Histological and biomechanical study of dura mater applied to the technique of dura splitting decompression in Chiari type I malformation.

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INTRODUCTION

Chiari malformation type I (CM-I) is a cerebellar tonsillar herniation through the foramen magnum, leading to a cerebro-spinal fluid (CSF) obstruction at the craniocervical junction. To restore the CSF flow, most of the surgeons enlarge this anatomical area (cisterna magna) by a suboccipital craniectomy associated with a dural graft[5,26,44]. We prefer an extradural approach, described by Isu et al.[20] in 1993, reducing the risks of CSF leak, in which the outer layer of the dura is separated from the inner layer and removed. Thus, a new cisterna magna is created, considering the dural inner part capability of expansion. From our clinical experience, we try to argue in this study the effectiveness of this technique using histological and biomechanical considerations. This work is divided in two parts : in the first one, we examine the posterior fossa dura mater (DM) composition and the orientation of its fibers; in the second one, we emphasize the difference of biomechanical behaviour between entire dura and split dura.

MATERIAL AND METHODS

1) Histological study

We removed 3 specimens (A, B, C) of craniocervical junction DM on fresh human cadavers (2 females, one male; all over 70 years old). For each specimen, 15 samples at precise zones (figure 1) and numbered from 1 to 15 were observed on transversal sections. We added 6 sagittal sections (from B and C specimens). All the samples were fixed in formalin and embedded in paraffin. Microtome sections were stained with hematoxylin-eosin, Masson trichrome for collagen and orcein for elastin. Four sections were not interpretable because of a wrong section plan, so that we examined 47 sections.

The characterisation of DM consists in examination of four criterions under optical microscopy: thickness, presence of two distinct layers with a define boundary, density and orientation of collagen and elastin fibers.

2) Biomechanical study

We removed 22 posterior fossa dural samples from 10 donors (6 females and 4 males; all over 70 years old). We took off 15 intact dural samples and 7 split dural samples (all the split samples had their intact equivalent from the same donor). For the latter, we removed the outer layer of the dura in condition of real surgery using a blade number 15 and a blunt dissector. No special direction was chosen. All samples were placed in physiological saline solution and kept refrigerated.

Each specimen of DM was cut into strips using a metallic pattern (figure 2) of 30 mm length by 20 mm width. To estimate the cross-section of the sample, thickness was measured by a digital micrometer gauge, at least five times. A pseudo-regular ink marking was made to assess the intrinsic deformation of the sample, by a Digital Imaging Correlation technique (DIC). Each mechanical test was performed at room temperature (20°C) on a uniaxial RAITH® testing device at monotonic displacement rate that is imposed constant. The tissue was held between small screw-tightened grips and displacement data were measured by CCD camera (2048 x 2048 pixels) (figure 3). From the images, DIC allowed the determination of the displacement between the ink marks deposited on the specimen surface or between the two grips of the testing device[1,19]. Force was measured with a loading cell of 100 N with an accuracy of 0.1% and the stress in the tissue was defined as the force (F) on the tissue divided by the cross-sectional area of the tissue under that force. Data (in plane strain tensor and load) were collected by a PC and analysed using the softwares CORRELMANUV® and AGNES® from LMS, Ecole Polytechnique[1]. When it was possible, we preconditioned the tissue samples to facilitate reproducibility of results by cycling between 0 and approximately 5% strain until a stable stress versus strain curve resulted on successive cycles. Five cycles had always been enough to reach the stabilised cycle.

We carried out two specific tests series. The first one focused on the dural macroscopic mechanical behaviour, at the scale of the whole sample. This allowed us to compare entire and split samples. The second test series consisted in the observation of the structural modifications of the tissue under strain thanks to strain field analysis, in order to understand the local deformation mechanisms during the test.

RESULTS

1) Histological study

The thickness of the samples was measured between 0.625 and 1.475 mm (mean thickness 1.106 mm, standard deviation 0.244 mm). The midline sections were thicker than the lateral ones (table 1). Sections number 5 and 8 had the largest average thickness; this can be explained by the remains of the posterior atlanto-occipital membrane, even if it was macroscopically removed during the dissection.

Two different layers occurred objectively on only 10 sections (specimen A n°5,11,12,13 / specimen B n°1,6,12 / specimen C n°6,12 + one sagittal section). Among these, a well define boundary between the two layers was observed in 6 samples. It seemed to be more a capillary network than a real interface (figure 4a). In the others samples, we could not distinguish the two layers structure. Moreover, we did not found some particular relation between the localization of the sections of the posterior fossa and the presence of two layers. Thus, we think that the classical description of two distinct layers constituting the DM[36] could be inconstant in the posterior fossa.

Concerning the DM composition, collagen was largely dominant with few elastin fibers (figure 4b). The collagen fibers were dense and strongly organized, distributed inside the tissue. However, we observed some degree of reciprocal density gradient between collagen and elastin, from one face of the dura to the other. There was a tendency of a decrease of collagen density, and conversely an increase of elastin fibers, from the inner to the outer layer (visible on 10 and 9 sections respectively). So the ratio collagen/elastin appeared higher in the inner dural part and lower in the outer one, but these considerations were inconstant.

To define the orientation of the fibers, we compared the transversal sections with the 6 sagittal sections. The collagen fibers appeared in a main cranio-caudal direction with probably some little degree of obliquity. On the 10 sections where 2 layers were identified, we did not observe a clear difference in collagen orientation between the inner part and the outer part.

From a scanning electron microscopic study, DM seemed to be composed of a multilayer structure, with piled up leaves. Each leaf was composed of lined up “big cables” (about 1 micrometer diameter) corresponding to collagen fibers and of a thinner spiderweb network with no preferential orientation corresponding to elastin fibers (figure 5). The observations of samples fracture surfaces obtained from liquid nitrogen fracture confirm that each leaf was separable from adjacent leaves. Therefore, we conclude that the dural splitting dissection does not occur between two macroscopic leaflets: it splits the DM between two layers of a microscopically divided multilayered structure.

2) Biomechanical study

a) Macroscopic tensile test

The first campaign consisted with twelve tensile tests. Figure 6 shows a typical mechanical response for tensile tests performed on entire and split DM (from the same donor) at room temperature and under a constant strain rate around 1.5 10^{-3} s^{-1}. As in previous studies[45,46], it appears that entire DM presents a large elastic domain (until some 10%) followed by occurrence of damage mechanisms what conducts to failure of the sample. For split DM, the mechanical behaviour is quite different than for intact one, with a smaller elastic domain followed by a larger irreversible strain domain. Partial unloadings were performed during the test to check the evolution of the Young modulus in the irreversible strain region. Decrease of the Young modulus could be considered as a signature of the damage occurrence inside the specimen: the more damaged is the specimen the weaker is the Young Modulus. So for the split dura the Young modulus evolves from 25 MPa to 19 MPa (corresponding to 0.78% of the initial value) even if the irreversible strain is quite large before failure: that means that damage occurs gradually inside the specimen during the test. On the contrary, from entire dura mater the evolution of young modulus seems to be quite different with an increase of the

Young modulus in the first part of the test from 44 MPa to 91 Mpa - that could correspond to a rearrangement of the fibers along the stress axis - then a quite constant value is reached until final rupture occurs as observed in figure 6.

b) Displacement and Strain field analysis

From a more local point of view corresponding here to the effect of the fibers distribution, second tensile tests campaign is performed to follow, thanks to optical microscope and DIC, the evolution of the displacement field inside the sample during the test. This campaign has consisted with ten tensile tests performed under optical microscope with a spatial resolution equals to 0.9 µm at room temperature for the same strain rate that the macroscopic ones. It appears from figure 7, obtained from intact DM for two samples coming from two different areas presenting different microstructure's configurations, that the displacement field seems to be homogeneous along the sample and quite independent on the fiber's axis versus the tensile axis. That means that, at this mesoscopic scale (corresponding to a few hundred of microns), the mechanical behaviour of DM could be considered as being homogeneous, with no effect of the orientation of the fibers on the mechanical behaviour. So it seems that the mechanical behaviour of the DM could be considered at first approximation as being isotropic.

c) Modeling of the DM expansion

We estimate the volume benefit from the DM splitting through estimation of the order of magnitude of its deformation. The geometry of the split dural zone is complex. As a first approximation, we assume that this region is a spherical cap with a diameter around 40 millimeters (figure 8).

Prior to deformation (just after bone removal), the curvature radius R_c of the DM cap is about the one of the head, which can estimated at 5 cm. Thus, the geometrical characteristics of the

spherical cap are: volume of 2.6 cm^3, surface of 13 cm^2 and maximum height z_{max} (measured between the support line and the top of the cap) around 4 mm.

In order to estimate the deformation in the DM, we need to know the stress in the tissue. The highest component $\sigma_{\theta\theta}$ of the stress tensor in the DM can be estimated from the mechanical equilibrium of the structure: if an inner pressure P_{ic} (intra-cranial pressure) is applied on the DM cap, the stress on the border (the supporting line) of the membrane has to equilibrate this pressure. Therefore, the stress is related to the applied pressure by:

$$\sigma_{\theta\theta} = \frac{R_c}{2h} P_{ic}$$

with *h* the thickness of the DM.

We assume that the DM splitting removes half of the thickness (h = 0.5 mm) and that the P_{ic} is about 10 mmHg in CM-I. In that case, the $\sigma_{\theta\theta}$ stress is around 70 kPa. The deformation associated with this stress in our uniaxial traction experiments is between 2 to 5%, remaining small. Such deformations are not big enough to bring us in the domain of irreversible strains, which implies that there is no risk of leaking of the DM due to the tissue stretching.

The DM membrane is under a biaxial solicitation, while we performed an uniaxial test. Relating both approaches is not straightforward in the general case, but we can consider, in first approximation, that the relative surface deformation is of the same order of magnitude of the relative length deformation. Therefore, the surface after deformation S_{def} is related to the initial surface S_{init} by $S_{def} = S_{init}(1+\varepsilon)$, where ε is the estimate of the deformation.

From this result, simple geometric considerations lead to the volume and to the maximum height after deformation.

A deformation of ε=2% (lowest estimate) leads to a volume of 3.3 cm^3, an increase of 22% of the initial one, and the maximum height is about 6 mm, increasing by 45% the original one.

The highest estimate of the deformation of ε=5% leads to a volume of 4 cm^3 (increase of 50%), and a maximum height of 9 mm, twice the initial one. This second estimate is more likely to be closer to the real case than the first one, considering the deformation data from our tensile experiments. This is also in agreement with the observation of the posterior fossa enlargement after dural splitting during surgery[20].

DISCUSSION

The dura mater is a connective tissue surrounding the nervous central system. It is known to be a membrane composed of collagen and elastin fibers with two different layers[36]. Actually, its structure remains controversial, especially concerning the orientation of its fibers. On the contrary of the lumbar DM[32,34], the posterior fossa dura mater has not been studied yet. From our observations, we think that the presence of two distinct layers on the posterior fossa dura mater is inconstant. The limit between the inner and the outer dural part sometimes appears as a vascular plan (capillary network), but is more often virtual. Previous studies on dura mater in the spinal region[38,48] have shown a well defined structure in two leaflets. We think that the difference may be due to the location, as the posterior fossa is making the transition between cerebral compartment, with a spherical shape, and the spinal one, with a cylindrical shape. Based on our observations, we conclude that the structure is closer from a multilayer material, stacking many sheets of fibers. However, the understanding of the fibers orientations of the dura remains difficult. Histological studies indicate more or less a cranio-caudal orientation of the fibers, but we use also other imagery methods, such as scanning electron microscopy and second harmonic generation microscopy (SHG)[10,47], which indicate that the different layers of fibers may have different orientations. SHG studies confirm histological studies about the fact that composition of the dura mater is inhomogeneous in the thickness, with more collagen

close from the inner face (figure 4b). Note that SEM and SHG were used in complement of the histological studies, and not in a systematic approach, due to the time they request. The difficulty of finding quantitative results based on histological approach indicates that more specific methods should be used in the future to improve the understanding of the dural structure.

Human DM has its own biomechanical properties, which have been studied in the past[38,39,45] . Most of these works concern the lumbar DM and are applied to the comprehension of the post dural puncture headache[13,32]. Dura has also been tested as a potential component of heart valves[24]. But, posterior fossa DM has not been examined until now.

In this work, we tried to highlight the difference of behaviour between the entire dura and the split dura, correlated to the surgical technique described by Isu et al.[20]. This biomechanical work is the first to evaluate the posterior fossa dura mater mechanical properties. Also, it is the first investigation of the biomechanical behaviour of split dura mater in association to the specific surgical technique of splitting decompression in CM-I. Our tests compared one entire sample and one split sample from each donor when it was possible. For the intact DM, we found an elastic behaviour, with a small domain where deformation is reversible with stress followed after the yield point by a fragile behavior, as indicated by others authors on lumbar dura[46]. On split DM, we observe the same type of behaviour at small strain, with a slightly smaller stress level for the same strain. The main significant difference between entire dura and split dura is after the yield point: split DM presents a large domain where the mechanical behaviour is elasto-plastic, with permanent strain and a lower stress level, instead of a fragile behavior for the intact DM.

We must nuance in our observations since the tests were performed on samples from elderly fresh human cadavers (over 70 years old). So it is reasonable to think that tests on specimens from younger donors would have shown different results, although no study has been made to

compare dural biomechanical behaviour between children and adults for example. Also, we worked on donors from the Ecole de Chirurgie Assistance Publique - Hopitaux de Paris whom we considered to be free of CM-I. We know from Nakamura et al.[30] that DM in CM-I can be thicker than a normal dura. This implies of course that the neurosurgeon must remove a significant thickness of dura to obtain a visual deformation of the split area and then a satisfying decompression. Also, it may modify the mechanical behaviour, as the tissue is inhomogeneous in its thickness.

These experiments concern the instantaneous behaviour of the dura mater. To confirm that they are in agreement with the decompression in CM-I, we build a model of DM expansion. It shows that reasonable stresses due to the malformation on the dura mater lead to a large structural deformation: the volume increases about 50%. But, this model simplifies the real situation: in particular, we did not introduce the head muscles that could prevent the dural extension. However, it shows that the immediate deformation frees enough space to relax the neuraxis constrain and to restore the CSF flow.

A clear extension of our approach should be to study the time-dependent extension by studying the mechanical answer to a creep test. Radiological findings, where an increase of cisterna magna size is observed[20,22], seems to support the hypothesis that the split dura could enlarge with time.

~~Moreover, a more complex model, including creep tests, would be useful to the neurosurgeon to plan the dural splitting decompression and to anticipate the results of the surgery.~~

Pourquoi cette suppression ?

CONCLUSION

This original study demonstrates the capability of the split dura mater to enlarge for suitable stress conditions and quantifies it by biomechanical tests. We have built an experimental

model that shows a significant volume benefit at the cranio-cervical junction after splitting. Thus, dural splitting decompression has a real biomechanical substrate to envision the effectiveness of this Chiari type I malformation surgical technique.

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