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Oral tadalafil reduces intra-abdominal adhesion reformation in rats

Mehmet Serdar Kutuk[1,*], Mahmut Tuncay Ozgun[1], Cem Batukan[2], Bulent Ozcelik[1], Mustafa Basbug[1], and Ahmet Ozturk[3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Erciyes University, Gevher Nesibe Hospital, 38039 Kayseri, Turkey [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Acıbadem University, Istanbul, Turkey [3]Department of Biostatistics, Faculty of Medicine, Erciyes University, Kayseri, Turkey

*Correspondence address. Tel: +90-5424722807; Fax: +90-3522222376; E-mail: mskutuk@erciyes.edu.tr

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BACKGOUND: Currently, there is no ideal agent to prevent adhesion formation. We have shown that sildenafil, a phosphodiesterase-5 (PDE-5) inhibitor, reduces post-operative adhesion formation by vasodilatation and increases fibrinolytic activity. Here, we evaluated whether tadalafil, a long-acting PDE-5 inhibitor, decreases post-operative adhesion reformation in rats.

MATERIALS AND METHODS: Standardized lesions were created in Wistar albino rats by cauterization of uterine horns and abrasion of adjacent peritonium. The extent and severity of adhesions were scored on the 14th post-operative day and adhesiolysis was performed at the second laparotomy. Animals were then assigned randomly into two groups. The study group ($n = 11$) received 10 mg/kg oral tadalafil by gavage 60 min before the second laparotomy and daily for 14 days afterwards. Controls ($n = 11$) received the same volume of tap water for 14 days by gavage. Animals were killed 15 days after adhesiolysis and adhesions were scored blind during the third laparotomy.

RESULTS: Basal adhesion scores at the time of the second laparotomy were comparable in the study and control groups. Scores for the extent of adhesion reformation in the study and control groups did not differ [median 1 (range 0–3) versus median 2 (range 1–3); *P*: 0.81] but tadalafil reduced the respective severity scores [median 0.5 (range 0–1) versus median 1 (range 0.5–1); *P*: 0.02] and total scores [median 2 (range 0–4) versus median 2.5 (range 1.5–4); *P*: 0.042].

CONCLUSIONS: Oral administration of tadalafil during the perioperative period reduces intra-abdominal adhesion reformation in rats.

Key words: adhesion reformation / tadalafil / rat / phosphodiesterase-5 inhibitor / post-operative adhesions

Introduction

Adhesion formation is one of the most common and troublesome complication of pelvic and abdominal surgery that affects 55–94% of patients having open surgery (Drollette and Badawy, 1992), and responsible for 50% of hospital readmissions (Parker *et al.*, 2001). It can also cause late complications, such as female infertility and chronic pelvic pain. Additionally, adhesion formation is the leading factor for the occurrence of intra-operative complications, including organ injury, in subsequent surgery (Dijkstra *et al.*, 2000). Adhesion-related conditions are common and diffficult to treat, and the financial burden of adhesiolysis is very high on healthcare systems (Ray *et al.*, 1998). Therefore, these facts have led to an acceleration of the description of studies trying to prevent adhesion formation. The methods suggested as means for preventing adhesions vary from meticulous surgical handling of tissues and mechanical barriers to intraperitoneal (i.p.) application of profibrinolotic substances and virus-mediated gene transfection (Rodgers *et al.*, 1996; Başbug *et al.*, 1998; Johns *et al.*, 2001; Özçelik *et al.*, 2003; Gutt *et al.*, 2004; Atta *et al.*, 2009). Unfortunately, the elusive nature of adhesion genesis and individual differences regarding tissue repair and fibrosis frustrate efforts to find a universally applicable means to prevent adhesion formation.

Adhesion formation can be defined as a tissue reaction to inciting stimuli, causing mesothelial injury and tissue ischemia that ultimately leads to the formation of fibrinous bands and connections that distort normal anatomy. It starts as a normal protective physiological mechanism to contain inciting stimuli and to repair tissue injury, and culminates in pathological wound healing and fibrosis. It is thought that the disturbed balance between procoagulant and fibrinolytic activity is an important factor determining the outcome of the wound-healing process (Ellis, 1971; Reijnen *et al.*, 2003). At the critical period of inflammation and wound healing, local tissue oxygenation mediated by local hormones and growth factors determines whether adhesion formation occurs.

Experimental studies showed that agents that increase local tissue perfusion, such as nitric oxide (NO), and those enhancing its biological

activity decrease adhesion formation (Kaleli et al., 1998; Ozden et al., 1999). NO is generated by certain NO synthases as a by-product of conversion of L-arginine to L-citruline. NO plays multiple roles in the initiation, maintenance and modification of inflammatory response. At least some of the biological actions of NO are mediated through the stimulation of guanylate cyclase. Subsequent formation of cyclic guanosine monophosphate (cGMP) leads to the activation of protein kinase G (PKG) which is thought to play a central role in NO effect. NO is produced constitutively by endothelial cells and plays an important role in the maintenance of local perfusion owing to its inhibiting effect on platelet adhesion, mast cell degranulation and free oxygen radical production by leukocytes (Kubes et al., 1991; Rodeberg et al., 1995; Lugnier et al., 1999). Aside from its effect on the inflammatory component of tissue injury, it was recently shown that inhibition of phosphodiesterase-5 (PDE-5), which catalyses the breakdown of cGMP, reduces collagen synthesis and induces apoptosis of fibroblasts and myoblasts (Chiche et al., 1998; Sirotkin et al., 2000). The beneficial role played by cGMP against adhesion formation is further supported by the experimental study of Batukan et al. (2007) which showed that the administration of oral sildenafil, a PDE-5 inhibitor, can inhibit adhesion formation in a rat modified uterine horn model (Batukan et al., 2007).

Once formed, adhesions can only be eliminated by operative adhesiolysis and there is a high rate of reformation (Gutt et al., 2004). There is no satisfying hypothesis for how adhesion reformation occurs and which factors determine its occurrence. Whether adhesion reformation goes through the same route as the initial formation and is governed by the same mediators, such NO is yet to be determined.

Although adhesion formation is a somewhat well-studied process, data about the prevention of adhesion reformation are limited. On the basis of these facts, the aim of this experimental study was to evaluate whether tadalafil (Cialis®, Lilly, USA), a long-acting PDE-5 inhibitor, can decrease post-operative adhesion reformation in rats.

Materials and Methods

Twenty-two, 4-month-old female Wistar albino rats weighing 170–210 g were used in this study. During the whole study period, the animals were kept under controlled conditions of temperature (21–24°C), humidity (40–60%) and light (12-h light/12-h dark regime) and fed ad libitum on rat cubes and tap water. All procedures contained in the study were approved by the Animal Care Laboratory of Erciyes University, Turkey, and approval was obtained from the Institutional Review Board before the study.

The study protocol used was adopted from Batukan et al. (2007). Ketamine (10 mg/kg i.p.; Ketalar, Eczacibasi, Istanbul, Turkey) and xylazin (3 mg/kg; Rompun 2%, Bayer, Germany) were used to anesthetize the rats. The lower abdomen was shaved and prepared with povidone iodine solution. Laparotomy was carried out through a lower midline incision, 3–4 cm in length. A modified rat uterine horn adhesion model was used to induce intra-abdominal adhesion formation. The peritoneal sidewalls were scraped and care was taken not to harm the retroperitoneal structures. Unipolar electrocautery was used to traumatize the antimesenteric surface of the ipsilateral uterine horn at 8–10 spots. The scraped peritoneal sidewalls and the uterine horn were then approximated from the proximal and distal ends with 4/0 polypropylene (Prolene, Ethicon, Inc.) sutures. The midline incision was closed with two layers of 4/0 prolene sutures. The abdominal wall scar was examined for 2 days post-operatively. Antibiotic prophylaxis was not administered during or after surgery.

On the 14th post-operative day, the rats were randomly assigned into two groups (Study and Control groups) and a second surgery was performed using the same protocol for entering the abdominal cavity. The first and second surgeries and adhesiolysis were performed by the same operator (M.T.O.) who was unaware of the animal allocation. After the first and second surgeries, the extent and severity of adhesions in the operation field were evaluated by the same observer (C.B.), who was blinded to the treatment regimen. The severity of adhesions were graded modifying the classification of Knightly et al. (1962): Grade 0, no adhesion; Grade 1, tiny filmy adhesions, easy to separate without tension or injury of the involved tissues; Grade 2, dense adhesion, which requires tension to divide them; Grade 3, dense adhesion, which leads to serosal injury during lysis or needs to be divided with scissors; Grade 4 adhesion, where other intra-abdominal organs were involved and a conglomerate was formed. The extent of adhesions across the uterine horn was defined according to the criteria of Leach et al. (1998): 0, no adhesion; 1, 1–25% involvement; 2, 26–50% involvement; 3, 51–75% involvement; 4, 76–100% involvement. The sum of both parameters was used as the overall (total) score for each uterine horn.

Tablets containing 20 mg tadalafil were crushed and suspended in tap water to yield a concentration of 2 mg/ml. The animals in the study group ($n = 11$) were administered 10 mg/kg tadalafil by gavage 60 min prior to the second laparatomy, and daily for 14 days thereafter; the same volume of tap water was administered daily for 14 days by gavage to the control group.

The animals were killed 15 days after adhesiolysis and adhesions were determined and scored by a blinded examiner, according to the aforementioned criteria, during the third laparotomy.

Data are expressed as median (min.–max.) and mean ± SD. Comparison of adhesion scores between the groups was made using the Mann–Whitney *U*-test. Statistical significance was set at $P < 0.05$. All analyses were performed with the statistical package for social science (SPSS) (version 15.0, Chicago, IL, USA).

Results

No perioperative complications occurred during the study period and 44 uterine horns were evaluated for adhesion formation. The rats of the control group ($n = 11$) formed extensive adhesion between the uterine horn and adjacent peritoneum as well as the bowel and intraperitoneal organs. Adhesion scores after the first surgery were comparable between two groups. Adhesion scores after the second surgery between the tadalafil and control groups is shown in Table I. Although the scores for the extent of adhesion reformation in the study and control groups, respectively, were not different [median 1 (range 0–3) and mean ± SD: 1.29 ± 0.85] versus [median 2 (range 1–3) and mean ± SD: 1.72 ± 0.63, *P*: 0.810], tadalafil reduced the severity [median 0.5 (range 0–1) and mean ± SD: 0.57 ± 0.36] versus [median 1 (range 0.5–1) and mean ± SD: 0.82 ± 0.25 ; *P*: 0.020] and total [median 2 (range 0–4) and mean ± SD: 1.86 ± 1.14] versus [median 2.5 (range 1.5–4) and mean ± SD: 2.55 ± 0.71; *P*: 0.042] adhesion scores. The adhesions were largely confined to the localizations where the adhesiolysis was carried out.

Discussion

PDEs are a specific family of serine proteases that lyse cGMP and thus inhibit the pathway through which NO operates (Michel and Feron, 1997). Sildenafil (Viagra®, Pfizer, USA), a specific PDE-5 inhibitor

Table I The effect of tadalafil on intra-abdominal adhesion reformation in rats, measured 15 days after adhesiolysis.

	Uterine horn (*n*)	Adhesion scores		
		Extent (median; min.–max.), $\bar{x} \pm$ SD	Severity (median; min.–max.), $\bar{x} \pm$ SD	Total score (extent + severity) (median; min.–max.), $\bar{x} \pm$ SD
Study group (10 mg/kg/day tadalafil, for 14 days)	22	(1; 0–3), 1.29 ± 0.85	(0.5; 0–1), 0.57 ± 0.36	(2; 0–4), 1.86 ± 1.14
Control group	22	(2; 1–3), 1.72 ± 0.63	(1; 0.5–1), 0.82 ± 0.25	(2.5; 1.5–4), 2.55 ± 0.71
P-value*		0.810	0.020	0.042

*Mann–Whitney *U*-test.

that augments NO activity by the way of PDE-5 inhibition has been approved for the treatment of male erectile dysfunction (Goldstein *et al.*, 2002). After demonstration of its clinical efficacy in erectile dysfunction, Sildenafil has emerged as the most popular subject of studies in the area of vascular diseases, including pulmonary hypertension (Galiè *et al.*, 2005), diabetes (Desouza *et al.*, 2002) and cerebrovascular disease (Royl *et al.*, 2009).

Following the discovery of a role of insufficient local perfusion in adhesion formation, Batukan *et al.* (2007) studied sildenafil, an NO enhancer, as a potential adhesion preventing agent in an experimental animal model. In their study, they used a modified uterine horn model. Results of the study showed that sildenafil, when given orally at 15 mg/kg doses, significantly decreases *de novo* adhesion formation, whereas lower doses were not effective.

The effect of NO in modifying the inflammatory response and adhesiogenesis is known to be correlated with intra-cellular concentration of cGMP. Short-acting nitrate derivatives may not elevate cGMP levels long enough to allow a sufficient effect to occur (Bult *et al.*, 2000). After the discovery of an adhesion preventing the effect of the orally administered sildenafil by Batukan *et al.* (2007), we aimed to study the effect of a longer acting PDE-5 inhibitor in the adhesion reformation process. For this purpose, we used tadalafil with a half life of 17.5 h, which is three times longer than that of sildenafil (Carson, 2006).

In order to create a proper experimental model to examine the adhesion reformation process, firstly, the kinetics of adhesion formation should be comprehended. Recently, an increasing body of work dealing with the subject had been published (Baptista *et al.*, 2000; Matthews *et al.*, 2003; Gómez-Gil *et al.*, 2010). According to Gómez-Gil *et al.* (2010), who examined the sequence of events in adhesion formation, peritoneal re-epithelization is completed by Day 3 and adhesions formed beyond this time should be taken as *de novo* adhesion. After Day 3, adhesiogenesis is largely confined to the organization of the preformed adhesion. Nevertheless, peritoneal injury created by cauterization and suture placement has different healing kinetics compared with other types of injury. According to some, electrocauterization causes more tissue damage and leads to carbonization of tissue and formation of foreign body reaction, thus prolonging the healing time (diZerega and Campeau, 2001). In accordance with our adhesion model, which includes cauterization and suture placement, we performed our second-look laparotomy and adhesiolysis on Day 14 in order to assess adhesion formation and create adhesion reformation.

Unlike adhesion formation, there are unfortunately few studies focusing on adhesion reformation in the current literature. In one of these studies, Prushik *et al.* (2007) showed that activation of the fibrinolytic system via Nourokinin receptor blockade decreased adhesion reformation after adhesiolysis. Similarly, Attar *et al.* (2011) showed that i.p. application of melatonin, which is a powerful endogenous antioxidant, reduced adhesion reformation. One can surmise from these studies that adhesion reformation, like formation, may follow the same pathological pathway and the same measures used for the prevention of adhesion formation may be effective in reducing adhesion reformation. However, the probability for adhesion reformation after adhesiolysis is greater than *de novo* formation owing to ischaemia of previously damaged tissue (Kamel, 2010). In line with this, our study showed that administration of a longer acting PDE inhibitor used for 14 days reduced adhesion severity without significant effect on extent. Taken together, it can be speculated that NO-related attenuation of adhesion formation is a late event in adhesion genesis that mainly affects proliferation and early remodelling phases (Derici *et al.*, 2010). Furthermore, an increasing body of evidence recently suggests that, besides platelets and leukocytes, fibroblasts also contain PDE-5 (Redondo *et al.*, 1998; Valente *et al.*, 2003). Experimental studies have shown that exogenous cGMP analogues can induce apoptosis and inhibit collagen synthesis (Lee *et al.*, 1997; Redondo *et al.*, 1998; Sirotkin *et al.*, 2000). It is likely that the apoptotic effect of NO might be mediated through the action of intra-cellular cGMP-PKG. Intensifying the activity of these downstream compounds in the NO–cGMP cascade by certain PDEs may be important for elimination of the adhesion fibroblasts during adhesion reformation. Given the relatively ischemic and fibrotic nature of adhesion tissue, a direct effect of NO on fibroblast receptor may be a determining factor in the prevention of adhesion reformation. It was also shown that PDE-5 inhibitors reverse fibrotic change in cell cultures induced by transforming growth factor-B1 (Valente *et al.*, 2003). Within this theoretical frame, based on our results, we suggest that after the formation of adhesion tissue, long-acting PDE-5 inhibitors reduce the tenacity of readhesions but whether this effect results from administering a longer acting agent, or from a longer duration of application, is yet to be determined.

We conclude that oral administration of tadalafil during the perioperative period attenuates adhesion reformation in a rat uterine horn model. Further studies are required to reach a definitive conclusion regarding the value of tadalafil in reducing adhesion reformation.

Authors' roles

M.S.K. helped design the study, prepared the manuscript and approved the final version. M.T.O. and C.B. helped design the study, participated in surgeries, edited the manuscript and approved the final version. B.O. helped design the study, edited the manuscript and approved the final version. M.B. helped design the study, reviewed the manuscript and approved the final version. A.O. helped design the study, performed all the statistical analysis, reviewed the manuscript and approved the final version.

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Conflict of interest

None declared.

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