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EXPERIMENTAL / LABORATORY STUDIES

Effect of Carotid Stenosis on the Development of Subarachnoid Hemorrhage: An Experimental Study

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Abstract: Subarachnoid hemorrhage (SAH) may be seen after the invasive procedures of occlusive carotid artery disease. The objective of this experimental study is to investigate the effect of bilateral common carotid artery ligation (BCCAL) on the development of subarachnoid hemorrhage (SAH).Twenty Wistar adult rats (150-200g) were randomly divided into two groups, as follows: (I) Control group (without BCCAL, n = 5), (II) BCCAL group (n = 15). All rats were sacrificed three months later. The basilar artery with surrounding tissue was removed from the cranium and processed for paraffin embedding. Histopathological and Stereological examinations of the basilar artery were made. During the experimental period, six rats died within twenty days after BCCAL, and SAH was detected in four of them. The mean cross sectional area of basilar arteries was 0.28 mm^2 in the control, whereas in the rats that did not develop SAH and These that developed SAH were 0.33 mm^2 and 0.23 mm^2 ($P < 0.01$), respectively. Luminal enlargement, vascular wall thinning, endothelial injury and flattening of inner elastic lamina were examined histopathologically. There were red blood cells in the cisterna magna of animals that developed SAH.

Carotid stenosis or invasive applications such as carotid angioplasty and carotid stenting may cause fatal SAH.

Key Words: Carotid stenosis, subarachnoid hemorrhage, Rat

Introduction

Common carotid arteries are the major supplying vasculatures of the craniocervical region. After BCCAL, vertebro-basilar blood flow increases. New anastomoses develop, nonfunctional shunts open or reform; angiogenesis, collateral circulation and retrograde blood flow mechanisms may appear to provide sufficient blood flow to cranio-cervical region. Blood flow is directed to the carotid system from the vertebro-basilar circulation and decreased cranio-cervical blood flow returns to normal levels in almost three months (1).

Bilateral internal carotid artery occlusion causes an increase in the blood pressure and diameters of posterior circulation arteries (2). It has been reported that SAH develops when both internal carotid arteries are ligated at the neck (3). Bilateral carotid artery occlusion may also rupture a Basilar artery aneurysm associated with MoyaMoya disease (4). In the histopathologic analysis, thinning of the vascular intimal layer, breakdown of elastic lamina and endothelial injury at the site of the aneurysm regions are frequently observed (5). Cerebral vasospasm has also developed following SAH (6). From the above data, it is proposed that hemodynamic stress

may be regarded as an important factor in the etiology of SAH. In the present study, we aimed to investigate the role of BCCAL on the development of SAH.

Material and Methods

Animals

All experiments were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of Atatürk University, School of Medicine Laboratory bred Wistar adult rats (150-200g) of either sex were maintained under standard laboratory conditions (temperature 25 ± 2 ºC, relative humidity 50 ± 15 %) and the normal photo period (12 h dark/ 12 h light) was used for the study. Rats were divided into two groups as follows: (ı) control group (none applied BCCAL, n = 5), (II) BCCAL group (n = 15).

Experimental BCCAL

All animals were anaesthetized with intramuscular xylazine injection (Rompun Amp, Bayer 30mg /kg) and Ketamine HCL, (Ketalar flk, pfzizer 30mg/kg). Both common carotid arteries were exposed, separated from their sheaths and nerves, and ligated with 3.0 silk sutures. The anterior midline neck incision was closed with surgical clips. Control animals had sham operations with carotid sheath opening, but without ligation. During the experimental period, six rats died within twenty days after BCCAL. The remaining animals were sacrificed under pentobarbital anesthesia at the end of three months and basilar arteries were dissected out immediately and transferred into 10% formaldehyde solution.

Conventional histopathology of the basilar artery

The cross sections of the basilar artery at midpoint level, together with the adjacent tissues were cut at a thickness of 5 mm using a rotary microtome (LEICA RM 2155). The sections were mounted on glass slides and stained with H & E for histopathological examination. A pathologist, blind to procedures, examined all tissue slices with the light microscope at 100 x , 200 x and 400 x original magnifications.

Morphometric analysis

The dissected materials were embedded in paraffin the medulla oblongata being placed at the top. The initial cross sections were cut at the opposite site.

Consecutive sections were taken from the paraffin blocks, and about 200 sections were obtained from a tissue block for determining the basilar artery encounter. The basilar artery was encountered between approximately the 20 th and 200nd slices.

By systematic sampling approach (7), 1/20 of the total sections containing the basilar artery was selected, and used to estimate the cross sectional area of the basilar artery. It is important to stress that the first section must be determined randomly between 1st and 20 th sections in order to get a systematic random sample and an unbiased estimate. The sections were analyzed with a modified light microscope, which has a 12030 mm^2 representing area per point (7) (Figure 1). The formula for the estimation of cross sectional area of the basilar artery is $A = a(p).\Sigma P$, where A is the cross sectional area of the artery , a(p) and ΣP is the area of a point and total number of points hitting the cross

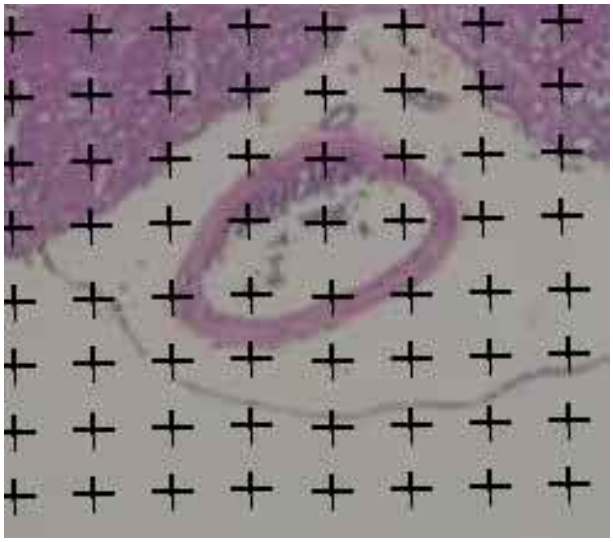

Figure 1. A schematic diagram and an explanation of the mean cross sectional area estimation of a basilar artery. The point grids randomly thrown on to the images of basilar artery in each sampled section. The sampled sections are selected using a systematic random sampling approach (1/20) from consecutive sections of the basilar artery. The point representing an area (a/p) which hits the image of the artery is counted. First, the mean point number that is superimposed on the image of artery in the sampled sections is found, and then this number is multiplied by *a/p*.Thereby, one can estimate an unbiased estimate of the mean cross sectional area of basilar artery for an animal. Points that hit the lumen, tunica intima and tunica media of the artery were evaluated for estimation (H& E, 400x).

sectional area of the basilar artery respectively. This estimation was done as described prevously.

Statistic

SPSS 8.0 for windows was used for the statistical analyses, and Chi square test was applied. $P < 0.01$ as considered statistically significant.

Results

Histopathological examination

During the experimental period, six rats died within twenty days after BCCAL, and SAH was detected in four of them. There were red blood cells in the cisterna magna of animals that developed SAH. In four of 15 animals, SAH developed after BCCAL, which was statistically significant ($P < 0.01$).

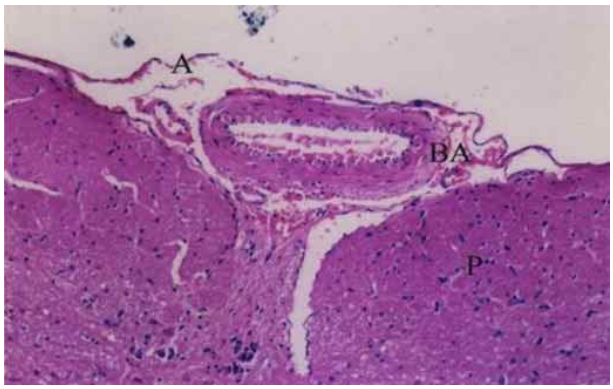

Figure 2a. Histopathologic appearance of BA in a normal rat. (A: Arachnoid, BA: Basilar artery, P:Pons).

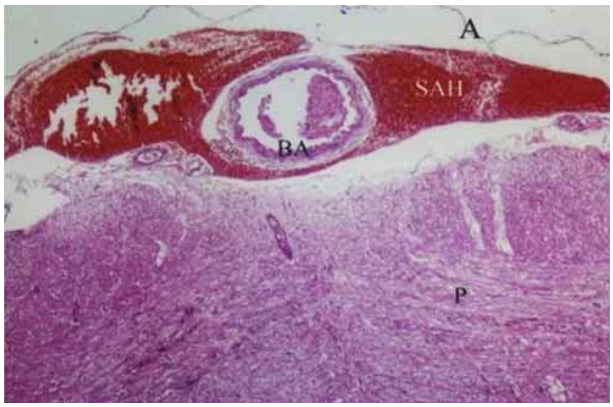

Figure 2c. A basilar artery is seen in a rat with SAH observed at periphery of the BA. (A: Arachnoid membrane, BA: Basilar artery, P:Pons).

Medial and adventitial layers, endothelial cells and internal elastic membranes were normal in the control group. It was observed that the lumen contours of basilar artery in the control group were more regular than with other groups (Fig.2a). It was determined that the lumen of the basilar artery in the animals that did not develop SAH were larger than the control group and also luminal enlargement, vascular wall thinning, endothelial injury and flattening of inner elastic lamina were observed (Fig.2b) but the lumen of the basilar artery was decreased significantly in the SAH developed animals due to vasoconstriction. (Fig.2c).

Stereological Analysis

The cross sectional areas of the basilar arteries were summarized in Table 1 A significant difference was observed between the groups ($P < 0.01$). The mean cross

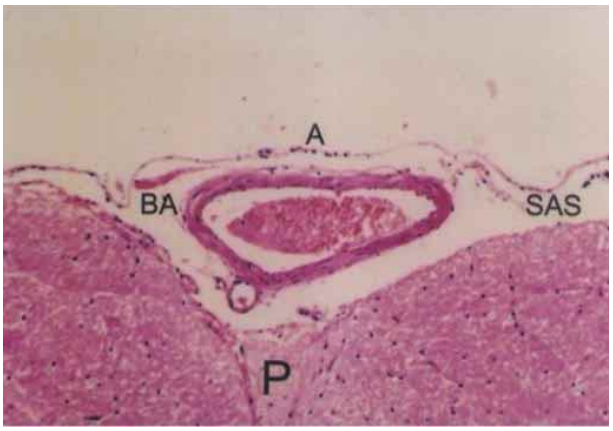

Figure 2b. The appearance of the BA in a animal that did not develop SAH. (A: Arachnoid membrane, BA: Basilar artery, P: Pons, SAS: Subarachnoid space).

Table1. Cross Sectional Area of the Basilar Artery .

Group	The mean sectional area of the basilar artery ± SD (mm^2)
Control (n = 5)	0.28 ± 0.012
Without SAH (n = 11)	0.33 ± 0.013*
SAH (n = 4)	0.23 ± 0.006*††

* $P < 0.01$, significant difference from the control group.
†† $P < 0.01$, significant difference from the animals that did not develop SAH.

sectional area of basilar arteries was 0.28mm^2 in the control, whereas in the rats that did not develop SAH and those that developed SAH were 0.33 mm^2 and 0.23 mm^2 respectively. The mean cross sectional area of basilar arteries was reduced remarkably in those animals who developed SAH.

Discussion

The carotid arteries conduct nearly half of the cerebral flow, with the remaining parts supplied by the vertebral arteries (8,9), but BCCAL alters the blood supply to the head. After ligation, the vertebral artery and basilar artery and the posterior cerebral circulation become the major arterial supply to the entire head and cervical region, and the vertebro-basilar arteries conduct all cerebral blood flow. These hemodynamic changes result in tortuosity of the basilar artery. After BCCAL, some features such as arterial elongation, convolution, luminal enlargement and vascular wall thinning at basilar arteries were assessed histopathologically and arteriographically by Oldendorf (1). He claimed that BCCAL relies on increase in the diameter of the basilar artery. We investigate the relationship between the development of SAH or not after BCCAL by the estimating the cross sectional area of the basilar artery. We found in this study that after BCCAL, the lumen of the basilar artery increased in the animals that did not develop SAH when compared with the control group.

The structural functions of an organ are not solely based on the molecular, biochemical, physiological or genetic activities, but are also based on morphometric parameters which describe the quantitative characteristics such as number, length, diameter, volume, surface area, pressure and retrograde flow. The objectivity and reliability of the morphological techniques come from the fact that they rely on reproducible results (10).

Histopathological analysis showed that increased intraluminal pressure caused thinning of the intimal layer of an artery and breakdown of elastic lamina at the site of an aneurysm (11,12). Histopathological indicators of the increased flow are vascular wall thinning, flattening of the inner elastic lamina and intimal defect, especially at an aneurysm region; Such conditions can cause arterial destruction and aneurysm formation (1). In this study, in the animals that did not develop SAH; luminal enlargement, vascular wall thinning, endothelial injury and flattening of inner elastic lamina were observed. However, in the animals that develop SAH the lumen of the basilar artery significantly decreased. It was shown previously that cerebral vasospasm developed following SAH (6).

Obstructive carotid artery disease increases the vertebro-basilar blood flow (13). It has been reported that carotid ligation causes rupture of a distal basilar artery aneurysm and results in SAH by increasing blood flow and intraluminal pressure (14). Kataoka et al. (15) reported that bilateral carotid occlusion caused SAH in two cases. In our study, six rats died after BCCAL, and SAH was detected in four of them. It is well known that carotid stenosis or occlusive carotid artery disease causes new aneurysm formations in the posterior circulation arteries of the brain (2,16,17,18,19,20,21). Because of BCCAL causing an increase in intraluminal pressure and vascular wall thinning, it may lead to aneurysm formation in those arteries especially in the vertebro-basilar arteries (1). Hemodynamic stress from long-lasting hypertension due to BCCAL can erode the mechanical resistance of the vessel wall. This kınd of hemodynamic stress is a known causative factor in SAH development in the vertebro-basilar artery. Balloon occlusions for unclippable giant aneurysms of the internal carotid artery lead to luminal enlargement and/or aneurysm development in the cerebral arteries, and consequently SAH may develop (3). We also believe that the vertebro-basilar blood flow velocity was higher in SAH developed animals than in the ones that did not develop this.

Batjer et al. (14) reported that an asymptomatic distal basilar aneurysm ruptured in a 12-year old girl after iatrogenic carotid artery occlusion. Hartmann et al.17 reported a case with fatal SAH after carotid angioplasty and stent placement. Also, Mubarek et al. (23) reported SAH following carotid stenting with the distal balloon protection. Several factors such as long standing severe carotid stenosis with contralateral occlusion and increased blood pressure after carotid angioplasty and stent placement, accompanied by the extensive use of antithrombotic agents may predispose to these fatal complications (17).

In summary: We concluded that SAH developed in rats in a significant ratio after BCCAL. So, Steno-occlusive

carotid artery diseases and surgical or invasive procedures such as carotid end-arterectomy, carotid angioplasty and carotid stenting may cause fatal SAH. We advise that physicians should be cautious in such interventions.

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