

Original Article**Effect of D-penicillamine in prevention of post operative pelvic adhesion formation in rat***Ata-Allah Ghahiri**, *Elahe Zarean***, *Mehdi Rasti****, *Shahriar Adibi*******Abstract**

INTRODUCTION: Adhesion formation after pelvic surgery is still a major cause of several morbidities such as infertility, pain, bowel obstruction and subsequent intra-operative complications. Our aim was to investigate the effects of d-penicillamine in preventing adhesion formation in rat uterine horn.

METHODS: This experimental trial was conducted on 30 rats. The rats were randomly assigned to two groups of 15. Measurable serosal injury was created using a standard technique. The rats in one of the two groups were given 17 mg/kg of d-penicillamine. All of the animals were operated on 4 weeks after surgery and adhesions were assessed and scored by an examiner who was blind to the test.

RESULTS: Frequency of severe adhesions in control and case groups were 76 and zero percent, respectively. Total scores of adhesions were found to be significantly reduced in the d-penicillamine treatment group when compared with control group (P = 0).

CONCLUSION: This study showed that penicillamine can decrease the chance of post-operative intra-peritoneal adhesion formation.

KEY WORDS: Adhesion formation, d-penicillamine, pelvis.

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Adhesion formation, the joining of two normally separate surfaces due to trauma or inflammation, can result in complications following surgical procedures¹. It is still a major cause of several morbidities such as infertility, pain, bowel obstruction and subsequent intra-operative complications^{2,3}. Pelvic adhesions are one of the major factors which significantly and adversely affect surgical outcomes due to intra- and post-operative morbidity and reduce future female fertility⁴. Thus, prevention of post-operative adhesion

formation is an issue of fundamental concern to the reproductive surgeon⁵.

Treatments of pelvic adhesions and their associated morbidities contribute to a significant portion of health care expenditure. In US hospitals, admissions related to adhesion-associated morbidities and surgical treatments of adhesions accounted for \$1.33 billion of health care expenditure in 1994⁶. In Sweden, a postal study administered via mail to all surgeons found the annual cost of treating adhesions in the whole country to amount to \$4.1 million⁷.

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Numerous adjuvants, including fibrinolytic agents⁸, crystalloid solutions, corticosteroids, heparin^{2,9}, hyaluronic acid^{10,11}, hydrogen¹², vitamin E¹³, and non-steroidal anti-inflammatory agents¹⁴ have been used for prevention of this complication. Intra-abdominal crystalloids and dextran have not been shown to be effective, while corticosteroids and anti-inflammatory agents have yielded inconsistent results¹⁵. Although non-steroidal anti-inflammatory drugs yielded good results in rodent and rabbit models^{16,17}, they were administered intra-peritoneally, a route which reduces the clinical applicability of these agents due to uncertainty about dosing and delivery¹⁸. Hyaluronic acid and polyethylene glycol/poly lactic acid barrier films reduce adhesion formation experimentally, as well as clinically^{19,20}. However, concerns about infection, intra-abdominal placement, and impaired healing of anastomoses remain²¹. Some agents like vitamin E had no effect in reduction of adhesion formation²². Calcium channel blockers show some potential favorable influences on peritoneal repair²³. Injury or inflammation of a serosal surface generally initiates the adhesion formation process²⁴. It has been shown that leukocyte-dependent inflammatory reactions may promote cellular and tissue injury and increase the use of melatonin, which is possibly the most powerful free radical scavenger².

Collagen cross-linking is known to form fibrils and fibers of collagen. The enzyme responsible for this step, lysyl oxidase, requires copper and can be inhibited by collagen cross-link inhibitors such as β aminopropionitrile (BAPN) and d-penicillamine²⁵.

In the present study, we used penicillamine to prevent adhesion band formation because it can inhibit cross-linking of collagen fibers (collagen is an essential element in adhesion bands). It also has anti-inflammatory and immunosuppressive properties which reduce fibroblast proliferation²⁶. We postulated that this medicine may reduce fibrin deposition and band formation.

Our review of literature from 1970 to date using different keywords returned no articles on d-penicillamine and adhesion band. Hence we were not able to compare our results with other studies. In other words, this article addresses adhesion prevention from a new perspective and intends to ascertain if penicillamine decreases the rate of adhesions in the post-operative period in animal models.

Methods

This study was an experimental double-blind randomized controlled animal trial. A total of 30 female Wistar-Albino rats weighting 190-200 grams (mean of 200 ± 30 grams) were randomly assigned to two groups of fifteen. Surgical procedure was performed by one surgeon for all subjects. During the procedure, each rat was anesthetized with ketamin hydrochloride (40 mg /kg/IM) before incision, the abdomen was shaved and prepared with povidine iodine solution using sterile technique, and the abdominal cavity was entered via a 4 cm vertical midline incision. Then, three 1-2 cm abrasions were made on the uterine horn and traumatized in 10 spots on bilateral pelvic side wall. Manipulation of other tissues was minimized and care was taken to avoid gross bleeding from the injured sites. At the end of the procedure, the abdomen was closed in double layer in a continuous method (muscle and peritoneum with 4-0 chronic suture, fascia and skin with 4-0 nylon suture). All rats were housed under controlled temperature (22 ± 20 °C) and 12/12 light/dark cycle with food and water.

In the study group, rats were given 17 mg/kg d-penicillamine (as 250 mg capsules made by Rubio factory, Spain) daily with their foods through feeding tube²⁶. Each capsule was solved in 35 cc of water. They received the medication for 10 days starting one day before operation. In the control group, the rats were given nothing but food and water.

After four weeks, long enough a period for adhesion formation, the animals were terminated and evaluated for adhesion and scoring. The extent and severity of adhesions in the op-

eration site for each uterine horn were evaluated using an established scoring system shown in table 1²⁷. The investigator (resident of gynecology) who performed the adhesion scoring had no knowledge of the subjects' grouping. Statistical comparison of the two groups was performed using Mann-Whitney U test.

Results

All rats were recovered without incident and resumed their preoperative physical activities and feeding, post-operatively. No residual viscous fluid or ascites was seen following laparotomy. The number of adhesion bands and the severity and total scores of adhesions are

shown in table 2. Severe adhesions were seen around the uterine horn and urinary bladder in the control group. Adhesions were found even around the intestine. In the case group, however, the entrance to the abdominal cavity was very easy because no adhesion was present around the laparotomy incision. Also, no adhesion was present between the uterine horn and other organs in severe pattern (score 4), as seen in figures 1 and 2.

Statistical data analysis showed significant difference between the two groups (Mann-Whitney U = 10, asymptomatic significance = 0, exact significance = 0 and P = 0). Adhesion band formation was significantly less severe and in lower grades in the study group.

Table 1. Adhesion measurement score described by M. Ahmet Canbaz

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 defused adhesion bands with vessels

Table 2. Severity and total scores of adhesions

Grade	0	1	2	3	4
Cases	4	8	2	1	0
Controls	0	1	1	2	11



Figure 1. Adhesion of uterine horn (UT) to other organs in the control group

A- Adhesion of uterine horn to mesentery (M) and bladder

B- Adhesion of uterine horn to intestine (INT)

C- Severe adhesion at laparotomy incision

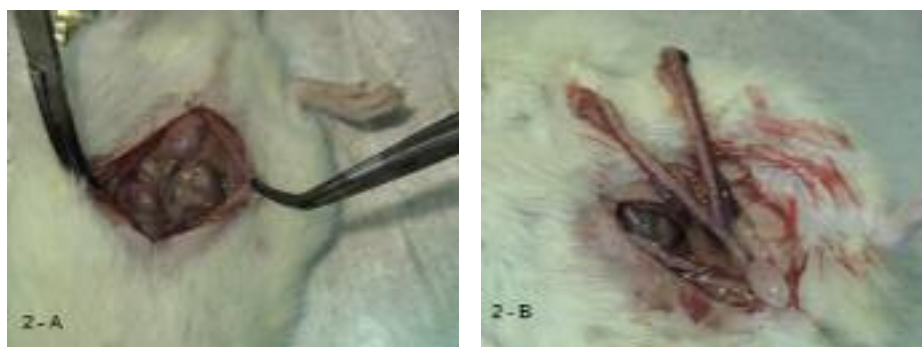


Figure 2. No adhesion is present between uterine horn and other organs in the experimental group

Discussion

Pelvic adhesions are truly the nemesis of gynecologists and abdominal surgeons and also troublesome from many aspects. Indeed, prevention of post-operative adhesion formation is an issue of fundamental concern to surgeons. A wide variety of adhesion-reducing substances have been evaluated in animal models, yet we are still far from the ideal adhesion preventing agent¹¹. In the best scenario, we can only reduce the rate of adhesion formation.

Some materials have produced good results; for example, one study showed that intrauterine application of auto-cross linked hyaluronic acid decreases intrauterine adhesions²⁸. Nonetheless, many of these agents are expensive and not readily available. Because collagen deposition has an important role in adhesion band formation, we used one of the major side effects of d-penicillamine as a favorable phenomenon for inhibition of adhesion band formation. Fibers become very unstable and cannot play a banding role in the absence collagen fiber cross-linking. As seen in figures, the prevalence of adhesion bands and their thickness were significantly less in the study group than in the control group ($P = 0$).

Physical barrier is also effective in preventing adhesions; for example, a multi-center double blind prospective randomized clinical trial performed at 11 centers, including Boston University Medical Center, showed that bioresorbable physical barrier seprafilm R (Genzyme Corporation, Cambridge, MA) was

efficacious in reducing adhesions in the site of application²⁹. Despite this significant advance in adhesion reduction, it is unclear whether physical barrier can provide protection in areas other than the site of application³⁰. A physical barrier alone applied to one area, may not completely eliminate adhesion formation. A systemically-administered drug which can reduce adhesions in all parts of the abdomen will obviously provide a better physical barrier.

The most important limitation in our study was the number of cases. Also, we were not able to evaluate the side effects of the drug on other parts of body. At this stage, we are not clear as to whether penicillamine at the administered dose can delay the wound healing process in the abdominal wall and increase the chance of dehiscence or abdominal wall hernia.

We did not observe any cases of evisceration or dehiscence. Another question we cannot answer at present is whether d-penicillamine decreases the tensile strength of adhesion bands, the number of bands, or both. Longer follow-up periods and histological evaluation of tissue samples are needed to address these questions.

The side effects of the drug are acceptable. A large number of studies have been published on the side effects of d-penicillamine; GI disturbances including anorexia, nausea and vomiting may occur. Hematological side effects including thrombocytopenia and less frequently leukopenia are usually reversible²⁶.

Many of these side effects do not occur at the mentioned dose, but the major effect of this drug which is the prevention of collagen cross-linking, has been observed at low doses³¹.

We were unable to find any published studies evaluating the use of d-penicillamine in prevention of adhesion formation. In conclusion, this experimental study seems to be the first on prevention of post-operative adhesion formation by d-penicillamine. Further studies with d-penicillamine on human subjects, as well as studies comparing this drug with other agents found effective in preventing adhesion formation are warranted. In addition, studies must be carried out to ascertain whether d-

penicillamine affects long-term adhesion reformation.

Surgical principles such as gentle handling, bleeding control, and avoidance of foreign materials are obviously of great importance in preventing adhesions. Finding an agent effective in reducing adhesion formation is bound to provide a boost to surgical procedures carrying this risk.

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