

# Burned spinal cord in acidotic cerebrospinal fluid during subarachnoid hemorrhage: Experimental study

*Médula espinal quemada en el líquido cefalorraquídeo ácido durante la hemorragia subaracnoidea: estudio experimental*

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## Abstract

**Objective:** We investigated the effect of carotid body ischemia-induced cerebrospinal fluid acidosis on spinal cord during subarachnoid hemorrhage (SAH). **Methods:** Twenty-three hybrid rabbits were divided into three groups: control (n = 5), Sham (injected with 0.5 ml isotonic) (n = 6), and the SAH (n = 12) (injected with 0.5 ml autologous blood into the 4<sup>th</sup> ventricle) and then monitored for 3 weeks. Cerebrospinal fluid pH and degenerated ependymal cell density and volume of cervical central canal were analyzed. **Results:** The mean cervical central canal volumes, degenerated ependymal cells densities, and cerebrospinal pH values were  $1.056 \pm 0.053 \text{ mm}^3$ - $6 \pm 2 \text{ per mm}^2$ - $7.342 \pm 0.034$ ,  $1.321 \pm 0.12 \text{ mm}^3$ - $35 \pm 9 \text{ per mm}^2$ - $7.314 \pm 0.056$ , and  $1.743 \pm 0.245 \text{ mm}^3$ - $159 \pm 24 \text{ per mm}^2$ - $7.257 \pm 0.049$  in the Control, Sham, and SAH groups, respectively. The more degenerated carotid body neuron density induced decreased cerebrospinal fluid pH values ( $p < 0.0001$ ) could result in the more ependymal cells desquamation ( $p < 0.0005$ ) and central canal dilatation ( $p < 0.00001$ ). **Conclusion:** Increased neurodegeneration of carotid bodies can reduce cause cerebrospinal fluid pH-induced ependymal cell degeneration and central canal dilatation following SAH.

**Key words:** Subarachnoid hemorrhage. Carotid body. Acidosis. Spinal cord.

## Resumen

**Objetivo:** El objetivo de este estudio fue investigar el efecto de la isquemia inducida del cuerpo carotideo por la acidosis de líquido cefalorraquídeo en la médula espinal durante una hemorragia subaracnoidea (SAH). **Metodo:** Conejos híbridos (n = 23) fueron divididos en Control (n = 5), Sham (inyectados con 0.5 ml de solución isotónica) (n = 6), y SAH (n = 12) (inyectados en el 4<sup>o</sup> ventrículo con 0.5 ml de sangre autóloga) y monitoreados por tres semanas. Se analizaron: El pH del líquido cerebro espinal, la densidad de las células ependimarias y el volumen del canal cervical central. **Resultados:** La media del volumen del canal cervical central, la densidad de las células ependimarias degeneradas y los valores de pH fueron  $1.056 + 0.053 \text{ mm}^3$ - $6 + 2 \text{ per mm}^2$ - $7.342 + 0.034$ ,  $1.321 + 0.12 \text{ mm}^3$ - $35 + 9 \text{ per mm}^2$ - $7.314 + 0.056$  y  $1.743 + 0.245 \text{ mm}^3$ - $159 + 24 \text{ per mm}^2$ - $7.257 + 0.049$  en los grupos Control, Sham y SHA, respectivamente. La mayor densidad inducida de la neurona del cuerpo carotideo

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degenerado, disminuyó los valores de pH del líquido cefalorraquídeo lo que podría dar como resultado un aumento en la des-camación de las células ependimarias así como la dilatación del canal central. **Conclusión:** Un aumento en la neurodegeneración del cuerpo carotideo puede reducir la degeneración de los ependimocitos y la dilatación del canal central siguiendo SAH.

**Palabras clave:** Hemorragia subaracnoidea. Cuerpos carotideos. Acidosis. Médula espinal.

## Introduction

Acid-base homeostasis is vital for the especially central nervous system. Aydin et al. showed that thermo-regulatory corpuscles regulate cerebrospinal fluid and brain temperature<sup>1</sup>, but pH regulating sensors have not been documented in the brain. The chemoreceptor network consists of carotid body/glossopharyngeal nerve is necessary for blood<sup>2</sup> and cerebrospinal fluid pH regulation. However, some pH-sensitive cell extensions discovered in the spinal cord, which regulate cerebrospinal fluid pH<sup>3</sup>. Acidosis is the most trouble complication of subarachnoid hemorrhage (SAH), as described by Ozmen et al.<sup>4</sup>. Furthermore, carotid body lesions in spine surgery can cause a decrease in blood pH<sup>5</sup>. A new cause of spastic disorders could be attributed to central canal hemorrhages<sup>6</sup>. Although anterior spinal artery<sup>7</sup> or Adamkiewicz artery vasospasm<sup>8</sup> has been accused of the spinal cord, peripheral nerve complex, and distal organ denervation injuries, we newly discovered that not only ischemic degeneration but also decreased blood and cerebrospinal fluid pH could be responsible for neurodegeneration which has been mentioned so far. The choroid plexuses involved in the production and reabsorption of cerebrospinal fluid<sup>9</sup> which regulate brain temperature<sup>1</sup> through deposited water vesicles<sup>10</sup>. If carotid bodies and choroid plexus undergo ischemic insult, then decreased pH/cerebrospinal fluid could cause irreversible dangerous neuronal injury in both brain and spinal cord networks mentioned by in this study.

## Material and Methods

This experiment was studied according to the Ethics Committee of Ataturk University.

### Animal selection

This experiment was done on 23 hybrid rabbits which five used control (n = 5), six of SHAM injection of 0.5 cc saline solution (n = 6), and the remaining used as SAH group (n = 12) with the injection of 0.5cc of autologous blood into their fourth ventricles. All

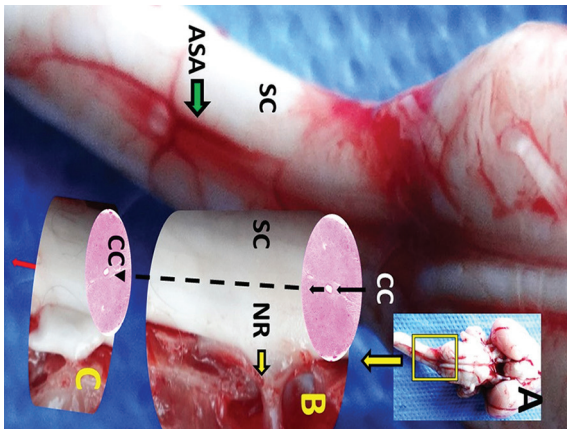
animals followed 3 weeks and humanely sacrificed under general anesthesia.

### Used experiment protocol

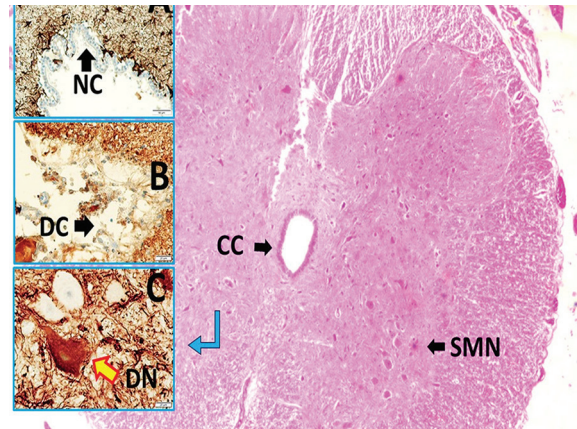
Routinely used injectable anesthetics were applied to reduce pain and mortality. Isoflurane (0.2 mL/kg) given by a facial mask with an anesthetic combination (Ketamine HCL, 150 mg/1.5 mL; Xylazine HCL, 30 mg/1.5 mL; and distilled water, 1 mL) injected subcutaneously at the beginning of surgery. Anesthetic combination of 0.1 mL/kg used during surgery if required. Auricular arterial autologous blood (1 mL) injected with a 22-Gauge needle into fourth ventricles to created SAH and saline solution (1 mL) injected to SHAM animals. The animals were sacrificed after 3 weeks and their carotid bodies, brains, cervical spinal cords, and dorsal roots at the level of C-4 were extracted and then kept in 10% formaline solutions. Cervical specimens and carotid bodies longitudinally but midcoronal brain sections embedded in paraffin blocks to observe brain ventricles and central canal to examine histopathological methods. All carotid body and brain tissues were sectioned of five micrometers consecutively but the spinal cord sectioned in numbers of 20-50 sections. They were stained with H and E and GFAP methods. The Cavalieri method was used to evaluate the volumes of the central canal. A number of consecutive sections obtained from tissue samples of spinal cords arranged over and over to obtain 3D appearances of the central canal as same as cylinders. All central canals were accepted as cylinder and their volumes were calculated as the cylinder methods using the required formula. To estimate the volume of their photographs were taken on the prepared forms of glass lamellae and surface areas of the central canal were calculated using mini squared papers described by Yolas et al.<sup>11</sup>.

### Main results

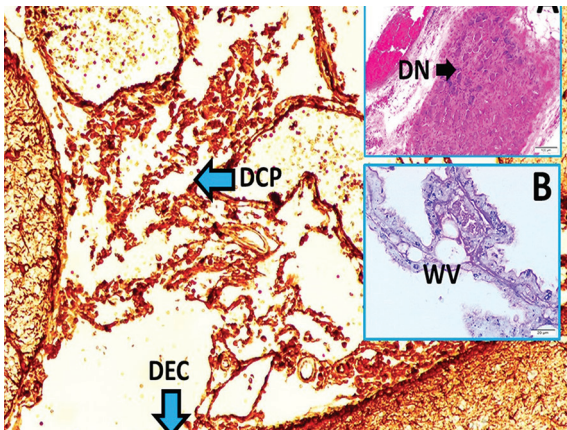
Cardiac and respiratory arrhythmias, unconsciousness, simple convulsions, and neurological reflex abnormalities occurred in SAH-created animals. Three animals



**Figure 1. A:** Macroscopical appearance of brain with cervical spinal cord (SC) and anterior spinal artery (ASA). **B:** magnified form of examined spinal cord part with nerve root (NR) of C-4. **C:** representative extension of spinal cord to shown central canal (CC) localization.



**Figure 3.** Transverse section of spinal cord with CC, SMN, (LM, H&E,  $\times 4$ /Base), normal ependymal cells (NC) (LM, GFAP,  $\times 20$ /A), deformed/degenerated ependymal cells (DC) (LM, GFAP,  $\times 40$ /B), and normal/deformed/degenerated second motor neuron (DN) in gray matter of low pH detected animal with subarachnoid hemorrhage (LM, GFAP,  $\times 40$ /C). CC: central canal; SMN: second motor neurons.



**Figure 2.** Degenerated neurons (DN) of carotid body (LM, H&E,  $\times 10$ /A), normal choroid plexus with water vesicles (WV) (LM, H&E,  $\times 40$ /B), and deformed/degenerated choroid plexus (DCP), deformed/degenerated ependymal cells (DEC) are seen in a subarachnoid hemorrhage created rabbit which owned low pH values (LM, GFAP,  $\times 10$ /Base).

dead in the SAH group. Subarachnoid inflammation, adhesion, blood collection, congested spinal cord vessels, and contracted anterior spinal artery observed in the SAH group in postmortem examinations.

### Histopathological results

To estimate the ependymal cell density of the central canal surfaces of the spinal cord, central canal volumes, all cervical spinal cords were sectioned coronally at the levels of the biggest part of intumescencia cervicalis (Fig. 1A). For the central canal volume and ependymal cell estimation, longitudinal spinal cord sections were

done at the mentioned levels (Fig. 1A). Histopathological appearances of central canal surfaces, choroid plexuses, and ventricles are seen in Figures 1 and 2 shows degenerated neurons of the carotid body, normal choroid plexus with water vesicles, and deformed/degenerated choroid plexus, deformed/degenerated ependymal cells are seen in a SAH created rabbit which owned low pH values. Transverse section of the spinal cord with central canal second motor neurons normal ependymal cells deformed degenerated ependymal cells and normal/deformed degenerated second motor neuron in the gray matter of low pH detected animal with SAH (Fig. 3). The findings of cervical spinal cords and dorsal roots at the level of C-4 of all animals resembled as our previous studies mentioned in the presented study at the level of lumbosacral regions<sup>12</sup>. Furthermore, carotid body examinations resembled that Ozmen et al.<sup>4</sup>.

The data were analyzed with the software package (SPSS® for Windows v. 12.0, Chicago, USA). The data analysis consisted of the Kruskal–Wallis and Mann–Whitney U tests. Differences were considered to be significant at  $p < 0.05$ . Cardiorespiratory disturbances cause 3 animals to exist in the SAH group. Subarachnoid inflammation, adhesion, blood collection, congested spinal cord vessels, and contracted anterior spinal artery observed in the SAH group.

### Numerical results

Mean cervical central canal volume was  $1.056 \pm 0.053 \text{ mm}^3$  and degenerated ependymal cells density

was  $6 \pm 2$  in control animals ( $n = 5$ );  $1.321 \pm 0.12 \text{ mm}^3$  and  $35 \pm 9$  in SHAM animals ( $n = 6$ ) and  $1.743 \pm 0.245 \text{ mm}^3$  and  $159 \pm 24/\text{mm}^2$  in low pH detected animals ( $n = 6$ ). The mean cerebrospinal pH values were:  $7.342 \pm 0.034$  in the control group;  $7.314 \pm 0.056$  in SHAM, and  $7.257 \pm 0.049$  in the SAH group. Low pH values cause the more ependymal cells desquamation ( $p < 0.0005$ ) with a direct linear link of degenerated carotid body neuron density ( $p < 0.0001$ ).

## Discussion

For life, acid-base balance is vital. Respiratory and renal systems are essential for pH control<sup>3</sup>. Acidosis is the most dangerous complication of SAH<sup>7</sup>. Glossopharyngeal nerve and carotid body web are known as chemoreceptors complex which essential for pH regulation. There is an inverse link between degenerated neuron numbers of carotid body and pH values<sup>2</sup>. Although the carotid body network is essential for pH regulation and neuronal degeneration of the carotid body could result in acidosis following SAH<sup>4</sup>, there is no information intracranial pH regulating cells. However, some pH sensors included neuronal networks discovered in the spinal cord, regulate cerebrospinal fluid pH<sup>3</sup>. A plenty of degenerated neuron density of the carotid body resulted in decreased blood pH and respiratory acidosis<sup>5</sup>.

The spinal arteries are innervated by vasodilatory dorsal root ganglia. SAH-induced spinal cord arteries vasospasm can be prevented by cervical dorsal root ganglions. Unfortunately, degenerated neuron density of cervical dorsal root ganglions causes severe anterior spinal arteries vasospasm in SAH<sup>7</sup>. Adamkiewicz artery vasospasm is responsible for both spinal cord and peripheral nerve insult<sup>8</sup> and abdominopelvic organs insufficiency<sup>13</sup>. Adamkiewicz artery spasm causes Onuf's nucleus linked pudendal nerve complex degeneration to also cause urinary retention<sup>11</sup> and gastrointestinal functional problems<sup>14</sup>. Caglar et al. discovered that Onuf's nucleus ischemia-related sacral parasympathetic network insult should be considered as Hirschsprung like disease<sup>12</sup>.

Spastic disorders have been considered as cerebral complications of SAH, but a recent study showed that de-synchronization of the flexor-extensor muscles innervating interneuronal structures is the uninvestigated agent following the central canal hemorrhage<sup>6</sup>. In the same manner, ciliospinal sympathetic center ischemia-induced neurodegeneration in the spinal cord can be responsible for permanent miosis following SAH<sup>15</sup>. Spinal cord tumors may have the same effect<sup>16</sup>.

The irrigation of subarachnoid spaces may decrease the percentage and severity of inflammatory complications by way of the excretion of inflamed purulent collection from the subarachnoid spaces<sup>17</sup>.

Furthermore, choroid plexuses are the significant foundation for cerebrospinal fluid in which cerebral immune surveillance, various endocrine-enzymatic activities, and cerebrospinal fluid-blood barrier functions impracticable unless choroid plexus. Hence, there may be a significant link between choroid plexus and cerebrospinal fluid pH regulation. SAH may extend to brain ventricles and causes ischemic degeneration of the choroid plexus by way of triggered choroidal artery vasospasm<sup>9</sup>.

## Limitations

Human samples, radiological, biochemical, and microbiological data were not investigated in this study.

## Conclusion

It should not be forgotten that the prevention of choroid plexus/functions can be inevitable for cerebrospinal fluid pH regulation.

## Future insight

In that article insight, we postulated that cerebrospinal fluid exchange could be used for cerebrospinal fluid pH restoration. In our study, systemic effects of acidosis were found to be irreversible. In the case of acidosis, cerebrospinal fluid exchange should be among the treatment approaches to obtain normal pH, provided that the clinical situation is considered.

## Conflicts of interest

The authors declare that no conflicts of interest.

## Funding

There is no financial support in the study

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and those of the Code of

Ethics of the World Medical Association (Declaration of Helsinki).

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

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