European Journal of Obstetrics & Gynecology and Reproductive Biology 148 (2010) 35–39

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Hydrogel protection: a novel approach to reduce bowel inflammation in experimental gastroschisis☆

Frances Lilian Lanhellas Gonçalves [a], Regiane da Silva [b], Augusto Frederico Schmidt [a], Marcelo Ganzarolli de Oliveira [b], Lourenço Sbragia [a,*]

[a] Division of Pediatric Surgery, Department of Surgery, School of Medical Sciences, University of Campinas, UNICAMP, CP 6111, Campinas, SP, Brazil
[b] Institute of Chemistry, University of Campinas, UNICAMP, CP 6154, Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form 14 September 2009
Accepted 5 October 2009

Keywords:
Hydrogel
Biomaterial
Gastroschisis
Scanning microscopy
Inflammation
Biocompatibility

ABSTRACT

Objective: In gastroschisis there is herniation of the fetal bowel into the amniotic cavity that results in severe intestinal dysfunction. In order to reduce bowel exposure to amniotic fluid we used a hydrogel of N-isopropylacrylamide copolymerized with acrylic acid (P(NIPAAm-co-AAc)) to coat the herniated bowel through the use of a fibrin adhesive (Beriplast®).

Study design: Gastroschisis was created in fetuses of 31 pregnant Sprague–Dawley rats by evisceration of the bowel through a right paramedian incision in the abdominal wall on day 18.5 of pregnancy. The fetuses were separated in four groups of 12 fetuses: control (C), gastroschisis (G), gastroschisis + fibrin adhesive (GA) and gastroschisis + fibrin adhesive + dry hydrogel (GAH). Animals were harvested at day 21.5 of pregnancy and the hydrogel was removed. Fetuses and bowels were weighed and morphometric analysis was performed. Isoelectric focusing of the amniotic fluid determined its electrical charge. We evaluated the hydrogel swelling ratio (Q) in the amniotic fluid. Histological analysis and scanning electronic microscopy (SEM) of the bowel and hydrogel were performed. Our primary outcome was bowel intactness after hydrogel removal and our secondary outcome was the effectiveness of the hydrogel in protecting the bowel against amniotic fluid and its components. Differences among the groups were tested by the ANOVA and Tukey–Kramer post-test method and the statistical significance accepted was for p values <0.05.

Results: The mass of swollen hydrogel was 34 times the mass of dry hydrogel. Isoelectric focusing of the amniotic fluid showed that most of its proteins are negatively charged as the hydrogel. SEM showed that removal of the hydrogel did not damage bowel serosa. Bowel weight, diameter and wall thickness were similar between groups C and GAH but bowel diameter and wall thickness was significantly reduced in C and GAH compared to G and GA ($p < 0.001$).

Conclusion: The P(NIPAAm-co-AAc) hydrogel does not harm the bowel and provides a safe effective protection with reduction of bowel damage in gastroschisis.

1. Introduction

Gastroschisis is a congenital abnormality in which the bowel herniates into the amniotic cavity and is exposed to amniotic fluid and its components [1]. Exposure of the bowel to amniotic fluid leads to morphological and histological changes of the intestinal wall causing bowel hypomotility and absorption deficiencies [2]. These deficiencies require the use of prolonged parenteral nutrition which increases the frequency of postoperative complications as well as morbidity, mortality and healthcare costs [3,4].

Protection of the bowel from the amniotic fluid and its components during gestation could decrease the frequency and severity of postoperative complications and could be performed using biomaterials like hydrogels. The suitability of mechanical and physical properties of the hydrogel for this use depends largely on its flexibility after hydration and resistance to compression [5].

A well studied thermo-responsive polymer is poli(N-isopropylacrylamide) (p(NIPAAm)) [6]. Based on these properties, we have developed nets of P(NIPPAm-co-AAc) that could work as a biomaterial for surgery, since when swollen they are soft and, because of the high amount of absorbed water, it presents

☆ This study was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) project 04/11204-4, 04/11204-4 and grant 08/51487-9 and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

* Corresponding author at: Experimental Laboratory of Fetal Surgery "Michael R. Harrison", Pediatric Surgery, Department of Surgery, Faculty of Medical Sciences, University of Campinas-UNICAMP, Rua Alexander Fleming, 40, Cidade Universitária "Zeferino Vaz", Campinas, SP 13083-970, Brazil. Tel.: +55 19 3521 9450; fax: +55 19 3521 9450.
E-mail address: sbragia@fmrp.usp.br (L. Sbragia).

0933-3657/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ejogrb.2009.10.009

biocompatibility. Our aim was to develop a novel strategy to reduce inflammation in experimental model of gastroschisis using a P(NIPPAm-co-AAc) hydrogel to coat the exposed bowel loops.

2. Materials and methods

Our primary outcome was to observe the hydrogel biocompatibility inside the womb and, consequently, the bowel intactness after removal of the hydrogel. Our secondary outcome was to observe the ability of the hydrogel in protecting the bowel loops from the amniotic fluid and its components and to compare intestinal damage with and without hydrogel protection.

2.1. P(NIPAAm-co-AAc) hydrogel preparation for bowel loops coating

P(NIPAAm-co-AAc) hydrogels were prepared as described elsewhere, modified according to the amniotic fluid pH (9.0) [7]. Hydrogel discs 1 cm in diameter, weighing approximately 5 mg, cut from 10 cm × 15 cm membranes with a thickness of ca. 0.4 cm were used.

2.2. Determination of the swelling ratio (Q)

In order to evaluate the biocompatibility of the hydrogel and the amount of space it would occupy inside the womb to allow fetal movement we calculated the swelling ratio. The swelling ratio of the P(NIPAAm-co-AAc) hydrogel (Q) in the amniotic fluid was calculated as the ratio of weight of swollen hydrogel (Ws), by the weight of dried hydrogel (Wd) in amniotic fluid (pH 9.0). Hydrogel samples were immersed in amniotic fluid harvested at 17.5, 18.5, 19.5, 20.5, and 21.5 gestational days from rats, kept at 25 °C for 2 days, until the swelling equilibrium was reached and subsequently weighed.

2.3. Assessment of the electrical charge of the amniotic fluid by isoelectric focusing (IEF)

Since components of the amniotic fluid are responsible for the intestinal damage in gastroschisis and these components are electrically charged, we assessed the ability of the hydrogel, which is negatively charged, to attract or repel these components by evaluating the electrical charge of the proteins in the amniotic fluid through IEF.

The amount of total protein in the amniotic fluid was measured as described by Bradford and Rapid [8]. IEF is a high resolution electrophoresis that allows protein separation in a pH gradient until they reach the final position where the net charge is zero, i.e. its isoelectric point (p*I*). An electrophoresis system (PhastSystem) with a homogeneous polyacrylamide gel PhastGel IEF 3–9 (Amersham Biosciences®) was used. A 3–9 pH gradient was created by using stable ampholytes (Pharmalyte®) isoelectrically charged. The gel run was performed at 15 °C, and 2.5 mA during approximately 30 min. The gel was revealed with silver nitrate.

2.4. Surgical procedure

This study was approved by the Ethics Committee on Animal Experimentation at the State University of Campinas (research project no. 1452-1). Thirty-one pregnant Sprague–Dawley rats (vaginal plug sperm ED0; term, 22 days) received food and water *ad libitum* and were kept in a 12 h day–night cycle. The gastroschisis model used was the one described by Correia-Pinto et al. [9]. Four groups with 12 fetuses each were created. Group C: Control fetuses were left undisturbed; Group G: Gastroschisis fetuses; Group GA: Gastroschisis fetuses plus fibrin adhesive (Beriplast®) and Group GAH: Gastroschisis fetuses plus fibrin adhesive (Beriplast®) and hydrogel.

Hydrogel discs 1 cm in diameter were adhered to the herniated bowel loops of the fetuses by using a surgical adhesive based on the polymerization of fibrinogen into fibrin. In order to promote the polymerization reaction only after the placement of the hydrogel disc on the herniated bowel loops, 15 μL of $CaCl_2$ solution were initially placed on the herniated bowel surface (Fig. 1D) using a micro-pipette. Immediately after placing the $CaCl_2$ solution, a fibrinogen solution was placed on one of the surfaces of the hydrogel disc. The disc was then immediately placed on the herniated bowel loop which had received the $CaCl_2$ solution

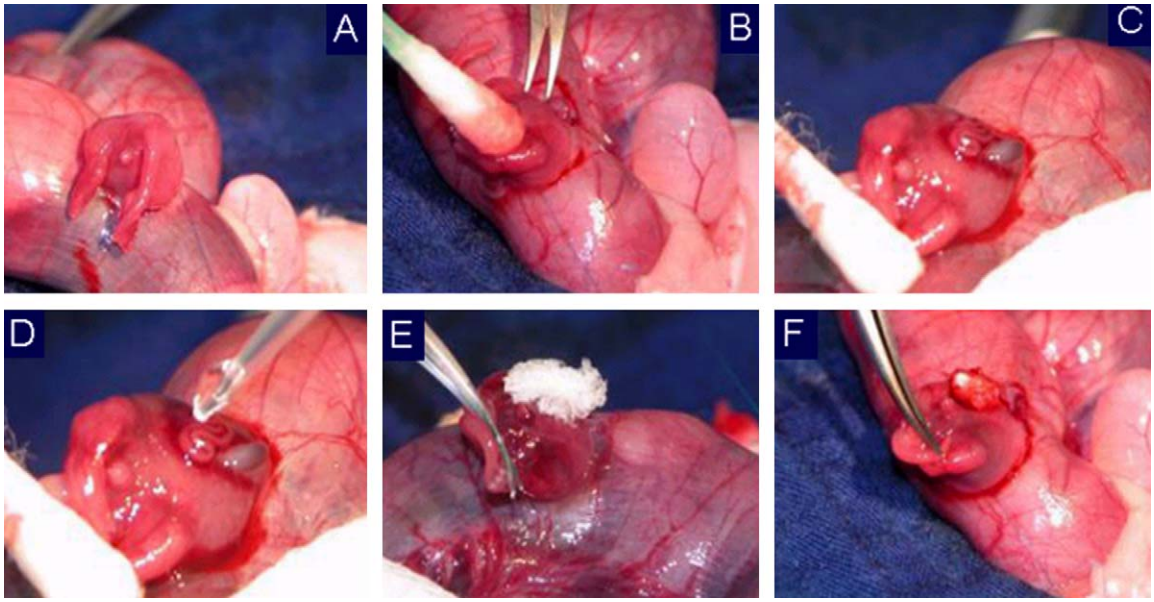

Fig. 1. Surgical procedure performed in the fetus at 18.5 days of gestation. (A) Exposure of the lower body of the fetus after opening of the womb and amniotic cavity, (B) incision at the right of the fetal umbilicus for bowel protrusion, (C) exposure of the fetal bowel through the incision in the gastroschisis group, (D) placement of the fibrin adhesive over the exposed bowel in the GA and GAH groups, (E) placement of the hydrogel over the bowel covered with fibrin adhesive in the GAH group and (F) adhesion and swelling of the hydrogel over the bowel.