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Arnold-Chiari in a Fetal Rat Model of Dysraphism

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Key Words
Myelomeningocele · Arnold-Chiari malformation · Fetal rat model

Abstract
Dysraphism is a defect in neural tube development, leading to dysplastic growth of the spinal cord and meninges. Myelomeningocele (MM) is just one of its forms. Hydrocephalus is among the most important alterations in MM and occurs as a consequence of Arnold-Chiari malformation (AC). Experimental models have been developed in sheep, rabbits and rats to study MM physiopathology, allowing a more detailed evaluation of clinical parameters involved in this anomaly. ***Objective:*** Using the experimental model of dysraphism in fetal rats, the aim of this study was to evaluate the relevance of AC malformations, clinical parameters and grade of histological lesions. ***Materials and Methods:*** Three groups with 16 fetuses in each were compared, MM, Control and Sham, after intrauterine surgical creation of MM on day 18.5 of gestation (term = 22 days). AC was evaluated by photographic comparison of sagittal cuts of fetal heads. Clinical and histological evaluations were also made. ***Results:*** 88% of AC (14/16) in MM fetuses were obtained, besides 100% of clinical alterations. Necrosis and erosion of the spinal cord exposed to amniotic fluid were verified in histology. ***Conclusion:*** The presence of AC in the dysraphism rat model was high. These results allowed the use of this model to study alterations and intrauterine evolution of MM in a fashion similar to those observed in humans.

Dysraphism is a defect in neural tube development, with incomplete fusion of the vertebral arches in the spinal column leading to dysplastic growth of the spinal cord and meninges. There are three main types of dysraphism: spina bifida, meningocele and myelomeningocele (MM) [1].

MM, the most severe form of dysraphism, is associated with critical motor handicaps, bone deformities, bladder and bowel incontinence and sensorial alterations below the level of the lesion. Hydrocephalus is among the most important alterations in MM and occurs as a consequence of Arnold-Chiari malformation (AC), which is characterized by permanent herniation of the brainstem and the cerebellum through the foramen magnum and into the cervical spinal canal.

Hydrocephalus, secondary to AC, in MM causes neuropsychomotor retardation, as a result of serious lesions to the spinal cord before birth and increasing intracranial pressure [2, 3]. As a consequence, patients born with MM present a mean longevity reduction to less than 40 years and a considerable decrease in quality of life, besides substantial personal and family difficulties and social costs [4].

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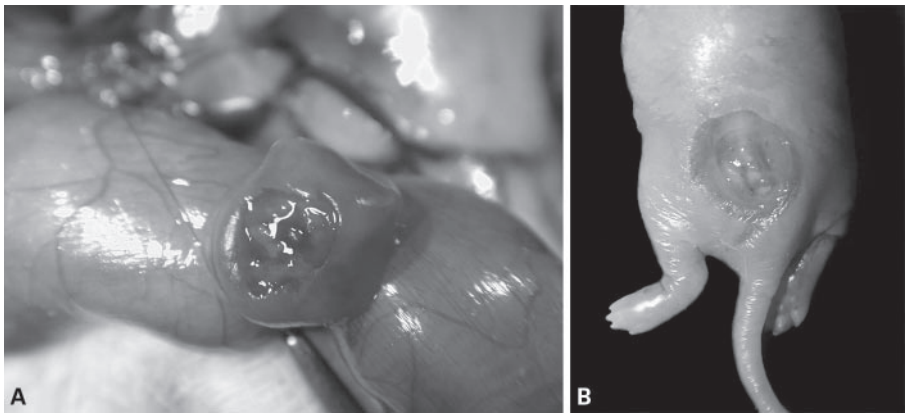

Fig. 1. A Dysraphism in fetal rat model. **B** Spinal lesion on the harvest day in MM newborn rat.

Intrauterine correction of MM is able to reverse AC, with the possibility of limiting hydrocephalus progression, reducing the use of ventriculoperitoneal shunts and ensuing complications caused by long-time use of the catheter.

Experimental models of MM with AC identification were developed in mice by Inagaki et al. [5] in 1997, and in sheep by Paek et al. [6] in 2000. Until then AC had not been identified in rats. Therefore, the aim of this study was to evaluate the resulting clinical alterations when using the experimental MM model in rat fetuses which emphasizes the presence of AC in this model.

Materials and Methods

Experimental protocols were reviewed and approved by the Animal Experimentation Committee of the State University of Campinas (UNICAMP) and the experiment had previously been approved as research project number 292-2.

Sprague-Dawley rats (~ 250 g) were mated and the females were checked daily for vaginal smear. The day of plugging was defined as gestational day 0 for time dating (term = 22 days).

Experimental Groups

Three groups of fetuses were studied after MM creation. Group I: 16 fetuses submitted to MM surgery on day 18.5 of gestation; Group II: 16 Control fetuses; and Group III: 16 Sham fetuses.

Experimental Model

The pregnant female rats were anesthetized on day 18.5 of gestation using ketamine 50 mg/ml (175 mg/kg) and xylazine 10 mg/ml (2.5 mg/kg). Surgery was performed under a 2.5× magnification microscope. Aseptic conditions were maintained. One horn of the bifid uterus was exteriorized through a midline abdominal incision. Individual fetal rats could be seen, each one in its amniotic sac. Open spinal dysraphism was created as described by Heffez et al. [7]. A hysterotomy was performed by placement of a 6-0 nylon purse-string suture including the amniotic membranes, followed by a 5-mm incision within the purse-string in the direction of the back of the fetal rat. For fetal immobilization, the purse-string was limited so that the fetus was kept partially inside the uterus. Under 2.5× magnification the paraspinal muscles were dissected, after which a 2-level laminectomy was performed. The dura mater was opened using a 26-gauge needle (fig. 1A). The fetus was then returned to the uterus and the amniotic fluid volume was restored. The Control Group was not touched. Fetuses in the Sham Group were only submitted to a dorsal skin incision, using the same technique as for the hysterotomy. The maternal laparotomy was closed in 2 layers with a continuous 4-0 silk suture. Fetuses were harvested by cesarean section on day 21.5 of gestation (fig. 1B).

Data Analysis

Liveborn pups were examined for evidence of neurological deficits, assessing the following clinical parameters: (1) kyphosis; (2) tail deformities; (3) leg deformities; (4) leg paralysis, and (5) pain and touch perception. Pain perception was assessed by pricking the pups' legs and feet with a needle, 5 min after the birth. Touch perception was assessed by pricking the pups' legs and feet, which have a finer sensitivity, with a Semmes-Weinstein® monofilament and 10 g pressure. Fetuses were weighed on a precision scale after clinical evaluation and then sacrificed. They were then fixed in 10% formalin and AC evaluation was performed. For this purpose fetal heads were sectioned along the sagittal suture. Sections were photographed with a digital Nikon camera, 5.4 megapixels, 8× magnification. Hindbrain herniation was then confirmed using the axis as parameter. Spine cross-sections were taken of all groups at the surgical site level and at the proximal and distal levels of the operative site.

Histological Analysis

The 7-μm-thick sections were stained with H&E (hematoxylin and eosin) and studied by light microscopy for evidence of spinal cord injury.

Statistical Analysis

Clinical parameters were evaluated for their presence or absence. Weight difference was evaluated by the Kruskal-Wallis method, with significant values of $p < 0.05$.

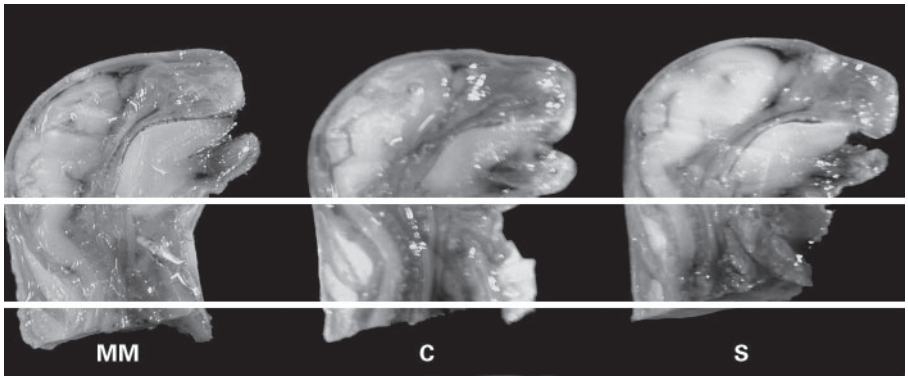

Fig. 2. Hindbrain herniation in MM fetus in relation to Control (C) and Sham (S).

Table 1. Clinical evaluation and Arnold-Chiari malformation

	Experimental MM (n = 16)	Control (n = 16)	Sham (n = 16)
Weight (± SD), g	5.31(± 0.70)	5.75(± 0.68)	5.25(± 0.77)*
Kyphosis	16 (100%)	0	0
Tail deformities	16 (100%)	0	0
Leg deformities	16 (100%)	0	0
Leg paralysis or paresis	16 (100%)	0	0
Perception of pain in the legs	0	16 (100%)	16 (100%)
Arnold Chiari	14 (88%)	0	0

* No statistical difference for Kruskal-Wallis (p = 0.07).

Results

Operative Results

There were no maternal deaths among the 8 pregnant rats operated in this study. Fetal survival rate was 94% (48/51).

Clinical Evaluation

The mean weights found for the fetuses in groups MM, Control and Sham were 5.31 g (± 0.70), 5.75 g (± 0.68) and 5.25 g (± 0.77), respectively; there was no statistic difference among the weights (p = 0.07).

The clinical findings for the 16 fetuses with MM were: 16 kyphoses (100%), 16 tail deformities (100%), 16 leg deformities (100%), 16 paresis/paralysis of the legs (100%), no fetus with pain perception in the legs or feet, and 14 AC (88%) (table 1, fig. 2).

Histological Evaluation

In the histological analysis by means of H&E staining of the spinal cord, sections of fetuses in Group MM, necrosis and erosion of segments exposed to the amniotic fluid were observed, in addition to an acute inflammatory infiltrate (fig. 3).

Discussion

MM is a consequence of incomplete closure of the posterior portion of the neural tube in the 4th gestational week and during the neurulation process, which is completed around the 30th day of gestation. The diagnosis of fetal MM is usually achieved through ultrasonography or magnetic resonance in routine prenatal exams, from week 16 of gestation onwards [8].

According to the *two-hit theory* hypothesis proposed by Heffez et al. [7], the primary event that leads to exposure of neural elements causes developmental defects of the spinal cord – myelodysplasia – while a secondary event that determines erosion and necrosis of the exposed region leads to increasing damage throughout the gestational period [9]. This hypothesis is corroborated by the

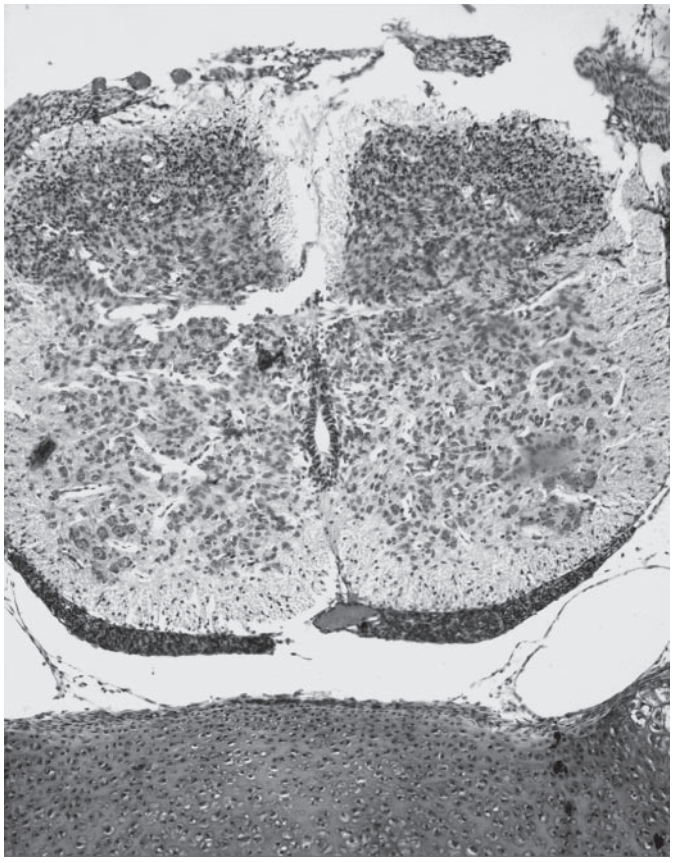

Fig. 3. Histological section of the spinal cord in the region of MM. H&E. ×40.

observation that the neural lesion acquired during fetal life is enhanced by mechanical trauma or by the chemical toxicity of the amniotic fluid [9].

The incidence of MM is variable when different regions are considered. The rate registered by the Program of Perinatal Genetics at UNICAMP was 2.3:1,000 births, while in the USA it was approximately 1 for each 1,000 liveborn infants [10].

The estimated health care costs for MM patients in 1985 was in excess of 200 million dollars per year in the USA [11]. In addition, even with the standard surgical treatment, approximately 14% of neonates with the anomaly do not survive beyond the age of 5, bringing this mortality rate to around 35% in those with dysfunction of the brain stem secondary to AC [12].

The first experimental MM model with surgical fetal repair was devised by Michejda [13], using primates, in 1984. A group of animals was immediately repaired with allogeneic bone paste and the Control Group was not submitted to repair. This last group developed neurological deficits and cystic lesions in the spinal cord similar to those found in human beings.

Heffez et al. [7] created a dysraphic lesion model in rat fetuses, carried out on the 18th gestational day (term = 22 days) to study the effect of the toxicity of the amniotic fluid on the spinal cord. The fetuses presented spinal cord damage similar to that seen in humans; lesion repair on the 20th gestational day prevented the anatomical and functional deficits observed when there was prolonged exposure of the spinal cord to the amniotic fluid. The same author, in 1993, obtained similar results using the experimental model in pig fetuses [9].

Meuli et al. [14, 15] created an MM experimental model in sheep. Sheep fetuses on the 75th gestational day (term = 145 days) were submitted to surgical creation of MM. Clinically speaking, the fetuses presented urinary and intestinal incontinence, flaccid paraplegia and loss of sensitivity in the extremities of the lower limbs. Histologically, the authors demonstrated the loss of neural tissue, interruption of nerve fascicles and necrotic areas in the exposed segments.

Inagaki et al. [5] developed the dysraphic lesion in mice embryos to study the importance of the hydrodynamic action of the cerebrospinal fluid and were able to demonstrate the presence of AC.

Paek et al. [6] studied the importance of MM intrauterine repair in AC reversal in sheep fetuses to protect them from hydrocephalus. The sheep were decapitated at birth and median sagittal sections of the head were performed in order to investigate the presence of herniation of the brainstem through the foramen magnum. The outcome revealed that fetal repair of MM prevented the occurrence of herniation of the hindbrain.

Due to the importance of AC in the development of hydrocephalus and its consequences for MM patients, until now AC has not been found in a fetal rat model, we regard the 88% AC rate in MM fetuses as an extremely satisfactory result, considering the participation of AC in the development of hydrocephalus and its deleterious consequences for MM neonates.

Moreover, the dysraphic model in rat fetuses has the advantages of low cost, short gestational period, a large number of fetuses per pregnancy and presence of control-fetuses in the same pregnancy.

The histological alterations found are in agreement with those already mentioned in literature on experimental models. The acute inflammatory infiltrate observed in MM confirms the hypothesis that the most serious aggression to the spinal cord occurs at a later gestational stage,

due to alterations in the composition of the amniotic fluid itself [16, 17].

In synthesis, we concluded that the experimental model of dysraphism (MM) in rat fetuses proved to be adequate to obtain clinical and histological alterations similar to those found in human MM, with special emphasis to the presence of AC in a significant number of MM fetuses.

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