REFERENCES


REZUMO – Objetivo – Testar a eficácia e segurança do pneumoperitônio com ar comprimido para cirurgias videolaparoscópicas em porcos em treinamento de residência médica. Métodos – Porcos da raça Dalland de peso variável de 15 a 17kg foram submetidos a anestesia geral e respiração controlada. Eles foram divididos em 3 grupos: A – 38 animais com pneumoperitônio feito com insuflador automatizado de CO2, usando este gás; B – 7 animais sujeitos ao mesmo procedimento exceto que o CO2 foi substituído por ar comprimido; e, C – 11 animais em que o pneumoperitônio foi feito com ar comprimido diretamente da rede hospitalar. Nos 3 grupos, a pressão intra-abdominal foi mantida entre 12 e 14mmHg. Os procedimentos realizados foram distribuídos proporcionalmente nos 3 grupos: nefrectomia bilateral – 20, pieloplastia desmembrada – 20 e nefrectomia parcial – 16. Antes e 2h após o pneumoperitônio foi colhido sangue arterial para gasometria em 5 porcos do grupo C. Resultados – Foram consumidos 25 torpedos de 4,5kg de CO2 a um custo total de R$ 3.150,00 no grupo A. A duração média da cirurgia nos grupos A, B e C foram respectivamente: 181±30min, 219±21min e 210±18min (p=0.05). Alcalose respiratória foi observada em 3/5 porcos testados do grupo C. Nenhum animal apresentou sinais de embolia gasosa ou faleceu durante o procedimento. Conclusão – O uso de ar comprimido para laparoscopias em porcos mostrou-se método seguro com redução de custos e tornou desnecessário o uso de insuflador automático.


Study of corpus callosum in experimental hydrocephalic wistar rats

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ABSTRACT – Purpose: Hydrocephalus causes countless cerebral damages, especially on the structures around the ventricles. Hydrocephalic children present deficiencies in the nonverbal skills more than in the verbal skills, and not always reversible with an early treatment. As the corpus callosum has an important role in the nonverbal acquisition it is possible that the injuries in this structure are responsible for the cognitive dysfunctions of these children. This present study tries to establish the alterations caused by hydrocephalus on the corpus callosum of developing Wistar rats, induced by intracisternal injection of kaolin. Methods: Seven, fourteen and twenty one days after the injection, the animals were killed, and the corpus callosum was dissected and prepared for the study of the axonal fibers. Results and Conclusion: The seven-day old rats in hydrocephalus development presented a delay in myelination in relation to the control rats. With the fourteen-day old rats in hydrocephalus development the corpus callosum showed a recovery of myelin, but with the twenty one-day old rats in hydrocephalus development the axonal fibers were damaged and reduced in number.

KEY WORDS: Corpus callosum, Hydrocephalus, Myelin, Wistar rat, Development.

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INTRODUCTION

Hydrocephalus is a complex disease, characterized by ventricular enlargement, secondary to the imbalance between the formation and absorption of the cerebrospinal fluid (CSF). The first structure that is affected by the increase of the CSF pressure and the expansion of the ventricles is the ependyma, by compressing and stretching of the structure, besides ruptures from isolated points until the nearly total destruction of the epithelium. In the white matter axonal degeneration is observed, as well as the loss of the myelin in the more accentuated degrees of ventricular enlargement. The distortion of the brain leads to stretching and distortion of the brain vessels, besides reducing the density of the capillaries. As a final result, there is a reduction of the cerebral blood flow. Hydrocephalus also affects the neurons and the synapse, it is not known if by direct compression or secondary suffering to the axons. The cytoarchitecture is affected in the density and neuronal size, with alterations in the cortical lamination. The affected in the density and neuronal size, besides ruptures from isolated points metabolism are increased, which mirrors the alteration of the extracellular space. Children suffering from hydrocephalus often present a retarding in the development of their cognitive functions, with a tendency of showing more faults in the development of nonverbal cognitive functions than in the development of the corpus callosum of the normal rats, and of those rats that were not killed after 7 days of the kaolin injection and that belonged to the groups of 14 and 21 days of hydrocephalus evolution, also presented staring eyes on the 10th day besides skull convexity. Some rats with accentuated skull convexity, became lethargic on the 3rd week of hydrocephalus evolution, and withdrew themselves to a corner of the cage, presenting trembling of the head and hyperexcitation, jumping without coordination.

METHODS

In the developmental and structural alterations are reverted with the treatment of hydrocephalus. Neurological residuary damage occurs on 10% to 74% of the children with hydrocephalus receiving treatment with shunts. The recovery of the white matter with remyelination and the neuronal recovery, and the restore of the levels of the neurotransmitters only occur when the treatment with shunts is precociously established. The objectives of this study were to determine the myelination progression in the corpus callosum development of the normal rats, and the alterations caused by hydrocephalus in different anatomic regions of this structure, according to the time of progression of the ventricular enlargement. THIS STUDY was done according to the CIOMS (Council for International Organization of Medical Sciences) ethical code for animal experimentation (WHO Chronicle 1985: 39(2):51–6) and the principles of the COBEA – Colégio Brasileiro de Experimentação Animal. Sixteen litters of 7-day old Wistar rats were used, without sexual distinction. Each litter was constituted of the mother rat and 6 to 8 newborn rats. Each pup rat was held by an auxiliary, who held the head with one hand and the body with the other, bending the animal’s neck, leaving the posterior cervical area free. By palpation the space between the posterior extremity of the foramen magnum, on the occipital bone, and the posterior arch of the first cervical vertebra was identified. With an Mise 0.3 odontologic needle, with a short bezel, the subcortical percutaneous injection was done, and 0.4 ml of a kaolin suspension (Merckâ) diluted in distilled water of 20% was slowly injected. One rat of each litter was not injected as it was used as a control. Following this the rats were put back into the cage with their mothers. All the injected animals were observed daily to see any possible clinical alterations. Those rats that did not develop hydrocephalus after the injection were dismissed and were not computed in the number of animals of each group. Brain perfusion and dissection of the structures. The animals of each group injected with kaolin and their respective controls of the same age, were weighed one by one, on a standard animal scale. Following anesthesia with vapors of sulphuric ether, they were submitted to a transcardiac perfusion with PBS solution (about 1ml/g of the animal’s weight), after the perfusion, with the same volume, with a 2% paraformaldehyde and 1% glutaraldehyde in a 0.1M, pH 7.4 phosphate buffer. The animals were decapitated, their brains removed in block cleavage plane, removing samples of the genu, mid-body and splenium. Preparing of samples for regional evaluation of the corpus callosum. The samples obtained from the corpus callosum were fixed in a 1% osmium tetroxide in a 0.2M phosphate buffer for 2 hours, at 4°C, washed with a 0.1M phosphate buffer and then dehydrated in an increasing series of acetone and embedded in araldite. The inclusion was done to permit that the corpus callosum could be sectioned transversally. After the polymerization of the resin, the blocks were cut in sections of 0.5 mm of thickness and the sections stained with 1% toluidine blue. The sections were analyzed in a light microscope and photographed on the Axiophot (Carl Zeiss) photomicrography system. The aspect, the distribution and the number of myelinated fibers and the neuropil cells were evaluated considering the region of the corpus callosum and the time of evolution of hydrocephalus. The maturity of the corpus callosum of the normal rats and of the hydrocephalus ones, were analyzed.

RESULTS

Clinic alterations of hydrocephalus. Around the 2nd day after the injection a slight convexity of the skull vertex was able to be observed on the animals that developed hydrocephalus. As the days went by this convexity was more evident. Those rats that were not killed after 7 days of the kaolin injection and that belonged to the groups of 14 and 21 days of hydrocephalus evolution, also presented staring eyes on the 10th day beside skull convexity. Some rats with accentuated skull convexity, became lethargic on the 3rd week of hydrocephalus evolution, and withdrew themselves to a corner of the cage, presenting trembling of the head and hyperexcitation, jumping without coordination.

Body and brain weight of the normal rats and the hydrocephalus ones. All the animals were weighed on the day that they were killed, immediately before the anesthesia to transcardiac perfusion. The results of the weights can be seen in Table 1. The average body weight of the hydrocephalus animals killed on the 7th day after the kaolin injection (group H1) was significantly less than that of the control (group C1). Although the difference between the average weight of the hydrocephalus rats killed on the 14th day after the injection (group H2) and their controls (group C2), it is not statistically significant, but there was a tendency of the hydrocephalus animals to present less weight. Finally in the animals killed on the 21st day after the kaolin injection (group H3) we noticed that there was not a statistically significant difference, but they also presented a tendency of less weight than the control rats of the same age (group C3). The result of brain weighing presented a similar relation between the control rats and the hydrocephalus ones. When the brain weights are related to the body weights we observed that the average decreases with age, in the normal rats, or better, in the younger animals, the brains present a bigger proportion in the animal’s total weight than the older animals. In the hydrocephalus rats of group H1, the brains were heavier than their controls. In the hydrocephalus rats of group H2, the brains continue to be heavier than the controls, but the difference is less than that of the rats of 1 week younger. In the rats of group H3, there is a tendency of the hydrocephalus brains being heavier than the controls, but the difference is not statistically significant.

Maturation of the brain in the control animals. The observation of the sections of the corpus callosum stained with toluidine blue showed that the myelinated fibers of the control animals of group C1 are disperse, in increasing number from the posterior extremity to the anterior of the structure, or better, the spilenum presents myelinated fibers in less number than the body, that in turn presents a smaller number of these
fibers than the genu of the corpus callosum. Neuroglia cells are present in a great number, with big nucleus and evident nucleolus. Some neuroglia cells appear withdrawn and dark (Figure 1A, B and C). In the rats of group C2, the number of myelinated fibers, are really increased, although compacted, but the crescent gradient of the splenium for the genu maintains. The neuroglia cells are in a reduced number in relation to the 14th day controls (Figure 1D, E and F). The controls of group C3 present myelinated fibers even more exuberant in number and density. The crescent gradient of the number of myelinated fibers of posterior to anterior callosal portion is still identified, but the number of fibers with myelin of the splenium increased considerably, and the fibers are bigger and better defined (Figure 1G, H and I).

**Lesions caused by hydrocephalus.** The observation of the sections of the corpus callosum of the hydrocephalic rats stained with toluidine blue showed that hydrocephalus increases the density of the neuroglia cells that however show up pyknotic and dark most of the time. The hydrocephalic animals of group H1 present a smaller number of myelinated fibers of the corpus callosum when compared to their controls. These fibers are reduced in the 3 areas of the examined corpus callosum, appearing more in the splenium as scattered groups. The splenium also shows vacuolization that appears as light areas (Figure 2 A, B and C). However the hydrocephalic rats of group H2, show a big number of myelinated fibers, more densely compacted than the controls. This compaction occurs in the more distant regions of the ventricular cavity, that is on the body and on the splenium of the corpus callosum, the immediate areas of the lateral ventricles roof have signals of edema with areas of spacing between the fibers (Figure 2D, E and F). The animals from group H3 also show important losses of myelinated fibers, which are disperse, with small areas of compacting (Figure 2 G, H and I).

![FIGURE 1](image1)

**FIGURE 1:** The development and maturing of the corpus callosum of the normal Wistar rat. A, B, and C: 14-day old animal (group C1); D, E and F: 21-day old animal (group C2); G, H and I: 28-day old animal (group C3). The first figure of each row is the sample of the genu of the corpus callosum, the second figure is the sample of the body of the corpus callosum and the third figure of each row is the sample of the splenium. Solid arrow in B: myelinated fiber of the corpus callosum. Open arrow in B: Neuroglia cell. Toluidine blue stain. 500 X.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
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<tbody>
<tr>
<td>Average of body</td>
<td>38.67 ±</td>
<td>54.8 ±</td>
<td>93 ±</td>
<td>37 ±</td>
<td>27 ±</td>
<td>3.98 ±</td>
</tr>
<tr>
<td>weight ± SD</td>
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<td>13.89</td>
<td>11.443 ±</td>
<td>11.126</td>
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<tr>
<td>Average of the brain weight ± SD</td>
<td>1.271 ±</td>
<td>1.419 ±</td>
<td>1.551 ±</td>
<td>1.425 ±</td>
<td>1.499 ±</td>
<td>2.038 ±</td>
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<tr>
<td>x100 ± SD</td>
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<td>0.382</td>
<td>0.237</td>
<td>1.199</td>
<td>0.543</td>
<td>0.846</td>
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**TABLE 1:** Averages of the body and brain weights and the brain/body rate of the normal rats with 14 (C1), 21 (C2), and 28-day old (C3), and the hydrocephalic ones, killed on the 7th (H1) 14th (H2) and 21st (H3) days after the injection.

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**FIGURE 2:** The development and myelination of the corpus callosum in hydrocephalic rats, killed on the 7th (H1) 14th (H2) and 21st (H3) days after the injection (moderate hydrocephalus). A, B and C: Group H1; D, E and F: Group H2; G, H and I Group H3. The first figure of each row is the sample of the genu of the corpus callosum, the second figure is the sample of the body of the corpus callosum and the third figure of each row is the sample of the splenium. Toluidine blue stain. 500 X.
DISCUSSION

Kaolin is an inactive chemical substance, composed of hydrated aluminum silicate, which causes an intense inflammatory response of the meninges, with an obstruction of the outflow of the cerebral spinal fluids of the 4th ventricle, without affecting the brain parenchyma lesions. One or two days after the intracisternal injection of kaolin, the ventricular enlargement begins, on the lateral ventricle, then advancing forwards. This model of experimental induction was chosen due to it being efficient, inexpensive, and it does not need surgical proceedings or cause anatomic modifications and does not even lead to other alterations unless those results that are totally of hydrocephalus.20,21

In this study, the induced hydrocephalus by transcutaneous, intracisternal injection, of the kaolin suspension, on 7-day old post-birth rats, lead to skull convexity, identified after the first days of the injection. This shows that the cranial sutures of these animals are still obvious therefore this model of experiment can simulate hydrocephalus that develops in very young children. The skull convexity was directly proportional to the degree of ventricular enlargement. Similar findings were obtained by Kiefer et al., although they used the H-Tx rats, genetically affected by hydrocephalus. These animals, however, are expensive and restricted to few researchers, besides not being able to guarantee that all the lesions found are entirely a result of hydrocephalus.

Besides the skull convexity, the hydrocephalic rats presented staring eyes that could correspond to “setting-sun phenomenon” of the hydrocephalic children. Similar findings were seen by Wright et al., although in kittens.19 Following this these animals begin to present apathy and trembling, also in the same way as hydrocephalic patients. Trembling is referred to by several authors, so much so as in hydrocephalic rats as in hydrocephalic cats.11

The hyperexcitability of some animals with moderate-severe ventriculomegaly could be explained by the compromising of the periventricular matter, compressed by the enlargement of the more peripheral levels of the ventricular system at this stage of hydrocephalus evolution. Nevertheless, detailed and directed studies for this structure have to be done. Nothing was found in the pertinent literature that something similar was discovered. The 14-day old post-birth animals with 7-day hydrocephalus evolution presented slightly more weight than its correspondent controls. But the 21-day old hydrocephalic animals, therefore 14 days of hydrocephalus evolution (group H2), although they presented a tendency of less weight when compared with their controls, did not have a significant difference of body weight gain. This can represent an adaptation of the animals with the new condition and then return to normal feeding. The same was observed with animals 1 week older, reinforcing this idea of readaptation. Several authors obtained similar data with hydrocephalic animals with less weight than the controls at the beginning of the ventricular enlargement process, recovering on the second week of hydrocephalus progression, but no explanations were given for these findings.22,23,24,25

As for the brain weight in relation to the body weight, the normal animals presented a progressive decrease of the relation, or better, at this phase of development the body gains more weight than the brain, indicating that the final brain weight is a lot affected before the animal reach body maturity. In the 2-week old hydrocephalic rats (group H1), the brains are heavier than their controls. These alterations in weight can reflect of brain edema and the proper accumulation of fluid in the enlarged ventricular cavities. However, this difference is accentuated with aging, as time goes by the brain begins to represent a smaller volume of the total body weight of the rat.

In rats the myelination of the corpus callosum begins around the 13th day of birth, increasing a lot at the end of the 1st month, but this continues until the 10th month of age.26 The presence of a big number of neuroglia cells on the corpus callosum of the younger animals indicates that these cells in the axonal myelination process, as with the increase in the number of fibers with myelin as time goes by, the neuroglia cells are inclined to reduce in number.

With the development of hydrocephalus the number of neuroglia cells of the corpus callosum increase in number, mainly in the posterior areas. This can be a sign of an attempt of the myelin of the damaged areas to repair. The apparent partial recovery of the myelin was also identified by Del Bigio et al.27 Hydrocephalus although it initially makes the body and the genu to become thinner, first goes to the edema and alters the myelination of the posterior areas of the corpus callosum. In this study an initial loss of myelinated fibers in the seven days of hydrocephalus evolution (group H1) was noticed, an important increase of these fibers with the 14 days of hydrocephalus evolution (group H2) and once again a drastic reduction of myelinated fibers in the 21 days of hydrocephalus evolution (group H3). This initial increase of the fibers with myelin, that still present themselves in a smaller number than in the control, is an indication of an attempt of the recovery of the corpus callosum damages. However, this recovery was not maintained while the ventricular enlargement persisted. These findings were similar to those of Del Bigio et al.28,29 An explanation for the increase of the damages of the corpus callosum fibers during the hydrocephalus time, could be an alteration of the tissue perfusion that hydrocephalus causes in the white matter that leads to the accumulation of toxic metabolites in this matter, as it suggested by Massicotte et al.27. Another explanation for this fact could be the maintenance of the biomechanical alterations in respect to the stabilization of the ventricular enlargement, as is proposed by Shapiro et al.29

It should be considered that the histochemical methods is qualitative and for a more criteria evaluation of the myelination, it is necessary a quantitative study, with an ultrastructural study, so much as for the number of existing fibers as the thickness of the myelin border of each fiber. The results of the present study allows us to conclude that the development of the corpus callosum of the Wistar rat, in relation to myelination, is essentially after birth following a standard of anterior-posterior sequence. Initially in hydrocephalus the myelination process tends to follow the normality standard, but as time goes by the myelinated fibers are slowly affected. Complementary studies are necessary, including morphometric and ultrastructural analysis for a better comprehension of the physiopathology of the damages produced by hydrocephalus in the corpus callosum and other structures of the brain parenchyma, close to the ventricular system.

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6–ARTIGO ORIGINAL

Brachial plexus variations in its formation and main branches

Valéria Paula Sassoli Fazan¹ André de Souza Amadeu² Adilson L. Caleffi² Omar Andrade Rodrigues Filho³


ABSTRACT – Purpose: The brachial plexus has a complex anatomical structure since its origin in the neck throughout its course in the axillary region. It also has close relationship to important anatomic structures what makes it an easy target of a sort of variations and provides its clinical and surgical importance. The aims of the present study were to describe the brachial plexus anatomical