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Original research

Prevention by rat amniotic fluid of adhesions after laparatomy in a rat model

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ABSTRACT

Background: Adhesion formation after abdominal surgery (especially multiple operations) is still a major cause of morbidities such as infertility, pain, bowel obstruction, difficult reoperation and other complications. Our aim was to investigate the ability of rat amniotic fluid to prevent adhesion formation after laparatomy in rats.
Methods: This experimental trial was conducted in 20 pregnant rats randomly assigned to two groups of 10 animals each. Measurable serosal and intestinal injuries were created with a standard technique. Rats in both groups underwent laparatomy and hysterectomy. Then amniotic fluid was poured into the abdominal cavity of animals in the case group. All animals were operated on 4 weeks after initial surgery to assess adhesions, which were scored by an examiner who was blind to the animals' group assignment.
Results: The frequency of severe adhesions was 30% in the control group and 0% in the intervention group. Total adhesion scores were significantly lower in the amniotic fluid treatment group than in the control group ($P = 0.002$).
Conclusions: Rat amniotic fluid can decrease the likelihood of post-operative intraperitoneal adhesion formation.

1. Introduction

Adhesion formation, the joining of two normally separate surfaces, causes major complications and morbidities after surgical procedures.[1] It can cause severe morbidities such as infertility, pain, bowel obstruction and subsequent intra-operative complications.[2,3] Adhesions also affect surgical outcomes because they can cause intra- and post-operative morbidities and decrease fertility in women.[4]

Peritoneal adhesions are fibrous bands between organs that are normally separated, or between organs and other areas such as internal body surfaces. Many intra-abdominal adhesions are the result of trauma or injury caused by a surgical procedure or intra-abdominal sepsis. Cadaver studies showed adhesions in 67% of patients with prior procedures and in 28% with a previous intra-abdominal infection. Abdominal adhesions are the most common cause of bowel obstruction (65%–75%), and lower abdominal procedures have a higher chance of producing adhesion and obstruction.[5] Thus, preventing adhesion formation is one of the most important concerns of all surgeons.[6] In our experience with different surgical procedures we have observed that in women who undergo a second operation after a previous cesarean delivery, the degree of adhesion formation is significantly decreased or even absent. This led us to hypothesize that the amniotic fluid that bathes the abdominal cavity during cesarean delivery may be responsible for reducing adhesion formation.

Factors such as hyaluronic acid and growth factor may be responsible, through different mechanisms, for the ability of amniotic fluid to prevent and treat adhesion formation. One mechanism may be by acting as a mechanical barrier between two injured peritoneal surfaces.[7,8] In the present study we evaluated the efficacy of rat amniotic fluid in preventing adhesion formation in an animal model.

2. Methods

The study was an experimental randomized controlled animal trial. A total of 20 pregnant female (gestational age 14 ± 2 days) Wistar-Albino rats weighting 250–300 g (mean 275 g) were randomly assigned to two groups of 10 animals each in accordance with the guidelines of the Animal Ethics Committee of Shiraz University of Medical Sciences. All surgical procedures were performed by the same surgeon. Each rat was anesthetized with ketamin hydrochloride (50 mg/kg i.m.), and before the incision, the abdomen was shaved and prepared with povidine iodine solution with a sterile technique. The abdominal cavity was opened via a 4-cm vertical midline incision. First hysterectomy was done and then multiple measurable

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Table 1
Adhesion scoring system according to Canbaz and colleagues.[9]

Degree of adhesion	Number of adhesion bands
0	No adhesion
1	One adhesion band, no vessel, easily separated
2	Two thin adhesion bands, no vessel, easily separated
3	Three thin adhesion bands, no vessel, easily separated
4	More than three thin adhesion bands, easily separated with no vessel or diffuse adhesion bands with vessels

abrasions were made on the different site of peritoneal surface and small intestine by nife. Care was taken to avoid gross bleeding from the injured sites. In the study group about 3–4 mL of the amniotic fluid from each rat was poured in to the abdominal cavity. Then the abdominal cavity in both groups was closed in a double layer with continuous suturing (muscle and peritoneum with 4-0 chromic suture, fascia and skin with 4-0 nylon suture). After that the rats were housed under controlled temperature conditions (21 ± 2 °C) and a 12/12 light/dark cycle with food and water available. After 4 weeks the animals were killed by KCl injection to evaluate adhesion formation.

The extent and severity of adhesions at the incision site, between bowel loops and between bowel loops and the peritoneal surface were evaluated with an established scoring system which it explain in Table 1.This scoring system utilized in different article for scoring of adhesion band. The investigator (surgery resident) who scored the adhesions had no knowledge of the animals' group assignment. The significance of the difference between the two groups was calculated with the Mann–Whitney U test Figs 1 and 2.

3. Results

All rats recovered and resumed their preoperative health and activities after surgery. No residual viscous or ascites fluid was observed in the abdominal cavity. The number of adhesions, their severity and total adhesion scores are shown in Table 2. In the intervention group, entry to the abdominal cavity was straightforward because there were no adhesions around the laparotomy incision. No score-4 adhesions were found between the bowel loops.

The statistical analysis showed significant differences between the two groups (Mann–Whitney U = 11, asymptomatic significance = 0.002, exact significance = 0.002, $P = 0.001$). Significantly fewer and significantly less severe adhesions were found in the intervention group.

4. Discussion

We tested the ability of rat amniotic fluid to inhibit the formation of peritoneal cavity adhesions, and found that the prevalence of adhesions and their severity were significantly lower in the intervention group than in the control group ($P = 0.002$). Postoperative adhesion formation has a considerable effect on quality of life and health. One of the greatest problems of gynecological and abdominal surgery is pelvic adhesions, and their prevention is of great concern to all surgeons. Different types of adhesion-reducing substances have been used in animal models but we are still far from the ideal substance.[10]

Some materials such as hyaluronic acid have yielded good results in reducing intrauterine adhesion formation.[11] Unfortunately, many of these agents are expensive and not readily available. An important factor in adhesion formation is collagen deposition, and materials such as d-penicillamine favor the prevention of adhesion band formation. This drug keeps the fibers loose and unstable so that they cannot perform their binding role in the absence of collagen fiber cross-linking.[11]

Physical barriers are another effective method of preventing adhesions. A randomized clinical trial involving11 centers in the USA

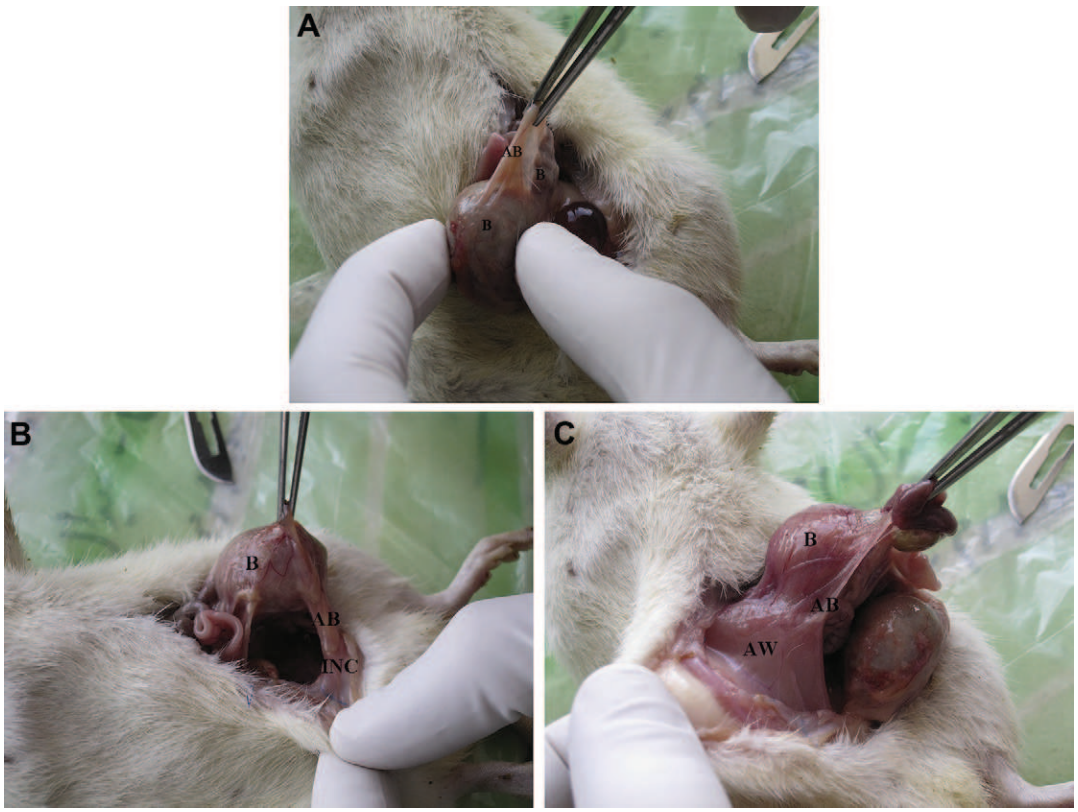

Fig. 1. Adhesions of bowel loops to each other and to other organs in the control group. A. Adhesion bands (AB) between bowel loops. B. Adhesion bands between bowel loops and the site of incision (INC). C. Adhesion bands between bowel loops and the abdominal wall (AW).

Fig. 2. No adhesions of bowel loops to each other, to the abdominal wall or to the site of incision in the intervention group.

demonstrated that the bioresorbable barrier Seprafilm R (Genzyme Corporation, Cambridge, MA) was effective in decreasing adhesions only at the site of application.[12] However, its efficacy in reducing adhesions in areas other than the site of use is unclear.[13] Therefore a physical barrier used in a limited area may not completely eliminate adhesion formation. In contrast, systemic drugs can reduce adhesions throughout the abdomen cavity and are therefore likely to be more effective than purely physical barriers.[11]

We use a safe, natural material to prevent adhesion formation, but at this time we can offer no explanation for its mechanism of action. One hypothetical mechanism is that amniotic fluid acts as a mechanical barrier between injured peritoneal surfaces.[14] Its healing characteristics may also be effective; for example, the amniotic fluid environment may act in scar-healing in the fetus.[7]

This fluid contains many factors such as hyaluronic acid(HA) and growth factor which may be responsible for this characteristic.[8] HA is glycosaminoglycan and a component of the Extracellular matrix, including connective tissue, cartilage, skin, vitreous and synovial fluids. HA is biocompatible, nonimmunogenic, nontoxic, and soluble.[17]

HA cover serosal surfaces and causes protection of serosal surface.[18] Because of the high content of HA in fetal wounds, it reduces collagen deposition and therefore lead to less scar formation.[19] This suggestion is similar to the research of West et al., who showed removal of HA results in fibrosis and scar formation.[20]

One of the mechanism of HA is decreasing the activity and proliferation of fibroblasts, and prevention of fibrin deposition on the injured serosal surfaces, and finally inhibit migration of inflammatory cells and cellular elements during repair.[21]

In orthopedic surgery human amniotic fluid is used immediately after tenorrhaphy to successfully prevent peri-tendinous adhesion formation in a rabbit model.[15] Bovine amniotic fluid may also be efficacious in burns.[16]

Another question we cannot answer at present is which component or components of amniotic fluid reduce the strength or number of the adhesion bands. Longer follow-up and further histological evaluations of tissue samples are required to answer these questions.

Table 2
Severity and total scores for severity of adhesions in rats subjected to surgery with (Case) and without (Control) intraperitoneal lavage with amniotic fluid.

Degree	0	1	2	3	4
Control	1	3	3	1	2
Case	7	3	0	0	0

In comparison to synthetic drugs, amniotic fluid has no side effects. In addition, no sign of evisceration or dehiscence were found after the use of amniotic fluid. We therefore suggest that if amniotic fluid is used to prevent adhesions in humans, it could be collected at birth and deposited in amniotic fluid banks for use in surgery. Another suggestion is to analyze amniotic fluid to identify the component or components that effectively inhibit adhesion band formation. These components could then be used as safe materials to prevent adhesions.

We were unable to find any published studies that evaluated the use of rat amniotic fluid to prevent adhesions. Our review of the literature from 1972 till now using different keywords returned no articles on amniotic fluid and adhesion formation similar to our study. In other words, the potential uses of amniotic fluid in surgery have not been studied widely, and this made it difficult to compare our results with those of other studies. Our study in a rat model of postsurgical adhesion formation and prevention approaches adhesion prevention from a new perspective that aims to test whether the animal's own amniotic fluid can decreases the rate and severity of post-operative adhesions.

In conclusion, this experimental study investigated the prevention of post-operative adhesion formation by rat amniotic fluid. Additional studies will be needed to ascertain whether amniotic fluid is effective in long-term adhesion reformation. Finding an effective agent to decrease adhesion formation would improve the post-operative course for surgical procedures with a high risk of adhesion formation.

Conflict of interest

The authors report no conflicts of interest.

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Ethical approval

The article is in accordance with the Animal Ethics Committee for the Shiraz Medical University.

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Author contribution

1. Sedigheh Tahmasebi: Study design.
2. Mehdi Tahamtan: Writing and editing.
3. Yahya tahamtan: Calculating and analyzing the data.

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