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Basic Science

Orientation and property of fibers of the myodural bridge in humans

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Abstract

BACKGROUND CONTEXT: Studies over the past 20 years have revealed that there are fibrous connective tissues between the suboccipital muscles, nuchal ligament, and cervical spinal dura mater (SDM). This fibrous connection with the SDM is through the posterior atlanto-occipital or atlanto-axial interspaces and is called the myodural bridge (MDB). Researchers have inferred that the MDB might have important functions. It was speculated that the function of MDB might be related to proprioception transmission, keeping the subarachnoid space and the cerebellomedullary cistern unobstructed, and affecting the dynamic circulation of the cerebrospinal fluid. In addition, clinicians have found that the pathologic change of the MDB might cause cervicogenic or chronic tension-type headache. Previous gross anatomical and histologic studies only confirmed the existence of the MDB but did not reveal the fiber properties of the MDB. This is important to further mechanical and functional research on the MDB.

PURPOSE: Multiple histologic staining methods were used in the present study to reveal the various origin and fiber properties of the MDB. Muscles and ligaments participating in forming the MDB at the posterior atlanto-occipital or atlantoaxial interspaces were observed, and the fiber properties of the MDB were confirmed. The present study provides a basis for speculating the tensile force values of the MDB on the SDM and a morphologic foundational work for exploring the physiological functions and clinical significances of the MDB.

STUDY DESIGN: Anatomical and histologic analyses of suboccipital structures that communicate with the SDM at the posterior atlanto-occipital or atlantoaxial interspaces were carried out.

METHODS: Multiple histologic staining methods were used to evaluate the histologic properties and composition of the MDB at the posterior atlanto-occipital or atlantoaxial interspaces in five formalin-fixed head-neck human specimens.

RESULTS: The results show that the MDB traversing the atlanto-occipital interspace originated from the rectus capitis posterior minor (RCPmi). The MDB traversing the atlantoaxial interspace originated mainly from the RCPmi, rectus capitis posterior major, and obliquus capitis inferior. These fibers form the vertebral dural ligament in the atlantoaxial interspace and connect with SDM. The MDB is mainly formed by parallel running type I collagen fibers; thus, suboccipital muscle could pull SDM strongly through the effective force propagated by the MDB during head movement.

FDA device/drug status: Not applicable.

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Hong-Jin Sui and Sheng-Bo Yu contributed equally to this work.

The authors declare no conflicts of interest associated with this study.

HJS and SBY conceived and designed the experiments. NZ, NXW, YLL, YYG, LXZ, TYL, YYC, and XHY performed the experiments. NZ and XYY analyzed the data. NZ drafted the manuscript. HJS and SBY were responsible for critical revision of the manuscript.

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CONCLUSIONS: Myodural bridge is mainly formed by parallel running type I collagen fibers; thus, it can transmit the strong pull from the diverse suboccipital muscles or ligaments during head movement. The results of the present study will serve as a basis for further biomechanical and functional MDB research.

Keywords: Atlanto-axial interspace; Atlanto-occipital interspace; Collagen I fibers; Histologic staining; Myodural bridge; Suboccipital muscles; Vertebral dural ligament

Introduction

Studies over the past 20 years have revealed that there are fibrous connective tissues between the rectus capitis posterior minor (RCPmi), rectus capitis posterior major (RCPma), obliquus capitis inferior (OCI), nuchal ligament, and cervical spinal dura mater (SDM). This fibrous connection with the SDM is at the posterior atlanto-occipital or atlantoaxial interspaces [1–10] and is called the myodural bridge (MDB). The concept of the MDB was first proposed by Hack et al. in 1995 [7]. Connective fibers between the posterior aspect of the SDM and the posterior wall of the spinal canal at the level of the atlas to the axis were also found. This fibrous connection was identified as the vertebral dural ligament (VDL) [11].

Researchers have inferred that the MDB might have physiological functions such as sensorimotor function [12], postural control [13], and maintenance of the integrity of the subarachnoid space and the cerebellomedullary cistern [2]. Sui et al. [14] proposed that the contraction of the suboccipital muscles may be a dynamic source of the cerebrospinal fluid (CSF) circulation via the MDB. In addition, clinicians have shown that the pathologic change of MDB might cause cervicogenic or chronic tension-type headache [15,16].

The physiological functions of the MDB such as maintenance of the integrity of the subarachnoid space and the cerebellomedullary cistern, and CSF propulsion are related to the tensile force on the SDM from the MDB. Myodural bridge with different histologic properties may stretch the SDM with different powers and may have different effects on surrounding structures. Based on gross anatomical study, Zumpano et al. [17] reported that the fiber property of the MDB could be divided into three types, which were tendon-like, muscle-like, and fascia-like; the most common being the tendon-like fibers. In 2005, Nash et al. [18] speculated that the MDB originating from RCPmi is composed mainly of collagenous fibers. These studies only make a conclusion through anatomical dissection research and observations on plastinated sections. Until now, detailed histologic studies about the fiber properties of the MDB have not been reported. In the present study, multiple histologic staining methods were thoroughly used to reveal the fiber properties and compositions of the MDB. The present study aims at providing a morphologic basis for the functional study of the MDB.

Materials and methods

Ethics statement

The present study was approved by the Ethics Committee of the Body and Organs Donation Center. The research involved five head-neck specimens of Chinese adults in middle and old age from the Body and Organs Donation Center. Written informed consent was obtained from the donors involved in the present study before death in accordance with the regulation of the ethics committee.

Five formalin-fixed head and neck human specimens were used for histologic study. Anatomical dissection specimens were dissected at the suboccipital region from superficial to deep, exposing the deep suboccipital muscles. The superior borders of the RCPmi and RCPma were separated from the occipital bone to expose the margin of foramen magnum. An incision was made at the junction between the cerebral and SDM. The lateral attachment of the OCI on the transverse processes of atlas was separated, and the muscle was reflected from lateral to medial side.

Sample drawing

A longitudinal incision was made between the lateral portion and the medial portion of the RCPma and OCI to expose the junction between the posterior arch of atlas, lateral mass of atlas, and pedicles of the vertebral arch of axis. A reciprocating saw was used to separate the posterior arch of atlas and the vertebral arch of axis from the rest of the vertebrae. Incising along the interspace between the posterior arches of the axis and the third cervical vertebra followed. A tissue block that includes the suboccipital muscles, posterior arch of atlas, vertebral arch of axis, and posterior part of SDM was separated from the specimens.

Histologic staining

The tissue blocks, which were about 5×6×7 cm^3 in volume, were placed into ethylenediaminetetraacetic acid for decalcification for about 2 months at 37°C. After that, the specimens were washed with flowing water for 12–24 hours. Regular dehydration, transparency, and paraffin embedding procedures were applied. The embedded tissue blocks were then cut into slices of about 13–15 µm thick.

These slices were divided into three groups.

Group 1: Hematoxylin and eosin (H&E) staining was applied to confirm the fibrous origin of MDB in the posterior atlanto-occipital and atlantoaxial interspaces.

Group 2: Fiber quad staining of reticular fibers, elastic fibers, collagen fibers, and muscular fibers was applied to confirm the fiber property of MDB [19].

First, deparaffinize and hydrate the slices, oxidate the slices in 0.25% potassium permanganate solution for 3 minutes, and then wash the slices with distilled water. Second, bleach the slices in 0.5% oxalic acid solution for 2 minutes and then wash the slices with distilled water. Third, place the slices in 5% ferric sulfate solution for 5–10 minutes and then wash the slices with distilled water. Fourth, for reticular fiber staining, place the slices in ammonium silver nitrate solution for 20–30 minutes. Fifth, place the slices in formalin solution for 1–2 minutes and then wash the slices with distilled water. Sixth, place the slices in sodium thiosulfate solution for 3–5 minutes and then wash the slices with distilled water. Differentiate in 70% alcohol for 1–2 minutes and then wash the slices with distilled water. Seventh, for elastic fiber staining, place the slices in aldehyde fuchsin solution for 15–30 minutes. Differentiate in 70% alcohol for 1–2 minutes and then wash the slices with distilled water. Eighth, for collagen fiber and muscular fiber staining, place the slices in Gomori solution for 5–10 minutes. Differentiate in acid water. Perform regular dehydration, pass through xylene, and then mount with balsam.

Group 3: Picric acid-sirius red staining was applied to further confirm the collagen fiber types of MDB using a polarizing microscope [20].

The course and relation of the fibers of the MDB originating from different suboccipital muscles was studied. The histologic properties of the MDB were also confirmed.

Result

MDB fibers originating from RCPmi as shown in the H&E sections

In these sections, most of the fibers of RCPmi terminated at the posterior tubercle of atlas. However, multiple fiber bundles originating from the ventral part of RCPmi pass through the atlanto-occipital interspaces in a parallel orientation. These fibers travel in three directions: first, some of these fibers run anteroinferiorly into the posterior atlanto-occipital membrane and terminated in the posterior atlanto-occipital membrane. Thus, through the connection between the posterior atlanto-occipital membrane and the SDM, the fibers originating from the RCPmi indirectly connect with the SDM (Fig. 1A). Second, some of these fibers pass through the posterior atlanto-occipital membrane and directly connect with the SDM (Fig. 1C, D). Third, part of the fibers that travel through the posterior atlanto-occipital membrane crossed over the posterior arch of atlas ventrally to participate in the formation of the atlas part of VDL (V1, the superior portion of VDL [8]) and indirectly connect the RCPmi with the SDM via the VDL (Fig. 1B). There are some dense fiber bundles originating from the dorsal part of RCPmi whose orientations were consistent with the muscle fibers. These fibrous bundles fused with the dense fiber bundles originating from the RCPma, and then parts of these fibers traverse the posterior atlantoaxial interspace to connect with V1(Fig. 1A, B). Parts of these fibers traverse the posterior atlantoaxial interspace and participate in the formation of the atlantoaxial part of VDL (V2, the middle portion of VDL [8]) and are thus indirectly connected with the SDM via the VDL (Fig. 1B, D).

MDB fibers originating from RCPma and OCI as shown in the H&E stain sections

Multiple dense fiber bundles arising from the ventral part of the RCPma pass through the posterior atlantoaxial interspace in a parallel orientation. A small amount of dense fibers originating from the superior border of the OCI run anteriorly. These fibers fused with the dense fiber bundles originating from the dorsal aspect of RCPmi and form the V2. The V2 and V1 fuse together and terminated in the SDM (Fig. 1B–D).

Fiber quad staining sections

In the fiber quad sections, the collagen fibers of the fibrous bundles of the MDB were stained deep green, muscular fibers were stained red, reticular fibers were stained black, and elastic fibers were stained purple (Fig. 2, Left).

Some thin collagen fiber bundles originate from the ventral part of the RCPmi. Near the atlanto-occipital interspace, these fibrous bundles converge together to form thick bundles, running in a parallel fashion. Then, they scatter slight collagen fibers and fuse with the posterior atlanto-occipital membrane. Thin collagen fiber bundles originate from the ventral part of posterior atlanto-occipital membrane and merge with the SDM (Fig. 2, Top right).

Near the atlantoaxial interspaces, the thin collagen fiber bundles between the RCPmi and the RCPma converged to form thick fibrous bundles. On traversing the atlantoaxial interspaces, most of the fiber bundles fuse with V1. V1 is composed of tight fibers. The inferior border of V1 is thin and merges with the SDM (Fig. 2, Left, Bottom right).

Sirius red staining

On observation under the ordinary optical microscope, the fibers of the MDB were stained dark red, which means that the MDB were composed mainly of collagen fibers. Under polarizing microscope, fibrous tissues of MDB were stained red or yellow. It means that MDB is mainly composed of type I collagen fibers. These type I collagen fibers are closely arranged and show strong double refraction (Fig. 3).

Discussion

Previous studies have shown that the dense fibers originating from the RCPmi reaches the posterior atlanto-occipital membrane only via the atlanto-occipital interspace, and the

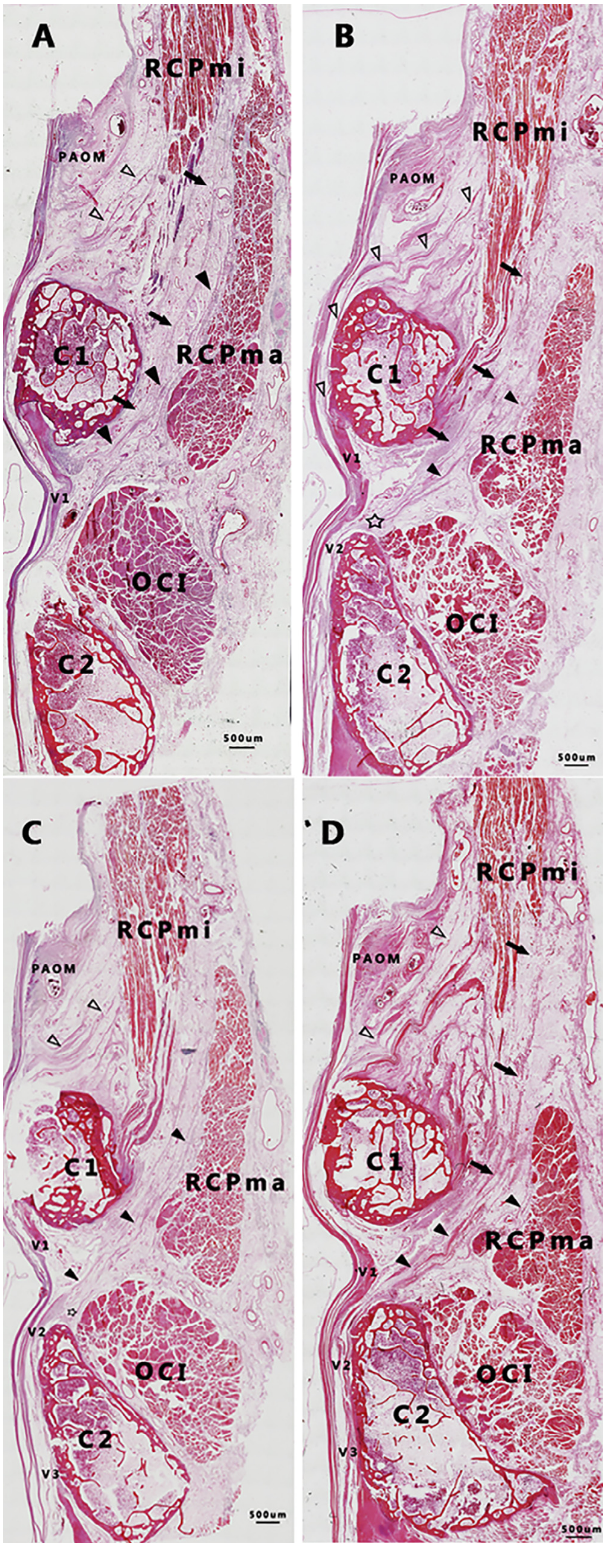

Fig. 1. Hematoxylin and eosin (H&E) stained sections of the suboccipital region structures (the parasagittal section): PAOM was closely connected with the spinal dura mater. In the atlanto-occipital interspace, the MDB originating from the ventral part of the RCPmi travel in three directions: first, these fibers run anteriorly and inferiorly into the PAOM and terminate in the PAOM. Thus, through the connection between the PAOM and the spinal dura mater, the fibers originating from RCPmi were indirectly connected with the spinal dura mater (A). Second, some fibers pass through the PAOM and are directly connected with the spinal dura mater (C,D). Third, part of the fibers traveled through the PAOM and crossed the ventral part of atlas to participate in the formation V1.These fibers of RCPmi were indirectly connected with the spinal dura mater by the V1 (B). In the atlantoaxial interspaces, fibers from three different sources converged and participated in the formation of the myodural bridge. They are (1) dense fibrous bundles originating from the dorsal RCPmi (→), (2) the dense fiber bundles originating from the ventral part of RCPma (▲), and (3) a small amount of dense fibers originating from the superior border of the OCI posterior to the posterior atlantoaxial space (☆). These three different fibers converged to form the atlantoaxial part of the VDL (V2); V2 and V1 eventually fuse together anterior to the C2 and finally terminated in the spinal dura mater (B,C). RCPmi, rectus capitis posterior minor; RCPma, rectus capitis posterior major; OCI, obliquus capitis inferior; C1, posterior arch of atlas; C2, vertebral arch of axis; V1, the atlas part of the vertebra dural ligament; PAOM, posterior atlanto-occipital membrane.

posterior atlanto-occipital membrane connects tightly with SDM [7,10,15]. Our present investigation supports the views of a previous study [10] and also shows some new MDB fibers originating from the RCPmi. In the present study, MDB fibers originating from the RCPmi show three different courses. First, fibers run anteriorly and inferiorly from the RCPmi to join the posterior atlanto-occipital membrane, and indirectly connect with the SDM via the posterior atlanto-occipital membrane, as previous studies depicted. Second, fibers of the MDB pass through the loose part of the posterior atlanto-occipital membrane to connect directly with the SDM. Third, fibers of the MDB pass downward anteriorly to the posterior arch of the atlas and finally attach to V1, which is connected to the SDM. These two latter MDB fiber courses were not mentioned in the previous studies. The MDB originating from the RCPmi, as shown in the present study, indicates diversity in the manner of attachment with the SDM and is found in both the atlanto-occipital and the atlantoaxial interspaces.

The MDB originating from the RCPma and OCI and attaching on the cervical dura mater was documented recently by Scali et al. and Pontell et al. in 2011 and 2013 [1–3,21]. In the anatomical studies by Scali et al. [1,2], MDB originating from the RCPma pass directly through the atlantoaxial interspace and attached to the posterior surface of SDM. Pontell et al. [3,21] documented that a continuous fibrous tissue originated at the anterior fascia of the OCI muscle belly and projected anteriorly across the atlantoaxial interspace and attached to the posterolateral aspect of the cervical dura mater between the first and the second cervical vertebrae. Although, studies in our previous research identified a dense ligament, connecting with the spinal dura mater in the epidural space at the level of atlantoaxial interspace, named the VDL [11]. Vertebral dural ligament

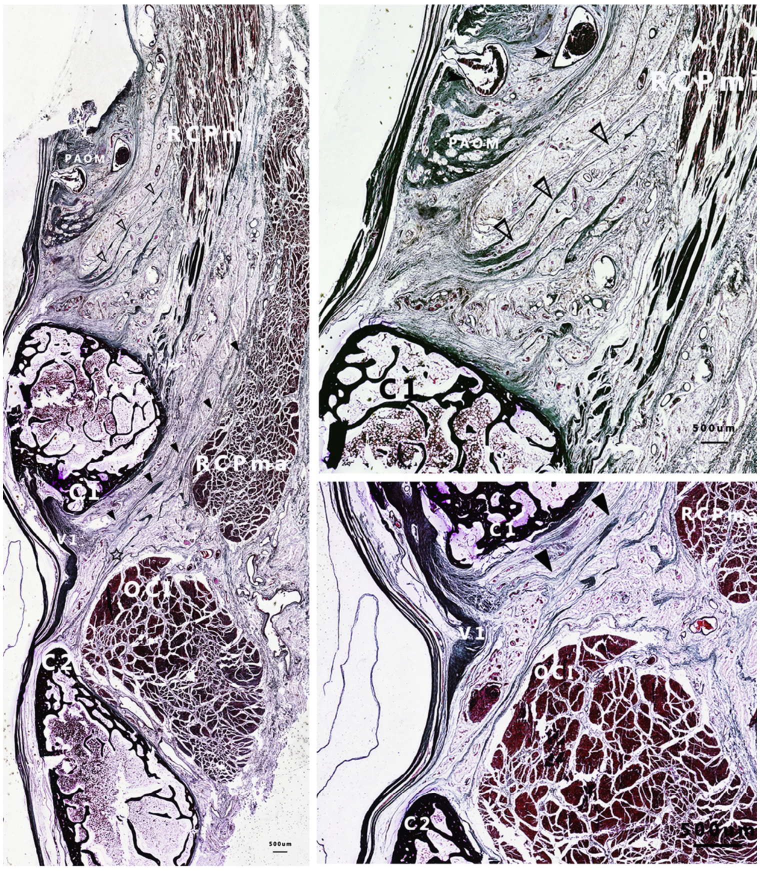

Fig. 2. Fiber quad staining sections of the occipital region tissue. In the section, the muscular fibers are stained red, and the collagen fibers green. Some thin collagen fiber bundles (△) are seen in the ventral part of RCPmi. Near the atlanto-occipital interspace, these fiber bundles converged to form thick bundles and cross parallel. Then they scattered slight collagen fiber bundles and fused with the PAOM. The thin collagen fiber bundles originating from the ventral part of PAOM merged with the spinal dura mater. The spinal dura mater was stained deep green and are in layers (Top right). Near the atlantoaxial interspaces, the thin collagen fiber bundles (▲) between the RCPmi and the RCPma converged to form thick fibrous bundles. On traversing the atlantoaxial interspaces, most of them fused with V1, a main part of the VDL, originated from the anteroinferior border of posterior arch of atlas (C1) and inserted into the SDM (Left, Bottom right). PAOM, posterior atlanto-occipital membrane; C1, posterior arch of atlas; C2, vertebral arch of axis; RCPmi, rectus capitis posterior minor; RCPma, rectus capitis posterior major; OCI, Obliquus capitis inferior; V1, atlas part of the vertebral dural ligament.

was demonstrated as a “common or final pathway” for the MDB in the atlantoaxial interspaces. This supports the result of Scali et al. [22]. In the present study, dense fibers originate from the dorsal part of RCPmi near the middle line, the ventral part of the RCPma and the superior part of OCI, and converge in the atlantoaxial interspace to form the MDB in this interspace. Then, these fibers traverse through the atlantoaxial interspace and form part of the VDL. The VDL later connects with the SDM. The present study further supports our previous result [8]. In addition, fibers originating from the dorsal and ventral parts of RCPmi participate in the formation of the MDB, making RCPmi an important contributor in the formation of the MDB among other deep suboccipital muscles. It was believed that the different fiber components of the MDB do not act individually. The present study reveals that the fiber of the MDB originating from the RCPmi, RCPma, and OCI “mixed” together in the atlantoaxial interspace to make a functional unit.

Few studies have focused on the histologic features of MDB since the MDB was first discovered. An anatomical study by Zumpano et al. [17] classified the fibers of the MDB into three categories: tendon-like, muscle-like, and fascia-like. Nash et al. [18] detected that MDB fibers have features of collagen fibers from autofluorescence. In-depth histologic research is needed to explain the microstructural features of the MDB, which will serve as a basis for further biomechanical and functional MDB research.

Multiple histologic staining was applied to study the MDB. First, MDB was shown to be made up of collagen fibers in the fiber quad staining sections. Second, MDB was confirmed to be made up of type I collagen fibers in the sirius red staining sections as observed under a polarizing microscope. It is known that type I collagen fibers are the most common component of tendons. So we suppose that the MDB is a high-tensile tendon-like structure. The biomechanical features of an organ depend on the organizational structures and

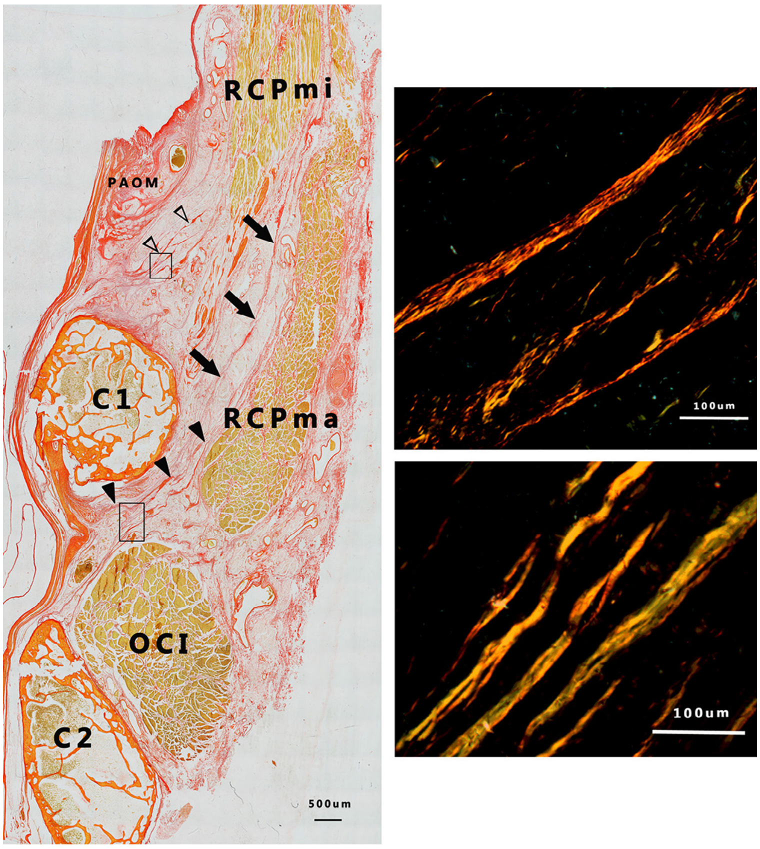

Fig. 3. Histologic sections of suboccipital region structures stained by sirius red BB. (Left) Sections observed under the ordinary optical microscope. (Top right, Bottom right) Sections observed under the polarizing microscope (the parasagittal section). (Left) The section was stained with sirius red BB and then observed under the ordinary optical microscope. In this section, collagenous fibers were stained red, and other structures stained yellow. (△) MDB fibers originating from the RCPmi in the atlanto-occipital interspace; (→) MDB fibers originating from the RCPmi in the atlantoaxial interspace; (▲) MDB fibers originating from the RCPma and OCI. (Top right) Fibrous tissues of MDB originating from the RCPmi were composed mainly of type I collagen fibers with strong refraction. (Bottom right) Fibrous tissues of MDB originating from RCPma and OCI were composed of type I collagen fibers with strong refraction. RCPmi, rectus capitis posterior minor; RCPma, rectus capitis posterior major; OCI, obliquus capitis inferior; C1, posterior arch of atlas; C2, vertebral arch of axis; V1, the atlas part of the vertebra dural ligament; PAOM, posterior atlanto-occipital membrane.

spatial arrangement of its structures [23,24], type I collagen fibers have high resistance to tension [25]. So it can be concluded that MDB could transmit force in a conducive manner during head movement because of the parallel arrangement of its type I collagen fibers.

Previous studies have demonstrated that MDB could prevent in-folding of the dura mater during head extension [7]; deliver the tension of SDM and adjust the posture of the head and neck through contraction and relaxation of the suboccipital muscles [14]; pull the SDM and play a role in maintaining the integrity of the subarachnoid space and cerebellomedullary cistern to ensure the smooth flow of CSF [2]. Recently, Sui et al. [26] found that the flow direction and flow velocity of CSF in the upper atlas level both changed after head shaking with phase contrast cine magnetic resonance (PC cine MR). So Sui et al. proposed that the contraction of the suboccipital muscles may be a dynamic source of the CSF circulation via the MDB. Our research proves that histologically, the MDB is a tendon-like structure consisting of type I collagen fibers. Myodural bridge could thus transmit the strong pull from the different suboccipital muscles or ligaments during head movement. The results of the present study will serve as a basis for further biomechanical and functional MDB research.

Conclusions

Myodural bridge is mainly formed by parallel running type I collagen fibers; thus, it can transmit the strong pull from the diverse suboccipital muscles or ligaments during head movement. The results of the present study will serve as a basis for further biomechanical and functional MDB research.

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