A quantitative analysis of the nerve fibres of the acetabular periosteum of dogs

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Summary
There are many techniques for the treatment of hip dysplasia, and novel research is currently being undertaken in the hope of obtaining more efficient and less traumatic techniques. The denervation of the hip joint capsule is a simple and effective technique that allows recovery of the functional activity of the affected limbs in significantly less time than other techniques. This surgical procedure consists of removing the acetabular periosteum, thus eliminating the nerve fibres with consequent analgesia. The aim of this investigation was to quantify the number of nerve fibres present in different regions of the acetabular periosteum. The knowledge of regional differences is potentially valuable for the refining of the denervation technique of the hip joint capsule. Thirty canine acetabular fragments were used to compare the nerve fibre density of the periosteum. The results showed a significant difference between the mean density of nerve fibres at the cranial and dorso-lateral portion (approximately 75 fibres/mm²) and caudo-lateral portion (approximately 60 fibres/mm²) of the acetabulum. Those fibres at the periosteum are almost positioned in a sagittal plane, pointing towards the joint capsule, suggesting the same density in the latter region. These results indicate a new approach to the articular denervation technique, thus obtaining even better results for the treatment of hip dysplasia in dogs.

Keywords
Acetabulum, nerve fibres, analgesia, hip dysplasia, dogs

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Introduction
The study of the hip joint in dogs has become important after the development of new surgical techniques that enable the sensory neurotomy of the acetabular region with the aim of reducing pain. In 2002, Staszky and Gasse (1) proposed a multiple innervation model based on segmented innervations, where the nerve fibres penetrate distinct areas of the articular capsule, as well as the ‘indirect’ nerve fibres that continually penetrate the muscle through the periosteum and the capsule. In contrast to the shoulder joint, where the innervations are not symmetrical, the hip joint has innervations that are bilaterally symmetrical and individually variable.

The close anatomical association between the nerve component and the joint is well established, since the gluteal (2, 3) and ischiatic (1, 4) nerve endings are frequently implicated in medical situations, such as fractures, dysplasia, and surgery.

The periosteum is a highly vascular (5) structure which contains innumerable nerve endings. There are various nerve fibres within the periosteum which are more frequently associated with blood vessels, either ramifying around or ending at blood vessel walls. Occasionally, isolated nerve fibres or small nerve terminations can also be found between cells, independent of blood vessels (6).

Articular denervation is a simple and effective method that is used to treat articular diseases of the hand and feet in human orthopaedics (7, 8). This technique is based on the selective neurotomy of the sensitive nerve fibres within the peri-capsular region, thus resulting in permanent analgesia and reactivation of the dynamic component of the joint. Initial studies in veterinary medicine have demonstrated that articular denervation could be useful for the treatment of diseases of the hip joint, such as hip dysplasia and arthrosis (9).

This technique consists of the surgical removal of the periosteum, in a semicircular fashion, beginning at the cranial-dorsal margin progressing to the ventral margin of the acetabulum. Additionally, a circular area is also removed at the insertion of the peri- articular muscles, thereby eliminating sensory nerve fibres in this area. Preliminary experiments performed in dogs obtained excellent results (9).

In 2002, Kinzel et al. (9) reported on 17 dogs that had been treated using this technique. 90.6% of these animals clinically recovered, while 56% showed remarkably decreased lameness scores three days after the surgery. One dog remained free of signs after 10 years of treatment.

With the aid of a radiofrequency percutaneous equipment, Kawaguchi et al. (10) used transitory block process of the sensory nerve response of the obturator and femoral nerves to treat articular pain in human hip joints and obtained satisfactory results in 86% of the patients during a period of one to 11 months.

However, the greatest advantage of denervation is the simplicity of the technique. There is no need for sophisticated equipment and the operation is restricted to the periosteum, thus eliminating the nerve fibres within this area, without disturbing the articular capsule or the joint. Such simplicity renders this surgical procedure feasible for young and old patients. Other advantages of this technique include the reduced surgical time and the shorter recovery period in comparison to other pre-existing procedures. Furthermore, in the authors’ experience, the low cost of the operation is yet another factor that easily persuades owners

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to proceed with the treatment. Even owners of older dogs seem to prefer this technique in comparison to other forms of treatment which are commonly associated with prolonged recuperation periods or onerous conservative treatments.

The objective of this study was to quantify the nerve fibres of the acetabular periosteum of dogs aiming to refine a low-cost, precise and efficient denervation technique for canine hip joints.

Material and methods

Thirty hip joints from eight male and seven female mongrel dogs, weighing between 22 to 62 kg, from two to eight years of age, without any evidence of bone disease, that had been sacrificed for reasons unrelated to the study (in accordance with the bioethics committee of the University of São Paulo) were studied. All of the specimens were collected and placed in fixative within three hours after euthanasia. The acetabular region was dissected, and released from all muscular and neurovascular insertions. The joint capsule and ligaments were then removed, followed by luxation of the femoral head at the point of incision and rupture of the femoral head ligament. A fragment of this region extending from 1 cm cranially and 1 cm caudally to the acetabular margin was removed.

This fragment was measured, divided in frontal plane sections into three equal parts and labeled division A (cranial region), B (dorsal-lateral region), and C (caudal region); these were further subdivided and labeled 1, 2, and 3 (Fig. 1).

From each subdivision, central 1 cm² fragments were obtained. Each fragment was obtained after removing the cancellous sub-cortical bone; only the periosteum and the cortical bone were utilized. These fragments were then identified based on their place of origin, denominated A1, A2, and A3, or B1, B2, and B3, or C1, C2, and C3. The segments were individually fixed in 10% buffered neutral formalin solution for five days.

After fixation, all of the fragments were decalcified in solution made up of 5% hydrochloric acid, 3% acetic acid, and 10% chloroform, 10% distilled water, and 73% alcohol at 95% (V/V). The time for decalcification varied according to each fragment, and was checked daily by penetration of the bone with a 6 metric (22 gauge) needle. Decalcification was assumed to be achieved when the needle passed freely through each section. The time for decalcification varied between six and 15 days. The fragments were then washed with water to remove the excess of decalcification solution and subsequently processed for histopathology.

Silver impregnation

Nerve fibres were identified by silver impregnation (Fig. 2) using the method proposed by Linder (11), with some adaptations. This method involved the use of a 1% silver nitrate solution with pH controlled by addition of a buffer solution of 0.5% borax/boric acid until a pH of 7.8 was obtained (6).

Randomization

This study was randomized, considering equally each region evaluated, independent of the animal studied. Of all of the animals only one of the areas (1, 2 or 3) from each of the regions (A, B or C) was selected and then analyzed. Consequently, in this study 30 acetabular samples were utilized (15 from the left and 15 from the right antimes-

Exclusion criteria

The following criteria for exclusion of dogs were established:
- Dogs that did not meet the previously mentioned requirements;
- Dogs whose clinical record revealed any presumptive or definitive diagnosis of any infectious disease;
- Dogs that demonstrated clinical signs of degenerative articular disease (12).
Negative controls

The specificity of the impregnation utilized in this study was verified by the usage of histological popliteal hyaline cartilage samples from the same studied animals, since this structure normally lacks nerve fibres (Fig. 3).

Measurements

Digitalized 400X photomicrographs of the area of each of the fragments were obtained using a digital camera (Canon E820®, Canon Inc., Tokio, Japan) affixed to a microscope. Each fibre was identified and determined by the software Image Pro Plus v 4.5® (Media Cybernetics, Silver Spring, MD, USA). Each fragment was analyzed in three different areas and the average number of nerves per square mm was calculated.

Statistical analysis

Statistical analyses were performed using a computer statistical package (Minitab, Release 13, 2000 – Minitab, Inc., State College, PA, USA). The data obtained were tested for normality using the Kolmogorov-Smirnov Test. Parametric distributions were obtained and the variance analysis was determined by one-way ANOVA with Tukey post-hoc test. The differences were considered as significant when \( P \leq 0.05 \).

Results

Significant statistical differences were not observed regarding the mean density of nerve fibres of the acetabular periosteum of the right antimer (69.84 ± 8.21), when compared to the left (68.27 ± 8.32), \( P=0.09 \). Furthermore, there was a lack of significant differences between each division of the stipulated regions in both antimeras. Nevertheless, marked significant differences were observed between regions A and B (cranial and dorso-lateral regions) of each antimer, when these were compared to region C (caudal region) (Table 1 and Fig. 4).

![Fig. 3](image1)

**Fig. 3** Photomicrography. Hyaline cartilage by silver staining. Note the absence of nerve fibres (magnification 400X)

![Fig. 4](image2)

**Fig. 4** Distribution of nerve fibres in the acetabular periosteum based on the analysis of each region. The regions A, B and C indicate anatomical cranial, dorso-lateral and caudal positions, respectively. The non-coincident letters, a, b, and c, indicate significant statistical difference between regions, independent of the antimerus analyzed – Tukey test (\( P \leq 0.05 \)). The non-coincident letters, A and B, indicate significant statistical difference between within the antimerus analyzed Tukey test (\( P \leq 0.05 \)). Horizontal lines represent medians; Vertical lines represent interval of data; The bar represents 75% of the values. Full circles represent averages; and * depicts discrepant value.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Antimer</th>
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<tbody>
<tr>
<td>Mean (fibres/mm²)</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Standard deviation (fibres/mm²)</td>
<td>8.21</td>
<td>8.32</td>
</tr>
<tr>
<td>Interval of data (fibres/mm²)</td>
<td>54–83</td>
<td>51–85</td>
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Note: Mean values do not present significant statistical differences by Tukey test.
Discussion

The relative sensitivity of a bone to a painful stimulus and its relation to the innervation density of the periosteum is well known and has been discussed in depth in the veterinary literature (6, 13, 14). Although the innervation of the periosteum is mainly responsible for the painful sensation of the bone tissue, it is certainly not the only factor associated with bone nociception (15, 16). Patients with painful bones, due to diseases restricted to cancellous bone or partial lesions that involve small portions of cortical bone, did not exhibit signs of periosteal damage (15, 16).

It has been reported that blood vessels and nerve fibres occur together in the periosteum (17–19). The silver impregnation technique that was used in this study was not specific enough to study the association of the direction of nerve fibres and blood vessels. Thurston (6) investigated the density of the nerve fibre population present at the periosteal area of long bones of human beings by using the same impregnation technique. Again, an association between the course of blood vessels and nerve fibres could not be found.

Silver impregnation of nerve fibres is a well-established technique (13). When silver impregnation was compared with immunohistochemical staining techniques for the identification of nerve fibres in the femur of rats, a marked difference was not observed (14). Moreover, the extreme specificity of each reagent used in immunohistochemical methods must be taken into consideration.

The silver impregnation technique used in this study did not stain any of the structures in the hyaline cartilage examined in this study. As aforementioned, the acetylcholinesterase was divided in three smaller fragments and, with the aim of minimizing the influence of potential artifacts provoked by osseous irregularities in order to get more precise results, these fragments were further divided into three, thus making nine portions from each antimeric. The final image was processed using the same software.

The results obtained in this investigation are similar to those described by Staszyk and Gasse in 2002 (1) but differ to those found by Kinzel and coworkers in 1998 (20). The former group demonstrated that by using acetylcholinesterase, the periosteal innervation present in the cranial-lateral and caudal-lateral regions of the dog’s acetabulum comes from nerve fibres which were originally innervating muscular fibres. Kinzel et al. (20) demonstrated the presence of nerve fibres only at the cranial-lateral region, showing an absence of nerve fibres at the caudal-lateral region. In our study, we were able to prove the presence of nerve fibres in all regions of the acetabulum, thus proving that the density of nerve fibres differs between the cranial-lateral (regions A and B of our study) and caudal-lateral (region C of our study).

The density of the nerve fibres obtained in this study, expressed as an average (fibres/mm²) has not previously been described in the literature in relation to the anatomical region analyzed.

Judging by the degree of sclerosis observed, in severe hip dysplasia cases, particularly at the cranial-lateral acetabular margin, with subsequent cranial-dorsal subluxation of the hip joint, it had been hypothesized that this region is the most affected by abnormal force behaviours caused by articular incongruence (9). Investigations of human hip joints demonstrated that the higher concentration of nerve terminations is localized where compressing forces are acting. Kinzel et al. (20) reported that the highest density of nerve terminations relative to pain is localized in the cranial-lateral region of acetabulum of dogs. At the time that this group published their results, there were not any reports about the innervation of the caudo-lateral and ventral portions of hip joint. Because of this, Kinzel et al. (9) only suggest a cranial-lateral acetabular periosteum removal as an efficient form of denervation of hip joint capsule. As a result, a 90% improvement is reported in patients on whom this technique was performed. In our study, we demonstrated the presence of a significant number of nerve fibres in the caudal-lateral region of acetabular periosteum in all analyzed areas.

With these data, Ferrigno et al. (21, 22) have demonstrated a new surgical approach to the denervation technique, thus improving freedom of movement and quality of life in 95% of the patients.

References


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