Successful Repair of Myelomeningoceles

Successful Fetal Surgery for the Repair of a ‘Myelomeningocele-Like’ Defect Created in the Fetal Rabbit

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Abstract

Objective: To study the correction of a 'myelomeningocele-like' defect in fetal rabbits. Methods: Twelve pregnant rabbits had a spinal defect surgically created in 40 of their fetuses at 23 days of gestation. Immediate repair was performed in 30 fetuses (group I), and 10 remained uncorrected (group II). After 30 days, the fetuses were harvested, and the anatomopathological aspects were compared using Fisher's exact test. Results: Three different techniques to apply a cellulose graft were used for correction in 8 (technique A), 7 (technique B), and 15 animals (technique C), but only one (technique C) was successful. The survival rate at 30 days was 66.7% (10/15) in group I and 80% (8/10) in group II. A 'myelomeningocele-like' defect was present in all fetuses in group II, while in group I the defect was successfully repaired in 80% of the surviving fetuses (p < 0.01). Conclusion: The surgically created spinal defect was successfully repaired, and also the fetal rabbit could be established as a model for the study of intrauterine correction of a myelomeningocele-like defect.

Introduction

A myelomeningocele (MMC), also known as spina bifida, is a common congenital defect with a low fetal/neonatal mortality, in which postnatal treatment has failed to significantly improve the neurological outcome, so far.

Children born with the defect can have variable degrees of impairment, including paraplegia/paresis, urinary and fecal incontinence, sexual dysfunction, and skeletal deformation. Hindbrain herniation occurs in the vast majority of them, often leading to a hydrocephalus that can cause intellectual impairment.

In the 1980s, Michejda [1] suggested that the neurological impairment observed at birth in cases of MMC was associated with progressive aggression (chemical and/or mechanical) to the exposed medulla during gestation, rather than a primary neural tissue maldevelopment. These findings were subsequently reinforced by other authors [2, 3], and, based on this assumption, attempts were made to protect the medulla before birth. The prevention of neurological damage was successfully reported after prenatal correction in the fetal monkey (nonsurgical correction) [4] and also in sheep [5].
The defect was successfully created in animal models like rat [2], monkey [6], sheep [5, 7, 8], and fetal rabbit [9, 10], but surgical techniques for the successful antenatal correction were only reported in the fetal sheep, so far [5, 7, 11].

In humans, the experience in the correction of MMC started with the endoscopic approach used by Bruner et al. [12], but soon the authors abandoned this approach because fetal survival rate was low and because the procedure was time-consuming. Recently, the same group [13] has reported their experience and also another group in Philadelphia [14], both using an open-surgery approach to correct the defect. Although a reduction of hindbrain herniation and/or in the incidence of shunt-dependent hydrocephalus was observed in both studies, not surprisingly, preterm delivery was a major problem. Bruner et al. [13] operated 29 human fetuses, 50% of them were admitted to the hospital with premature uterine contractions. Five cases delivered before 30 weeks of gestation and 1 uterine rupture occurred. The mean gestational age at delivery was 33.2 weeks. Sutton et al. [14] operated 10 cases, and 4 of them were delivered prior to 32 weeks of gestation.

We believe that if we could develop a new ‘easy-to-apply’ endoscopic technique to antenatally correct MMC, we could perhaps reduce preterm birth and also maternal morbidity [15]. To develop this ‘new technique’, we considered the rabbit to be the ideal model because of the short gestation and the high number of fetuses in each pregnancy. This would allow techniques to be rapidly adjusted, if necessary, during the course of surgery.

Materials and Methods

Twelve time-dated pregnant New Zealand white rabbits arrived at the laboratory 7 days prior to surgery to permit acclimatization. They were housed in individual cages in a quiet room with continuous air flow and natural daylight cycles. Food and water were permitted until 30 min prior to surgery. On gestational day 23, all does underwent surgery for the creation of an ‘MMC-like’ defect. They were premedicated with ketamine 20 mg/kg (Parke-Davis, São Paulo, Brazil) and acepromazine 1 mg/kg (Univet, São Paulo), half of the dose administered intramuscularly 20 min prior to surgery. After shaving, the animal was brought to the operating room, and the other half of the premedication dose was given through a lateral ear venous access. Also, endovenous cefazolin 25 mg/kg (Lilly, São Paulo) was used for antibiotic prophylaxis, and the venous access was maintained for saline infusion until the end of the procedure. The rabbit was placed in a supine position with four-limb restraint over a heating pad. Halothane 1–2% was delivered by a rabbit-modified ventilation mask. Medroxyprogesterone (4.5 mg i.m.; Pharmacia & Upjohn, São Paulo) was given to obtain uterine relaxation. All animals were maintained under spontaneous ventilation and had electrocardiac monitoring throughout surgery. An inferior laparotomy was performed, and the uterus was then exposed to count the number of viable fetuses. The uterus was returned to the abdominal cavity and only the fetus to be operated on was left exposed.
A maximum of 4 fetuses were operated in each doe. A total of 40 animals were operated on, using a previously described technique [16], briefly described as follows: After gentle palpation of the fetus, two sutures (4–0 silk) were placed in the myometrium, and an uterine incision was performed. Chorion and amnion were also incised; the fetus was manipulated to permit solely the fetal back and tail to be outside the uterine cavity. Using a loupe (magnification ×4), the skin over the spine was then removed (approximately 0.5 cm²). A longitudinal sharp incision of the posterior vertebral arch (including four to five vertebrae) was performed at the level of the pelvic crest, deep enough to include the dura. This incision was performed, while the fetal posterior limbs were externally compressed against each other (at the level of their insertion in the fetal pelvis), leading to an accentuated bulging of the medulla, once the dura was incised. In 10 out of the 40 fetuses operated, nothing else was performed, and the fetus was reinserted (control group). After a continuous ‘invaginating’ suture of the uterus (including the membranes), 5 ml of warm saline solution was injected, and the suture was tied.

The remaining 30 fetuses were submitted to immediate correction of the created defect. Three different techniques were subsequently used. The first technique (A) consisted of a cellulose graft (Biofill®; Fibrocel, Paraná, Brazil) applied directly to the area where the skin had been previously removed, sized approximately 5 mm larger than the area previously excised, to the edges of the graft were placed over the normal skin. The second technique (B) consisted of dissecting the skin around the defect below the dermis, approximately 5 mm around the defect, applying the cellulose material underneath the normal skin, completely covering the defect. The third technique (C) consisted of the same procedure as described for technique B, followed by a single suture (6–0 mononylon) placed in the middle of the skin defect, only to approximate the skin edges (not including the graft; fig. 1). The fetus was reinserted and the uterus closed, as in the control group. The maternal abdominal wall was closed in layers, and halothane administration was stopped. The animals were then placed in lateral decubitus position on the operating table until superficial recovery from anesthesia which took approximately 20–30 min. The animals were returned to their cages for full recovery and were maintained with the previous amount of food and water (ad libitum). Vaginal bleeding and the behavior were monitored on a daily basis for the next 7 days.

At 30 days of gestation maternal euthanasia was performed by injecting intrathoracically 0.5–1.0 ml/kg of T61® (Hoechst Roussel Vet, São Paulo). After maternal death, the fetuses were harvested through laparotomy and also killed through injection of T61 into the thorax. The operated fetuses were weighed, photographed, and fixed in 10% formol. Transverse sections of the fetal spine (hematoxylin and eosin stained) were analyzed, comparing the anatomopathological aspects of the corrected versus the noncorrected groups.

The project was approved by the local animal research ethical committee. Statistical analysis was performed using the Fisher exact test and Student’s test to compare the groups.

Results

In the group submitted to correction (group I), technique A was used in 8 fetuses (group IA), technique B in 7 fetuses (group IB) and technique C in 15 animals (group IC). No maternal deaths occurred during or after the procedure. Successful repair was achieved only in the group in which technique C was used. The 10 fetuses in which correction was not attempted belonged to the control group (group II).
### Survival

After 30 days, in groups IA and IB, 11 out of 15 fetuses survived (73.3% survival rate). In group IC, 10 out of the 15 fetuses submitted to correction survived (66.7% survival) and 8 out of 10 fetuses survived (80% survival) among controls (group II; table 1). The mean fetal weight at 30 days was 38.18 ± 9.55 g in groups IA and IB, 34.4 ± 5.27 g in group IC, and 41.4 ± 6.3 g in group II. There was a statistically significant difference (p < 0.05) in mean fetal weight between groups IC and II.

### Control Group

In group II, macroscopically the separated posterior vertebral arch could be seen and also the spinal artery (fig. 2a). Histological examination showed that a ‘MMC-like’ defect was successfully created in all controls. The skin was absent, the posterior vertebral arch was opened, the spine was exposed, and pia and dura mater were fused (fig. 3a). All surviving fetuses in groups IA and IB presented the same defect described in the controls.

### Successfully Corrected Group

In group IC, 80.0% (8/10) of the surviving fetuses submitted to correction showed significant signs of repair. Macroscopically, we could observe the closure of the skin (fig. 2b), and histologically we could observe the skin covering the defect, the cellulose graft in place, with no signs of rejection, and new connective tissue present between the spine and the graft (fig. 3b). There was a statistically significant difference when comparing groups IC and II (p = 0.001) regarding the successful correction of the defect (table 2).

### Discussion

Inspired by the success reported by Bruner et al. [12], using biological glue to place maternal skin over the MMC defect in 4 human fetuses, we decided to test a ‘self-adherent’ skin substitute product (Biofill) that would obviate the use of glue and the need of air injection into the uterine cavity, hopefully reducing the risks and mortality observed in Bruner’s initial series.

Biofill is a pure cellulose film (not derived from plants) produced by Acinetobacter that is similar to human skin. It is a low-cost material that has been approved by the Food and Drug Administration of the United States in 1995. It was developed for the treatment of skin burns, demonstrating a self-adherence characteristic in the damaged/exposed skin.
Applying the cellulose over the defect, we expected the skin to grow underneath the graft and that the new fetal skin formed would cover/protect the spinal defect. As the graft detached in our first attempt (technique A), we decided to test the cellulose as a ‘base’ where the skin would grow over it (technique B). As the graft also detached, we decided to approximate the skin over the defect. A single suture was used just to hold the cellulose in place, and this technique (C) was successful. We assume that Biofill facilitated the skin-healing process, as well as the ‘self-repair’ occurring in the spinal structures below it.

The isolation of the medulla from the skin is a desirable effect, since tethered spinal cord was reported by Sutton et al. [14] during his initial experience in the human antenatal correction of the defect. With this purpose, his group started using Alloderm®, but only recently it was tested in animals for the correction of the defect [12]. Alloderm is also considered a skin substitute; it is made from cadaveric skin samples and has a different mechanism to heal the damaged skin when compared with Biofill. Alloderm provides a collagen matrix for the migration of the dermal cells that is incorporated to the host tissue when used as a skin substitute. In the study performed by Paek et al. [11], it has failed to produce the expected effect of migration of cells when used to protect the medulla.

We could observe in our series a statistically significant difference in fetal weight at harvesting among the group corrected versus the noncorrected controls. We believe that a possible explanation for this difference could be the catabolism involved in the healing process or the more intense manipulation the corrected fetuses were submitted to.

Although we could demonstrate significant signs of repair after 30 days, definitive repair of the medulla could not be observed, presumably because of the short period of time between correction and harvesting (7 days). We believe that a longer period would be needed to demonstrate the long-term effects in the neoformation of dura and pia mater, but in the rabbit model, this will be probably difficult to demonstrate because of the offspring cannibalism the animals can present.

We believe the fetal rabbit can be used as an experimental model to study the correction of a surgically created MMC. The cellulose graft we tested was suitable for this correction. Especially for testing new materials and techniques for the correction of MMC, the rabbit is an adequate experimental model. This is true particularly because of the short gestational period and the high number of fetuses. This ‘easy-to-apply’ technique may permit, in the future, the utilization of an endoscopic approach to correct the defect in humans. This could reduce preterm delivery and maternal morbidity associated with an open-surgery approach. If the long-term effects of this correction prove to be effective, maybe this can be the future for
the correction of MMC in the human fetus.

Acknowledgments

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References

Fig. 1. Schematic illustration of the surgical techniques used to repair the ‘MMC-like’ defect created at 23 days of gestation in the fetal rabbit. Technique A: dissecting the skin around the defect. Technique B: the cellulose material is covering the defect. Technique C: single suture is placed to approximate the skin edges (not including the cellulose).
Fig. 2. a Macroscopic aspect at 30 days of gestation of the ‘MMC-like’ defect created at 23 days of gestation in the fetal rabbit (group II). Note the separation on the posterior vertebral arch and the intact spinal artery. b At the same gestational age, we can observe the macroscopic aspect of the repaired defect (group I, technique C). Note that the skin is healed and the single suture.
Fig. 3. Microscopic aspect of the ‘MMC-like’ defect at 30 days of gestation. a The defect is present; lack of skin covering and dura and pia mater fused (asterisk). b Skin (S) covering the defect, cellulose graft (CG) in place with no signs of rejection, and new connective tissue present between medulla (M) and graft in a fetus submitted to correction of the defect.
### Table 1. Survival rates

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<th></th>
<th>Operated</th>
<th>Survived</th>
<th>Total %</th>
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<tr>
<td>Group I (corrected)</td>
<td>15</td>
<td>10</td>
<td>66.7</td>
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<tr>
<td>Group II (controls)</td>
<td>10</td>
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<td><strong>Total</strong></td>
<td>25</td>
<td>18</td>
<td>72.0</td>
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### Table 2. Number of fetuses submitted to correction of the MMC-like defect

<table>
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<tr>
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<th>Group I (corrected)</th>
<th>Group II (controls)</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>Defect present</td>
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<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Defect corrected</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>8</td>
<td>18</td>
</tr>
</tbody>
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