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Research article

Histological examination of the human obliquus capitis inferior myodural bridge

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SUMMARY

This study was designed to examine the anatomical relationship between the obliquus capitis inferior (OCI) muscle and the cervical dura mater at the histological level. Eight human cadavers, with an average age of 65 ± 7.9 years were selected from a convenience sample for suboccipital dissection. Twelve OCI muscle specimens were excised, 100% of which emitted grossly visible soft tissue tracts that inserted into the posterolateral aspect of the cervical dura. These 12 myodural specimens were excised as single, continuous structures and sent for H&E staining. One sample also underwent immuno-peroxidase staining. Microscopic evaluation confirmed a connective tissue bridge emanating from the OCI muscular body and attaching to the posterolateral aspect of the cervical dura mater in 75% of the specimens. Microtome slices of the remaining 25% were not able to capture muscle, connective tissue and dura within the same plane and were therefore unable to be properly analyzed. The sample sent for neuro-analysis stained positively for several neuronal fascicles traveling within, and passing through the OCI myodural bridge. This study histologically confirms the presence of a connective tissue bridge that links the OCI muscle to the dura mater and the presence of neuronal tissue within this connection warrants further examination. This structure may represent a component of normal human anatomy. In addition to its hypothetical role in human homeostasis, it may contribute to certain neuropathological conditions, as well.

1. Introduction

Anatomical reports of the suboccipital region have revealed what appears to be a network of fibrous tissue structures that link several suboccipital muscles to the outermost layer of the meninges (Kahn et al., 1992; Hack et al., 1995, 1996, 1997; Humphreys et al., 2003; Scali et al., 2011, 2013; Pontell et al., 2012). Specifically, it is the rectus capitis posterior minor (RCPmi), rectus capitis posterior major (RCPma), and obliquus capitis inferior (OCI) muscles that contribute to this cervical myodural nexus (Kahn et al., 1992; Hack et al., 1995; Scali et al., 2011; Pontell et al., 2012). The RCPmi myodural bridge has been verified via gross (Hack et al., 1995; Kahkeshani and Ward, 2012), histological (Nash et al., 2005; Zumpano et al., 2006) and imaging (Humphreys et al., 2003; Hack and Hallgren, 2004) studies. The RCPma myodural bridge has also been confirmed by gross (Scali et al., 2011) and histological (Kahn et al., 1992; Scali et al., 2013) reports. The RCPma myodural bridge has also been found to contain neuronal tissue (Scali et al., 2013) and it may be visible on T-2 weighted MRI images as it courses through the atlantoaxial interspace and inserts in the posterior dura mater (Scali et al., 2012).

While RCPma and RCPmi muscles are receiving increasing amounts of attention, the OCI muscle has received relatively little consideration. The OCI myodural bridge was first mentioned in a study on the posterior intervertebral spaces in 1992 (Kahn et al., 1992). It was briefly noted in a study on the RCPma myodural bridge (Scali et al., 2011) and it was the subject of a gross anatomical report in 2012 (Pontell et al., 2012). To the best of our knowledge a report focusing exclusively on the histological nature of the OCI myodural bridge has yet to be conducted. The OCI myodural bridge is distinct from that of the RCPmi as it appears to merge with the RCPma myodural bridge on gross observation (Pontell et al., 2012;Fig. 1). While the RCPma myodural bridge has been confirmed histologically (Kahn et al., 1992; Scali et al., 2013), the OCI myodural bridge requires further microscopic analysis to determine whether

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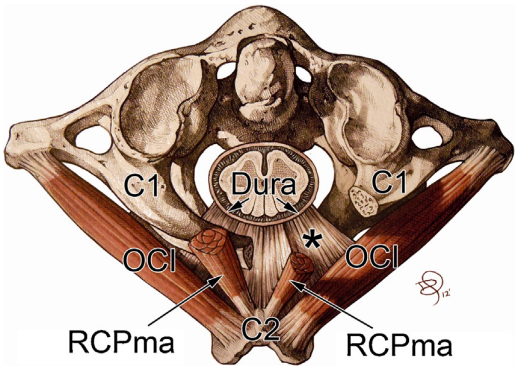

Fig. 1. Illustration depicts a superior to inferior view of the first and second cervical vertebrae. The posterior arch of the atlas (C1) is sectioned in order to illustrate the myodural bridge of the atlantoaxial interspace (*). This soft tissue communication bridges the rectus capitis posterior major (RCPma) and obliquus capitis inferior (OCI) to the posterior sleeve of the dura mater (Dura) at the level of the second cervical vertebra (C2) (original anatomical illustration produced by Danny Quirk).

the OCI connective tissue bridge inserts into the RCPma myodural bridge, or, in fact, directly links the OCI and the cervical dura mater. Additionally, while the RCPma myodural bridge has been found to contain neuronal tissue (Scali et al., 2013), the OCI myodural bridge has yet to be the subject of neuro-analysis.

The objective of this study is to microscopically investigate the bi-directional continuity of the OCI myodural bridge with both the dura mater and the OCI muscular belly. Additionally, the myodural bridge is to be further analyzed for the presence of neuronal tissue. This study aims to contribute to the current understanding of the anatomy, physiology and pathophysiology of the atlantoaxial region.

2. Materials and methods

A convenience sample of eight cadavers, (four male and four female, ages ranging 49–81 years) were selected from the Department of Anatomical Sciences at Logan College of Chiropractic. The specimens obtained had been treated with a formalin–alcohol–phenol solution and did not show signs of cervical surgery and/or trauma.

All specimens were denuded of soft tissue structures from the level of the third cervical vertebra to the level of the sixth cervical vertebra. The suboccipital muscles were exposed and the origin and insertion sites of the OCI and RCPma muscles were identified. Their tendons were subsequently excised from their respective bony attachment points. The OCI/RCPma myodural bridge was isolated with its attachment to the cervical dura mater preserved. A laminectomy was then performed from the third through sixth cervical vertebrae in order to reveal the contents of the spinal canal. Using a Stryker 810 oscillating saw, the second cervical vertebra was hemisected at its bifid spinous process and cuts were made bilaterally through the laminae, just medial to its articulations with adjacent vertebrae. The second cervical vertebra was then completely detached in order to reveal the components of the atlantoaxial interspace. Using a surgical scalpel, a section of the OCI, RCPma, the atlantoaxial myodural bridge and a 2 cm × 2 cm section of dura mater was excised as one continuous piece. The RCPma was then excised leaving only the OCI, the myodural bridge and a piece of the cervical dura mater as a single unit. When RCPma and OCI specimens presented with excessive ventral adipose tissue and no visible cleavage plane, a cleavage plane was introduced manually by surgical scalpel, bisecting the approximate midpoint of the two individual muscles. These samples were then fixed in 10% neutral buffered formaldehyde and sent to the Department of Pathology at St. Louis University for histological analysis and interpretation.

Tissue samples were fixed in formalin, dehydrated through ethanols and embedded in paraffin wax. The blocks were sectioned at 6 μm, placed on glass slides and dried overnight at 60 °C. Sections for hematoxylin and eosin staining were rehydrated, stained with hematoxylin and eosin, dehydrated again and mounted in Permount. Stained sections were photographed with an Olympus BX41 research light microscope and a DP72 digital camera at 10× magnification. Multiple images from each section were stitched together using Microsoft Image Composite Editor.

For immuno-peroxidase analysis, sample sections were de-paraffinized and rehydrated. After antigen retrieval (Diva Decloaker), slides were washed twice for 3 min each in phosphate buffered saline (PBS) and endogenous peroxidase and blocked in 3% H_2O_2 in 100% methanol for 30 min at room temperature. After washing twice for 3 min at a time in PBS, the sections were blocked for 1 h at room temperature in block solution in a humidified and sealed container. The slides were then washed in PBS for 5 min and the sections incubated in anti-human neurofilament protein antibody (Dako clone 2F11) diluted 1:50–1:250 in 1/10th block solution for 2 h at room temperature and kept overnight at 4 °C in a sealed and humidified container. Controls were normal mouse IgG diluted 1:100. The slides were washed three times for 15 min at a time in PBS, and the sections incubated in goat anti-mouse IgG conjugated to HRP (Sigma) diluted 1:400 in 1/10th block solution for 2 h at room temperature. After three 15 min washes in PBS, the sections were incubated in peroxidase substrate solution (Sigma DAB tablets) for 5–15 min. The reaction was terminated with a distilled water wash and the slides dehydrated through graded ethanols and xylene, and then mounted in Permount. Photography was conducted with an Olympus 41BX research light microscope and a DP72 digital camera at 10× and 40× magnifications. In control experiments, the primary antibody was replaced with normal mouse IgG diluted 1:125 in 1/10th block solution.

3. Results

Eight human cadavers were dissected (4 male and 4 female) with an average age of 65 ± 7.9 (range 49–81) years. From these dissections, twelve atlantoaxial myodural tissue complexes were isolated (5 male and 7 female). In 100% of the isolated atlantoaxial myodural specimens, both the RCPma and OCI muscles emitted connective tissue strands that, when covered by ventral adipose tissue, grossly appeared to coalesce within the atlantoaxial interspace prior to insertion in the cervical dura mater (Fig. 2). Upon dissection and removal of the RCPma muscle, 100% of the OCI muscles remained firmly anchored to the dura mater by a connective tissue bridge that passed anterior, downward and medially through the atlantoaxial interspace, inserting in the posterolateral aspect of the cervical dura mater.

These twelve OCI muscle-connective tissue-dura mater complexes were excised and sent for microscopic analysis (Table 1). Histological examination of the OCI myodural bridge specimens revealed a connective tissue bridge that emanated from the ventral OCI muscular fascia and inserted directly into the posterolateral aspect of the cervical dura mater in 75% of the specimens (Fig. 3). In these specimens, the bidirectional continuity of the OCI myodural bridge was verified in transverse and en face sections. Of these 75%, 56% were male specimens and 44% were female specimens. Microtome slices of the remaining 25% were not able to capture muscle, connective tissue and dura within the same plane and were therefore unable to be properly analyzed. However, there were no

Table 1
Obliquus capitis inferior myodural bridge sample analysis.

Sample number	Age (years)	Sex	Sample side	Histologically verified myodural connection	
				TV	EF
1	49	Male	–	Yes	Yes
2[a]	51	Male	–	Yes	Yes
3	57	Male	Right	No	No
4	57	Male	Left	Yes	Yes
5	65	Female	–	Yes	Yes
6	69	Female	Right	Yes	Yes
7	69	Female	Left	No	No
8	71	Male	Left	Yes	Yes
9	79	Female	Left	Yes	Yes
10	79	Female	Right	No	No
11	81	Female	Right	Yes	Yes
12	81	Female	Left	Yes	Yes

Abbreviations: TV, transverse section; EF, en face section.
[a] Immuno-peroxidase studies were performed on this specimen.
– Information not collected.

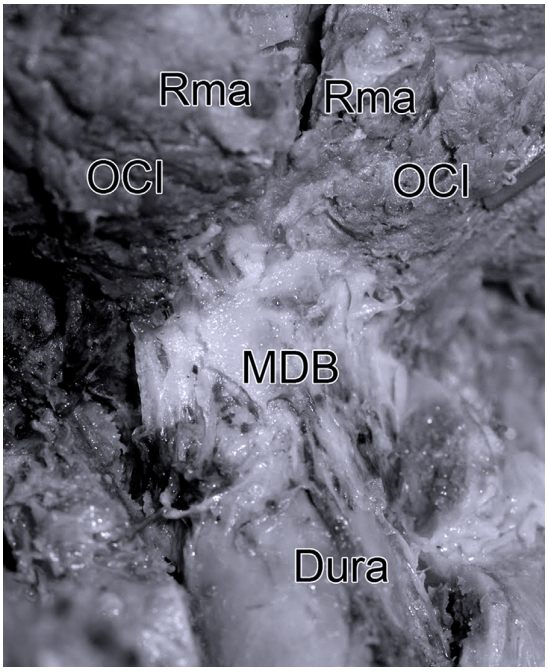

Fig. 2. Photographic evidence of the atlantoaxial myodural bridge (MDB) between the rectus capitis posterior major (Rma), obliquus capitis inferior (OCI), and the cervical dura mater (Dura) in a cadaveric specimen post-laminectomy.

specimens that when analyzed, discounted the myodural bridge from a histological standpoint.

A single specimen was sent for immuno-peroxidase staining using Dako's neurofilament protein monoclonal antibody. Neuro-analysis of this specimen revealed fascicles of neurons traveling perpendicular to, and in parallel with the OCI myodural bridge (Fig. 4).

4. Discussion

The results of this study histologically confirm the presence of a fibrous soft tissue structure that links the OCI muscular belly to the posterolateral aspect of the cervical dura mater. These results support the gross observations reported in an earlier study on the OCI myodural bridge (Pontell et al., 2012). This study confirms that the OCI muscle is now one of three suboccipital muscles that exhibit connections with the dura mater and care should be taken not to mistake these communications for posterior epidural ligaments or dural adhesions. The complexity of these myodural linkages alludes to the fact they may be more than just an incidental anatomical finding.

Earlier reports on the RCPmi and RCPma myodural bridges hypothesize that these communications may play a role in normal human physiology (Hack et al., 1995; Hallgren et al., 1997; Rutten et al., 1997; Alix and Bates, 1999; Hack and Hallgren, 2004; Tagil et al., 2005; Fernandez-de-las-Peñas et al., 2007; Grgic, 2007; Scali et al., 2011, 2013). These studies speculate that the myodural communications function dynamically to prevent dural infolding during movements of the upper cervical spine (Hack et al., 1995; Hallgren et al., 1997; Rutten et al., 1997) Changes in dural tension may activate a sensory relay, thereby inducing compensatory dural realignment via muscular contractile forces transmitted through the myodural connections (Hack et al., 1995; Hallgren et al., 1997; McPartland and Brodeur, 1999; Scali et al., 2011, 2012, 2013). This hypothesis may be supported by the presence of a neuronal component within the myodural bridges (Scali et al., 2013). Furthermore, studies suggest that these communications may aid in the maintenance of CSF outflow from the cisterna magna by

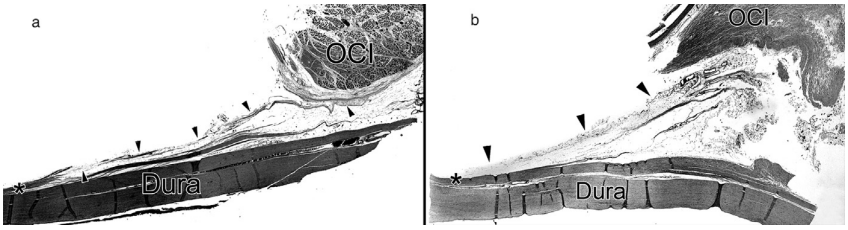

Fig. 3. Hematoxylin and eosin stained slides depicting female (a) and male (b) obliquus capitis inferior (OCI) myodural bridges. A communicating bridge (single arrowheads) extends from the OCI, traverses the epidural space, and attaches (*) to the posterior sleeve of the dura mater (Dura). 10×.

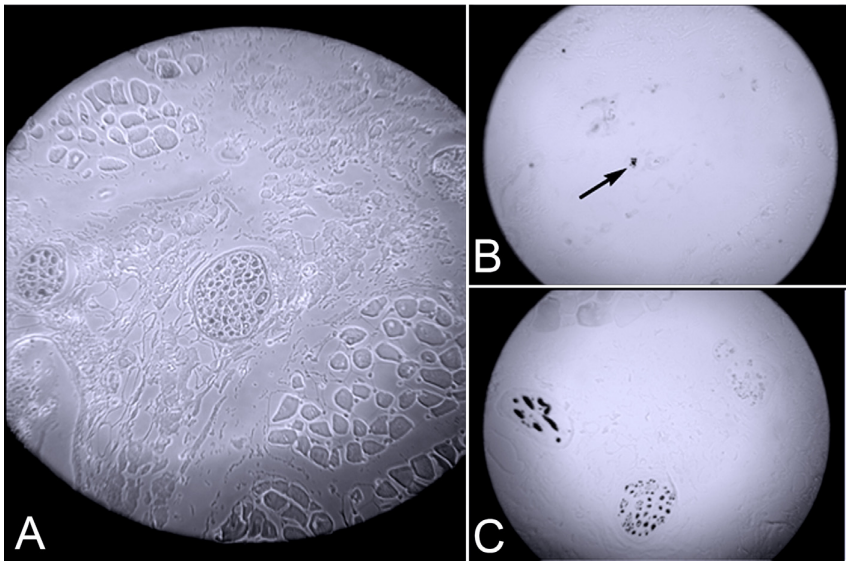

Fig. 4. Immuno-peroxidase staining of male obliquus capitis inferior myodural bridge using Dako's neurofilament protein monoclonal antibody. (A) Phase contrast image showing two nerve bundles within connective tissue, alongside collections of muscle fibers. 40×. (B) Bright field image with arrow pointing to small cross-sectioned nerve bundle. 10×. (C) Bright field image of two nerve bundles, the left bundle obliquely sectioned and the right bundle cross-sectioned. 10×.

maintaining the patency of the subarachnoid space (Hallgren et al., 1997; Kahkeshani and Ward, 2012; Scali et al., 2013). This postulation is in accordance with Becker's hypothesis, which states that voluntary muscles may influence CSF flow by acting on dural membranes via fascial continuities (Becker, 1977). Nevertheless, at this point we can only theorize and further physiologic testing is warranted to determine the true functionality of these structures.

From a clinical perspective, studies postulate that excessive tension on the dura mater transmitted through the RCPma and RCPmi myodural bridges may manifest as cervicogenic cephalalgia (Hack et al., 1995; Alix and Bates, 1999; Tagil et al., 2005; Fernandez-de-las-Peñas et al., 2007; Grgic, 2007; Scali et al., 2011, 2012, 2013). In support of this hypothesis, Hack and Hallgren published a case report in which surgical release of the RCPmi myodural bridge provided relief of chronic, intractable cervicogenic cephalalgia (Hack and Hallgren, 2004). The area of the dura that receives the myodural connections is innervated by the first three cervical nerves, which converge on the spinal trigeminal nucleus (Bogduk, 2001). Given that dural tension manifests as pain (Bogduk, 2001), excessive tension transmitted by these myodural connections may result in nociceptive signals referred through the trigeminal distribution. Conversely, decreased RCPma and RCPmi myodural tone may result in dural infolding with resultant compression of the underlying subarachnoid space (Hack et al., 1995; Hallgren et al., 1997; Rutten et al., 1997). This may impede CSF outflow from the cisterna magna resulting in alterations of intracranial pressure (Becker, 1977; Hallgren et al., 1997). Collectively, studies on the OCI muscle have revealed the presence of a myodural bridge that appears identical to the myodural bridges of the RCPma and the RCPmi (Kahn et al., 1992; Scali et al., 2011; Pontell et al., 2012). For this reason, it is conceivable that the same physiological and pathological hypotheses may apply to the OCI myodural bridge.

Limitations of this study include the average patient age and the small sample size, which were both the result of a scarcity of resources. Only one sample was analyzed for the presence of a neuronal component and the staining techniques implemented could only detect the presence, not the type, of nerve fibers. Further examination is warranted to determine the specific type of neurons that travel within the OCI myodural bridge. The results of this study cannot be generalized and warrant confirmation in studies of a larger sample size. Moreover, studies should be conducted to determine the prevalence of the OCI myodural bridge and whether or not it should be considered as a part of normal human anatomy.

Although grossly the RCPma and OCI myodural bridges appear to coalesce within the atlantoaxial interspace, the results of this study suggest that the OCI is in direct connection with the cervical dura mater. Immuno-histology has revealed the presence of a neuronal component within the OCI myodural bridge; however, at this time we can only speculate as to its importance. Given the similarities between the OCI, RCPma and RCPmi myodural bridges, it is reasonable to suggest that they all may have similar physiologic and pathologic implications. The cervical myodural system has only recently been defined in anatomical literature and further examination is indicated to clarify its physiological and pathological contributions to the human body.

Authors' contributions

Matthew Pontell led this study. He contributed to the study design, statistical analysis, data interpretation, manuscript preparation, literature search and project coordination. Dr. Frank Scali contributed to the study design, data interpretation, manuscript preparation and literature search. Dr. Dennis Enix assisted in data collection, manuscript preparation and literature search. Dr. Patrick Battaglia assisted in data collection, manuscript preparation and literature search. Dr. Ewarld Marshall assisted in data interpretation, manuscript preparation, literature search and project oversight.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aanat.2013.04.013.

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