Stenoocclusive Carotid Artery Disease As A Facilitating Factor Of Neurogenic Lung Edema: An Experimental Study.
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Citation

Abstract
Aim : Steno-occlusive carotid artery disease and their treatment strategies may be cause various cardiopulmonary pathologies. The purpose of this study was to evaluate if there is effects of bilateral common carotid artery ligation (BCCAL) on the development of neurogenic pulmonary edema (NPE).

Material and Methods: Study included 25 adult rabbits (3.5 ± 0.5 kg). Five animals (Control Group, n = 5) were used to evaluate normal lung tissue. Remaining animals were randomly divided into two groups as the SHAM group (n = 5) and the experimental group (n = 15). In the SHAM group, longitudinal neck dissection was performed and carotid sheathes were incised, but BCCAL was not performed. In the experimental group both common carotid arteries were exposed; separated from their sheaths and nerves, and ligated by 3 / 0 silk sutures. 6 rabbit dead in first weeks and other rabbit were followed three months and then sacrificed. Their brains, lungs and carotid arteries were examined histopathologically. Results were analysed by using Mann Whitney U test. Results: Six of the rabbits in study group dead within first weeks. Macroscopic examination of the dead animals revealed subpleural petechial hemorrhage, intratracheal hemorrhagic fluid collection, and subarachnoid hemorrhage (SAH) was noticed five of the dead animals . In the lungs, there were perivascular and subintimal edema and hemorrhage, intra-alveolar hemorrhage and alveolar wall destruction. SAH developed animals have more severe lung injuries.Conclusion: Steno-occlusive carotid artery disease and/or their treatment strategies may be considered as predisposing and aggravating factors on the occurrence of NPE, SAH and sudden death.

INTRODUCTION
Common carotid arteries are the major vessels of cranio-cervical region. Vertebro-basilar blood flow is increased in severe bilateral steno-occlusive carotid artery disease. New anastomoses develop; non-functional shunts open or reform; and angiogenesis, collateral circulation and retrograde blood flow mechanisms may provide sufficient blood flow to the cranio-cervical region. Bilateral carotid artery occlusion causes an increase in the blood pressure and also in the diameter of the arteries of the posterior circulation (1-4). This hemodynamic stress is known to induce subarachnoid hemorrhage (SAH) in vertebro-basilar artery. SAH has been reported after ligation of both internal carotid arteries at the neck (1,5).

Although its pathogenesis is not completely understood, NPE is defined as a life-threatening disease associated with dangerous hypoxia following several hours after mortal neurological status such as SAH (6). Systemic neuromediators released from central nervous system cause pulmonary vasoconstructuon and pulmonary edema without an increase in the pulmonary capillary wedge pressure. Pulmonary interstitial and alveolar fluid collections are the most important cause of hypoxia. Baumann et al (7) reported that SAH is the main cause of NPE. Pulmonary edema (7) and fat embolism (8) are major complication of SAH. NPE may also be the first sign in patients with steno-occlusive carotid artery disease (9). There have been limited reports describe pulmonary edema (10,11) and sudden death after endarterectomy and stent placement in the literature (12). To our knowledge, there is no experimental study about NPE development as a complication of steno-occlusive carotid artery disease. The purpose of this study was to evaluate the impact of bilateral ligation of the carotid arteries in rabbits as the first step for development of NPE.

MATERIALS AND METHOD
In this study, all procedures were conducted in accordance with the guidelines established by the Experimental Animal Laboratory and approved by the Animal Care and Utilization Committee. Twenty five adult rabbits (3.5 ± 0.5 kg) from both sexes were included in the study and preserved under
standard laboratory conditions (temperature 25 ± 2 °C, relative humidity 50 ± 15%) with normal diurnal rhythm (12 hours dark / 12 hours light). Normal lung tissue was examined in five animals (Control Group, n = 5). Twenty animals were randomized into two groups as the SHAM group (n = 5) and the experimental group (n = 15). All animals received anesthesia by intramuscular xylazine injection (Rompun Amp, Bayer 30mg/kg) and Ketamine HCL (Ketalar flacon, Pfizer 30mg/kg). Both common carotid arteries were exposed and dissected from their sheaths and nerves via anterior longitudinal skin incision and the arteries of the study group were ligated with 3.0 silk sutures (n = 15). The animals in the SHAM Group (GI, n = 5) also underwent carotid sheath opening through longitudinal neck dissection. However no BCCAL was performed. The SHAM group was used because hypothetically, surgical procedure performed might lead to irritation, adhesion and vascular or pulmonary injury. Permanent BCCAL was performed in the remaining 15 animals (experimental group). This procedure may be considered as a model for steno-occlusive carotid artery disease. Remaining animals were sacrificed under pentobarbital anesthesia at the end of three months and their lungs were resected immediately and transferred into 10% formaldehyde solution.

For the histopathologic analysis, the dissected materials taken from the lungs, brains and common carotid arteries were embedded in paraffin blocks. sections from these specimens were prepared at 5 micrometer thickness using a rotary microtome (LEICA RM 2155). These sections were placed on glass slides and stained with H&E for histopathologic examination. A pathologist, blinded to the procedures, examined all of the tissue specimens under the light microscope with various magnifications. On each slide, the specimen was divided into two distinct zones representing the dependent lateral and the non-dependent medial regions of the lungs. Ten microscopic fields, five in each region were randomly selected. Lung injury was scored under light microscopy between 0 and 5 modified form of Tassiopoulos AP et al. No damage was graded as Gr-0, the presence of pulmonary alveolar edema Gr-1, Gr-1 + intraparenchymal hemorrhage Gr-2, Gr-2 + alveolocapillary membrane ruptures & alveolar hemorrhage Gr-3, Gr-3 + SAH developed animals scored as Gr-4 and dead animals scored as Gr-5 (13). Scores of each rabbit was recorded. Data was analyzed by SPSS for Windows. Histopathologic scores of non-survivors and survivors were compared by Mann-Whitney U test. A p value less than 0.05 was accepted as significant.

RESULTS
Surgical complications such as hematoma, infection, and tissue necrosis were not observed at the incision sites of animals at the postoperative period. Six animals in experimental group dead within the first week and they were examined urgently after dead for the prevention of autolytic processes. Macroscopic examinations of the dead animals revealed subpleural and intraparenchymal hemorrhagic spots, turbid fluid collection in the intrapleural compartments and intrabronchiolar hemorrhagic fluid collection were observed in the lungs of these six animals (n = 6) (Figure-1). SAH developed in dead some animals (n = 5) (Figure-2). For the understanding of the lung injuries, normal histological appearance of the lungs is seen in figure-3. Extensive pulmonary tissue edema, abundant intraalveolar hemorrhage, intrabronchiolar hemorrhagic fluid collections (Figure-4) and alveolar wall rupture (Figure-5) were noted in the lungs of the five of the six animals that died. Highest scores of lung injury were observed in animals that dead or SAH developed animals (P < 0.05). However, histopathological appearance of the lung tissues of SHAM group did not include NPE findings excluded two of them have minimally lung edema and intraalveolar hemorrhage (Figure-6) and there was no significant difference between the scores histopathological of the SHAM and survivors (P < 0.05). Macroscopical appearance of a rabbit belong to SHAM group is nearly normal (Figure-7). In the postmortem analysis of the lungs of survivors were nearly normal. But some major cerebral vascular changes were observed. Basilar, posterior communicating and posterior cerebral arteries revealed convolution, luminal enlargement and vascular wall thinning (figure-2).

In histopathological examinations of the common carotid arteries of the dead animals thrombus formations were noticed both pre and postligational parts of the common carotid arteries. Whereas, the examinations of the common carotid arteries of the survivors (Figure-8); luminal enlargement and thinning of the vessel walls were detected in the pre-ligated cranial parts of the common carotid arteries (Figure-8 A-). At the post-ligation parts of these arteries shown minor luminal enlargement and wall thinning (Figure-8 A+).

Distribution of lung injury injury scores according to experimental group are summarized at the table-1. High lung
injury scores were important dangerous factor on the progression of BCCAL. There was a meaningful relationship between the lung scores and mortality rate (P < 0.05).

**Figure 1**
Figure-1: Macroscopic appearance of a lung belong to a dead animal with SAH&NPE.

**Figure 2**
Figure 2: A brain with subarachnoid hemorrhage and its histopathological representation are shown. Massive subarachnoid hemorrhage at the basal cisterns and subarachnoid spaces (SAH), arachnoid thickening (A) and blood collection in the subarachnoid spaces are shown (LM, H&E, x100).

**Figure 3**
Figure-3: Histopathological appearance of a rabbit belong to normal lung tissue (PA-Pulmonary artery; A-Alveolus; B-Bronchiole; LM, H&E, x40).

**Figure 4**
Figure-4: Alveolar wall destruction (EI), intralveolar hemorrhage (H) and alveolar fluid collection&hemorrhage; (IAH) were observed in dead animals (LM, H&E, x100).
Figure 5
Figure 5: Alveolar ruptures (AR, Arrows) are shown in a dead rat after BCCAL (LM, H&E, x 40).

Figure 6
Figure 6: Histopathological appearance of an animal belong to SHAM group. Lungs nearly normal except some degree transudative fluid collection (TF) in the bronchioles and alvolar stasis (S).

Figure 7
Figure 7: Macroscopical appearance of a rabbit belong to SHAM group. Lung is nearly normal.

Figure 8
Figure 8: Histopathological appearance of a normal common carotid artery (A), preligational cervical part (A-) and postligational cranial part (A+) are illustrated. Postligational part of the common carotid artery is more expanded and vascular wall is more thinned in comparison to other parts (LM, H&E, x40).
Figure 9
Table -1: Distribution of lung injury injury scores according to experimental group are shown at the following table. High lung injury scores were important dangerous factor on the progression of BCCAL. There was a meaningful relationship between the lung scores and mortality rate (p<0.005).

<table>
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<th>Gr-1</th>
<th>Gr-2</th>
<th>Gr-3</th>
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<tr>
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<td>1</td>
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DISCUSSION

BCCAL cause retrograde blood flow from posterior cerebral circulation to carotid circulation together with increased intra luminal pressure in these arteries. It was experimentally shown that retrograde blood flow and increased blood pressure cause luminal enlargement, doligoectasia, collateral circulation and new collateral formation, endothelial hyperplasia, intimal proliferation and even aneurysm formation (3,4). Also, clinical studies shown that there has been increasing incidence of lung pathologies, stroke, SAH and sudden death due to carotid stent placement (12). Many authors have reported SAH as the main cause of NPE (7). Acute pulmonary edema has been documented in approximately 90% of the sudden deaths due to spontaneous SAH (7,14). Weir et al (15) shown that fatal ending SAH due to ruptured aneurysm may produce NPE in 71% (7,16).

We experimentally shown that BCCAL may be responsible for SAH (1). Developing fat embolism may be another dangerous factor on the fatal outcome of SAH (8). In this study, it was examined if BCCAL cause NPE as a model of steno-occlusive carotid artery disease or stent application because experimental model has not been published so far.

Vertebro-basilar blood flow increases in obstructive carotid artery disease (17). It is well known that stenosis of carotid arteries lead to new aneurysms formation in the the posterior circulation arteries of the brain (18,19). Ligation of carotid arteries cause distal basilar artery rupture by increasing blood flow and intraluminal pressure at the basilar arteries (20). Batjer et al (20) have reported an asymptomatic distal basilar aneurysm rupture after iatrogenic carotid artery occlusion. Several factors such as long standing carotid stenosis, contralateral carotid artery occlusion, increased blood pressure after carotid angioplasty or stent placement and extensive use of antithrombotic agents may predispose to aneurysm rupture of brain arteries (20). Kataoka et al (21) have reported two cases cause SAH after bilateral occlusion of carotid arteries. We have observed similar results in our study. Histopathologic findings of NPE were determined in all animals that has dead or experienced SAH (22,23).

Neurogenic pulmonary edema can be produced by administration of adrenergic agents in the laboratory animals (24). NPE may occur within 1st to 7th day after various acute neurologic disorders, particularly SAH (7). Possible hemodynamic mechanisms involved in the pathophysiology of NPE is intense pulmonary vasosconstriction resulted from adrenergic response to the cerebral insult. Adrenergic impulses cause increased pulmonary hydrostatic pressure and increased capillary permeability (6,24,25). The activation of these centers leads to massive blood pressure increase as well as decrease in heart rate by baroreflex, leading further to increase in pulmonary capillary hydrostatic pressure, damage to the alveolar wall and the leakage of fluid into the intraalveolar space. The baroreflex response has been currently shown to be more important in the pathogenesis of NPE than the blood pressure increase (26).

In our experimental study increased adrenergic discharge secondary to vagal nerve injury cause NPE. Developing SAH may also be the another reason of NPE at the dead six rabbits with SAH. Vascular congestions, intraparenchymal lung edema, interstitial hemorrhage, inrabronchial hemorrhage, alveolar membrane ruptures and fat accumulation were observed abundantly in the dead animals with SAH than those of the others. Survivor animals shoved no NPE findings. These results suggest that there is a relation between SAH and pulmonary injury after the BCCAL procedure.

Carotid angioplasty, stent placement and endarterectomy are the main treatment methods for steno-occlusive carotid artery disease. Because there have been some reports describing the development of NPE due the surgical and endovascular treatment of steno-occlusive carotid artery diseases (7,8), all physicians should be considered SAH and NPE development during endarterectomy and endovascular applications procedures. Thus it should be remembered that NPE may develop within hours after detection of neurologic symptoms in patients with steno-occlusive carotid artery disease. More importantly, clinical picture of the patients may be improved if neurologic problem is resolved. In addition, prompt diagnosis and treatment of NPE is necessary to minimize SAH-related mortality. Before the endovascular treatment of steno-occlusive carotid artery
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disease, cerebrovascular angiography should be done not to cause fatal complications such as NPE and SAH.

CONCLUSION

NPE and SAH may be a significant complications of steno-occlusive carotid artery disease and its’ treatment strategies. Furthermore, all physicians should be evaluate whether cerebral aneurysm or lung pathologies at all patients with steno-occlusive carotid artery disease before the treatment.

References

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