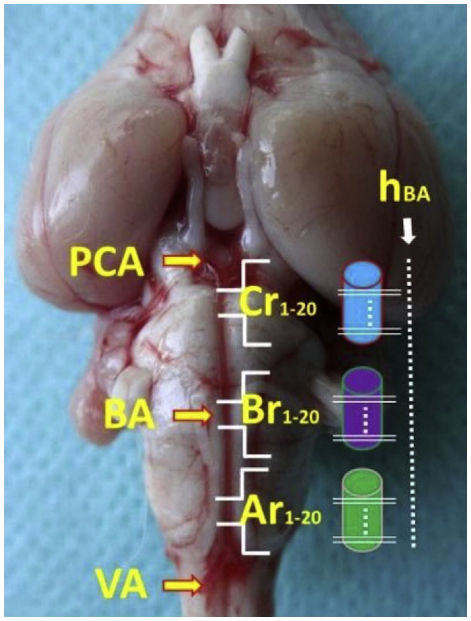

Figure 1. Macroscopic view of a basilar artery (BA) in a normal animal. The height (hBA) was measured as 20 mm ± 5. The volume was determined by a cylinder volume estimation method, in which micrographs of the same field of view were taken from 20 parallel and adjacent thin sections separated by a distance of 1 mm (e.g., Ar_{1-20}, Br_{1-20}, and Cr_{1-20}). These were fused and considered as a 20-unit cylinder with hBA, and sections A, B, and C were defined as the postfusion level of the vertebral arteries, mid—basilar artery level, and prebifurcation level of the posterior cerebral artery. The volume was calculated with the following formula: $V = \Pi r^2_{ABC}{}^{BC}$.

Stellate ganglia on both sides were dissected and embedded horizontally in paraffin blocks in a manner that permitted observation of all the roots during histologic examination. The physical dissector method was used to estimate the number of neurons in the stellate ganglia.[11,12] Two consecutive sections (dissector pairs) obtained from reference tissue samples (BAs at levels A, B, and C in Figure 1) were mounted on each slide. The order of paired reference sections was also reversed to double the number of dissector pairs without the need to cut new sections. The mean density of normal neurons in the stellate ganglia (Nv/Gv) per mm^3 was estimated using the formula $Nv/Gv = \Sigma Q^-/\Sigma A \times d$, where Q^-N is the total number of counted neurons appearing only in the reference sections, d is the section thickness, and A is the area of the counting frame. ΣA was estimated for the set of dissectors by $\Sigma A = \Sigma Pa$, where ΣPa is the total number of counting frame set points, and a is a constant area associated with the set points. The areas of the counting frames are shown in Figure 2A and B, and specimens were blindly evaluated by 2 examiners (M.D.A. and C.G.) (specimen Y13). Figure 2A and B were consecutive sections taken 5 μm apart in which a neuronal nucleus present in Figure 2A was absent in Figure 2B (specimen Y14). The Cavalieri volume estimation method was used to obtain the total number of neurons in each specimen, which was calculated by multiplying the volume (πm^3) and neuron density in each stellate ganglion. The number of normal and degenerated neurons in the stellate ganglia was counted for each animal. Differences in BA volume and neuron density in stellate ganglia were analyzed with SPSS for Windows version 12.0 (SPSS, Inc., Chicago, Illinois, USA), using Kruskal-Wallis and Mann-Whitney U tests. Differences were considered significant at $P < 0.05$.

RESULTS

Three animals in the BCCAL group died within the first week following surgery, after experiencing ischemic attacks, loss of consciousness, convulsions, cardiac arrhythmia, and breathing disturbances. The remaining animals ($n = 12$) were followed for 2 months. Gross examination of the brains showed that BAs localized to the basilar sulcus and extended from the fusion point of vertebral arteries and the origin of the posterior cerebral arteries (Figure 1). The mean BA length was 19.50 mm ± 1.20. The lumen diameter, inner elastic membrane convolutions, endothelial cell structure, vascular wall muscles, and adventitia of normal BAs from nonoperated animals are shown in Figure 3. In the BCCAL group, minimal inner elastic membrane flattening, greater luminal surface and BA expansion, wall thinning, and increased BA volume were associated with high neuron density in the stellate ganglion (Figure 4). In the remaining animals, leptomeningeal thickening and dolichoectasia of the BA (i.e., elongation and convolution) were observed macroscopically. On histologic examinations, inner elastic membrane flattening, thinning of the intimal flap, endothelial cell shrinkage, desquamation, cell loss, luminal enlargement and dilatation,