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Original Article

Hydrogen sulfide prevents postoperative adhesion in a rat uterine horn model

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ABSTRACT

Objective: Abdominal adhesions are primarily severe postoperative complications that can cause gynecological problems such as infertility and chronic pelvic pain. Inflammatory mediators are significantly related to adhesion formation, and hydrogen sulfide plays a significant anti-inflammatory role in multiple physiological processes. Therefore, the effect of NaHS, a hydrogen sulfide donor, on postoperative adhesion formation was examined in a rat uterine horn model.

Materials and methods: A rat uterine horn model was created to evaluate whether NaHS, a hydrogen sulfide donor, could decrease postoperative adhesion formation. Rats were randomly grouped and administrated with different doses of NaHS, where DL-propargylglycine and low-molecular-weight heparin acted as negative and positive controls, respectively. The extent and severity of adhesions were assessed on the 14th postoperative day. Serum of rats was sampled for the determination of 27 cytokines using a chip.

Results: The severity and total scores of adhesion in rats given 112μM/kg and 56μM/kg NaHS were significantly less compared with those of the control group ($p < 0.01$). Scores for the extent of adhesion re-formation in the DL-propargylglycine and control groups did not differ ($p > 0.05$). At least six cytokines were involved in the procedures for the prevention of adhesion formation, as they varied significantly among different groups.

Conclusion: Administration of NaHS could apparently reduce postoperative adhesion in the rat uterine horn model. This preventive effect may be associated with the variation of cytokine that is related to inflammatory.

Introduction

Abdominal adhesions are primarily severe postoperative complications, developed by internal scars that misconnect tissues when organs are handled and shifted temporarily from their normal positions after laparotomy. Adhesion-related twisting and pulling of internal organs can result in quite a lot of common complications such as abdominal pain or intestinal obstruction, as well as gynecological problems such as infertility and chronic pelvic pain [1,2].

An unfortunate fact is that adhesions are difficult to prevent and unavoidable in surgery, and currently the main treatment for adhesions is mostly through surgery. Inflammatory reactions are generally assumed to play an important part in abdominal adhesions, despite the fact that their pathogenesis has not yet been revealed. Many important substances are involved in adhesion formation. For instance, certain mediators, such as interleukins and transforming growth factors, increase the formation of adhesions through decreasing the fibrinolytic capacity of the peritoneum [3,4]. To the best of our knowledge, some of the drugs were reported to effectively suppress inflammatory reactions through inactivation of the nuclear transcription factor (NF)-κB signaling pathway and subsequently prevent abdominal adhesions [4,5].

Hydrogen sulfide (H_2S), originally considered to be a malodorous and toxic gas, is produced endogenously from cysteine

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by cystathionine-β-synthase, cystathionine-γ-lyase, and 3-mercaptopyruvate sulfurtransferase. A rapidly expanding body of studies has reported the critical role of H_2S in a multitude of pathophysiological processes, including maintenance of cardiovascular homoeostasis [6–8], oxidative stress, mitochondrial function, inflammation, apoptosis [9,10], vasodilatation, angiogenesis, ion channel signaling, and interaction with NO. Although H_2S has a significant anti-inflammatory effect in a multiple physiological processes, and inflammatory mediators are remarkably related to adhesion formation, a potential effect of H_2S on abdominal adhesions has not been investigated. Thus, a rat uterine horn adhesion model was prepared to explore the effect of H_2S on adhesion prevention. The antiadhesion effect of NaHS on the rat uterine horn adhesion model was investigated, with the low-molecular-weight heparin (LMWH) as a positive control and DL-propargylglycine (PAG) as the inhibitor. The standard score system was used to evaluate the adhesion on the rat uterine horn adhesion model, and the effect of H_2S on the animal model was further examined by scores of adhesion. Moreover, to test the connection between the inflammatory process and the effect of H_2S in the model, the serum of rats was sampled for cytokine detection by a chip. As a result, we first found that H_2S reduced postoperative adhesion in the rat uterine horn model through its interaction with specific inflammatory mediators.

Materials and methods

Animals

All procedures during the feeding and experiment complied with the Regulations of Experimental Animal Management and Care of Fudan University, and approval was obtained from the Institutional Review Board before conducting the study.

For this study, 64 nonpregnant 8-week-old female Wistar-Albino rats of special pathogen free grade (SPF grade), weighing 180–210 g, were provided by the Animal Center of the Pharmacy School, Fudan University. They were used as model animals for the experimental induction of postoperative intra-abdominal and uterine horn adhesions. Prior to and after the surgery, the animals were housed in cages (4 per cage) with free access to water and food in an SPF environment. They were kept under controlled conditions of temperature (21–24°C), humidity (40–60%), and light (12-hour light/12-hour dark regime).

Prior to the induction of the animal model, the rats were randomly assigned to eight groups, with each group containing eight rats. All the groups were given 2 days of continuous medication in advance before the day of model induction. The normal group, the model group, and the sham-operation group received physiological saline before the surgery. Three groups for model induction were given different concentrations (112μM/kg, 56μM/kg, and 28μM/kg) of NaHS solution by hypodermic injection (i.h.). One of the groups for model induction received LMWH of 1.5 mg/kg (i.h.). The last group was applied with PAG of 10 mg/kg (i.h.).

Establishment of animal models

After 2 days of different medications in these groups, a modified rat uterine horn adhesion model was used to induce intra-abdominal adhesion formation [11,12]. All the operations were performed by the same person who was blind to animal allocation. All the rats were fasted for 12 hours before the operation. Each rat was anesthetized with chloral hydrate (7%, 0.4 mL/100 g, intraperitoneal injection) and fixed in the supine position for surgery. The operation was limited to 10 minutes for each rat to control the effect of room-air tissue drying, and handling of other tissues was minimized and care taken to avoid gross bleeding from injured sites. The middle lower abdomen of each rat was shaved and sterilized with iodine solution. A lower midline vertical incision, approximately 3 cm in length, was made. The peritoneal cavity was kept moist with copious amounts of saline solution throughout the surgery. Both peritoneal sidewalls were scraped using blades 10 times, taking care not to harm the retroperitoneal structures except mild bleeding. Unipolar electrocautery at a temperature of 380°C was used to traumatize the antimesenteric surface of the bilateral uterine horns at nine to 10 spots. The scraped peritoneal sidewalls and the cauterized uterine horn were then sutured approximately, from the top and bottom ends, at intervals of about 1 cm. The midline incision was closed with two layers of 4/0 Prolene sutures.

The sham-operated group, which contained eight rats, was prepared using the same procedures as those conducted for the model groups, except for the nondamage to the tissues by unipolar electric cautery and blades.

Scores standards

To ascertain the objectivity and impartiality, the scoring criteria of Linsky et al [13] and Knightly et al [14] were modified and applied for the evaluation of adhesion formation. The severity of adhesions to the uterine horn was measured as follows: 0, no adhesion; 1, tiny filmy adhesions, easy to separate without tension or injury of the involved tissues; 2, mild adhesion, moderate force for separation; 3, dense adhesion, which leads to serosal injury during lysis or needs to be divided with scissors; and 4, severe adhesion, cohesive attachment of the uterine horn to the ipsilateral abdominal sidewall. Scores for the extent of adhesions was evaluated (characterized, accessed) as follows: 0, no uterine adhesion; 1, 1–25% of traumatized area; 2, 26–50% of traumatized area; 3, 51–75% of traumatized area; and 4, 76–100% total involvement. The sum of both parameters was used as the overall score for each uterine horn.

Score and cytokine determination

After model induction, all the rats of the different groups continued to be administered the daily doses in the same way as that in the initial period, until the second laparotomy. No antibiotic prophylaxis was applied during or after the surgery.

Two weeks postoperation and after the administration of different experimental agents, the rats were anesthetized with chloral hydrate (7%, 0.4 mL/100 g, intraperitoneal injection) and fixed in the supine position for second laparotomy. Then, the abdominal wall scar was examined. Adhesions of the peritoneal sidewall with the uterine horn were scored according to the scoring system, which was performed by a person who was unaware of the allocation and medication of the rats.

The blood was collected through the aorta abdominalis after scoring the adhesions during the second laparotomy. Different batches of serum were prepared through centrifugation of these clotted blood, and stored in a refrigerator at −20°C. Measurement of cytokines inside the serum was performed using the determination kit of Raybiotech (Norcross, GA, USA) according to its standard procedure.

Statistical analysis

Statistical evaluation was performed using SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA). Data are expressed as median (min.–max.). Statistical analysis was performed by the Kruskal–Wallis test for multiple independent groups except the normal and sham groups, and by Wilcoxon rank sum test with