THE JOURNAL OF
Obstetrics and Gynaecology Research

The official Journal of Asia and Oceania Federation of Obstetrics and Gynaecology
Japan Society of Obstetrics and Gynecology

doi:10.1111/j.1447-0756.2010.01168.x J. Obstet. Gynaecol. Res. Vol. 36, No. 3: 639–645, June 2010

Efficacy of glycerol in preventing postoperative peritoneal adhesions

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Abstract

Aim: To evaluate the efficacy of glycerol in preventing postoperative peritoneal adhesions.

Materials and Methods: Forty Wistar albino female rats were randomly assigned into four groups of 10 rats each. Rats in group 1 were each injected intraperitoneally with 0.1 mL glycerol. In group 2, the adhesion model was created with no injection of glycerol. In group 3, the adhesion model was created and the area was covered with 0.1 mL glycerol. In group 4, 0.1 mL glycerol was used to cover the area where the model was to be formed and the adhesion model was created. The rats were killed on postoperative day 10, and the size and severity of adhesions were evaluated, together with histopathological fibrosis parameters.

Results: Mean macroscopic adhesion scores in groups 1–4 were, 0, 5.8 ± 0.42, 0.30 ± 0.95 and 0, respectively ($P = 0.0001$), with the score in group 2 higher than those of groups 1 ($P < 0.001$), 3 ($P < 0.01$) and 4 ($P < 0.001$). Mean histopathological fibrosis values were 0, 2.8 ± 0.32, 1.60 ± 0.70 and 0.60 ± 0.51, respectively ($P < 0.0001$). Group 3 and 4 scores were different than group 2 ($P < 0.0001$) and group 3 was also different than group 4 ($P < 0.001$).

Conclusion: Covering peritoneal surfaces with glycerol, both before and after peritoneal trauma, is effective in decreasing peritoneal adhesion formation. The efficacy of glycerol covering was greater in the group receiving glycerol prior to trauma because it decreased the direct effects of trauma on the surface.

Key words: adhesion, glycerol, infertility, peritoneal, postoperative, preventing.

Introduction

Postoperative peritoneal adhesion (PPA) is a major complication in abdominal surgery. It occurs in more than three quarters of all laparotomies and is one of the most common causes of intestinal obstruction, infertility, and abdominal and pelvic pains.[1–3] PPA also represents a major financial issue. A multicenter study covering all abdominal surgery units in Sweden demonstrated that the burden of small bowel obstruction alone on the national economy was more than $US6 million annually.[4] Although efforts have been made to reduce the effects of PPA, no effective remedy for this condition has been found.

Glycerol (synonym: glycerine, glycol alcohol) is a viscous liquid alcohol with a molecular weight of 92.09 daltons. Its chemical formula is $C_3H_5(OH)_3$. It dissolves in water and alcohols, but not in liquid hydrocarbons.[5] Being one of the most common molecules in living organisms, it is also a central component of lipids. Most fats consist of one molecule of glycerol combined with three molecules of fatty acids.[6,7] Glycerol is used in medical, pharmaceutical and personal care preparations, mainly as a means of improving smoothness, providing lubrication and as a humectant (a hygroscopic substance). It is found in cough syrup, elixir and expectorant, toothpaste, mouthwash, skin care products, shaving cream, hair care products and soap.[6,8]

Despite the wide use of glycerol in medical applications, to our knowledge, its efficacy in preventing PPA has not been assessed. We therefore investigated the efficacy of glycerol in preventing PPA. Glycerol was

Received: October 3 2008.
Accepted: June 18 2009.
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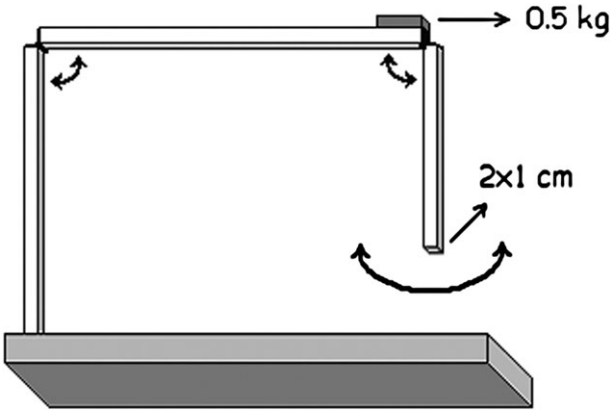

Figure 1 Standard peritoneal adhesion creation apparatus.

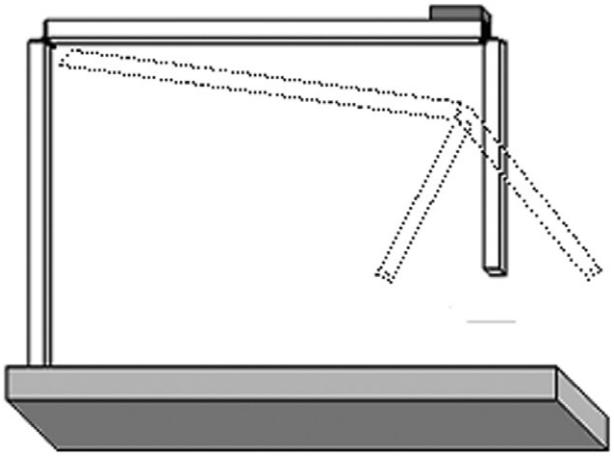

Figure 2 Standard peritoneal adhesion creation apparatus movement simulations.

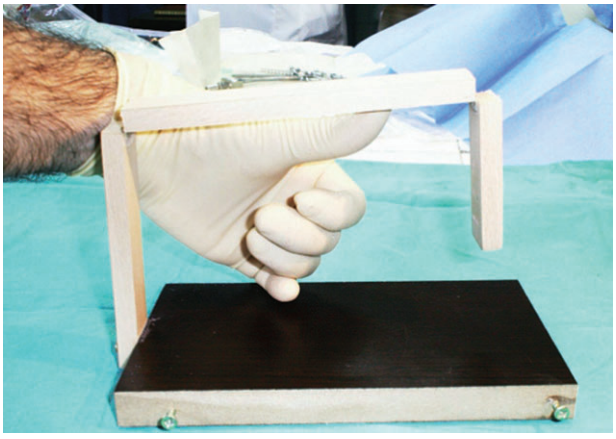

Figure 3 Standard peritoneal adhesion creation apparatus.

applied before and after trauma induction in a traumatic peritoneal adhesion model in rats. Consequently, it was possible to separately evaluate its effects on surfaces exposed to trauma as well as on surfaces that were not.

Materials and Methods

The study protocol was approved by the Animal Ethics Committee of the Istanbul University Cerrahpasa Medical School. Female Wistar albino rats (mean weight, 180 ± 25 g; mean age, 6 months, out bred) were randomly assigned into four groups of 10 rats each, calculated to yield results with 0.9 power and 0.05 confidence interval. Each rat in group 1 was injected percutaneously with 0.1 mL glycerol into the peritoneal cavity using a 22 French diameter needle. In group 2, a standard adhesion model was created through laparotomy. In group 3, a standard adhesion model was created and the area was covered with 0.1 mL glycerol. In group 4, the area where the model was to be formed was covered with 0.1 mL glycerol and the adhesion model was created. Animal housing, methods of anesthesia and adhesion model (Figs 1–5) are similar to our previous manuscript currently in press.[9]

Group 1

Following short-term general anesthesia with ether, 0.1 mL sterile glycerol (Gliserin, Arifoglu Co., Istanbul, Turkey) was percutaneously injected into the peritoneal cavity using a standard plastic syringe with a diameter of 22 French through a point above the midline of the anterior abdomen wall and 3 cm below the xiphoid. After six hours the rats were allowed food and water.

Group 2

Rats under general anesthesia were placed on their backs on the operating table, and their extremities were fixed on the surface by wound plasters. The midline of abdomen was shaved, and the area was coated with povidone iodine solution (Betadine, Kurtsan Co., Istanbul, Turkey). The peritoneal cavity was penetrated through a vertical midline incision of 3 cm. The cecum was pulled out of the abdomen and the anterior wall was placed face upwards over the left index finger of the surgeon. While in this position the adhesion setup was placed just below the vertical moving arm. The surgeon moved this arm back and forth, initiating free oscillation of the pendulum, which was repeated ten times. The cecum was replaced into the abdomen and

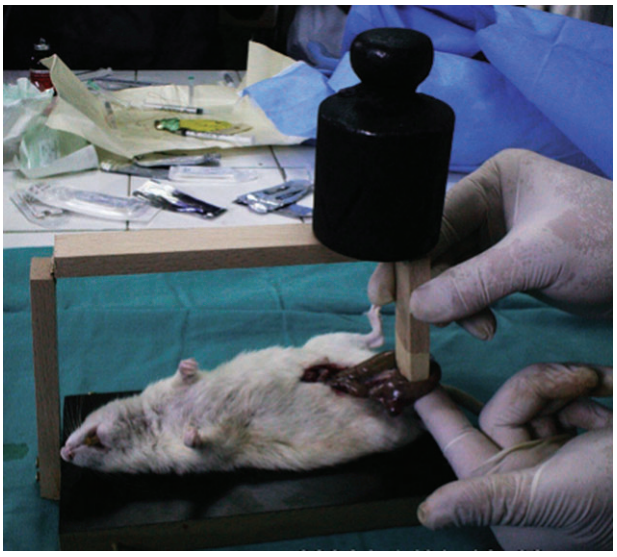

Figure 4 Standard peritoneal adhesion creation apparatus on movement.

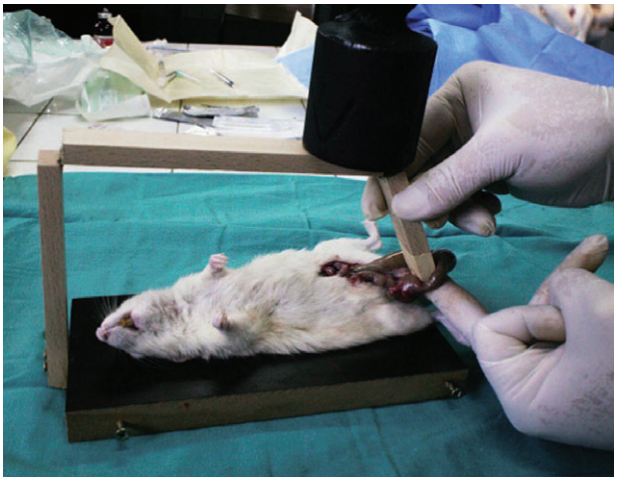

Figure 5 Standard peritoneal adhesion creation apparatus on movement.

the incision was closed with 000 polypropylene sutures (Prolene, Dogsan Co., Trabzon, Turkey) using a continuous suture technique. After 6 h, the rats were allowed food and water.

Group 3

The protocol was identical to that in group 2 except that, following the induction of the adhesions, 0.1 mL sterile glycerol was applied to this area.

Group 4

The protocol was identical to that in group 2 except that the surface of the cecum was covered with 0.1 mL sterile glycerol before induction of adhesions.

All rats were sacrificed 10 days postoperatively by ether inhalation for about 10 min. The peritoneal cavity of each rat was penetrated via a 'reverse U' incision. Without damaging the formed adhesions, the anterior abdomen wall flap was pulled caudally. Adhesions were graded according to size and severity (Table 1). Other intraperitoneal pathologies were also recorded. The 2 cm^2 area of cecum, where the model was formed, was resected for histopathological evaluation and conserved in glass bottles containing formol.

The primary outcome measure of this study was adhesion score, which is the sum of adhesion severity and adhesion size grading (Table 1). The secondary outcome measure was fibrosis grading of the tissue samples extracted from the adhesion model area (Table 2).[10,11]

Histopathological evaluation

The model formation area of the cecum and any adhesions over this area were fixed in formol. After dehydration, they were embedded in paraffin, and 5 mm cross sections were stained with hematoxylin-eosin. The samples were histopathologically evaluated by a pathologist blinded to the study protocol, using light microscopy at a magnification of x100. Fibrosis was graded as described in Table 2.

Statistical evaluation

Statistical analyses were performed using GraphPad Prism V.3 software. Results were evaluated with a confidence interval of 95% and $P < 0.05$ level. In addition to descriptive statistical methods (mean, standard deviation and median), the Kruskal–Wallis test for intergroup comparisons, the Dunn's multiple comparison test for comparison of subgroups, and the chi-squared (χ^2) test and Fisher's exact test for comparison of nonparametric variables were used for evaluation of the data.

Results

We observed statistically significant differences among groups in the size and severity of adhesions ($P = 0.0001$). Adhesion sizes in group 2 were significantly greater than those of the other groups ($P = 0.0001$). In contrast, adhesion sizes did not differ

Table 1 Definitions of size and severity grades of the peritoneal adhesions

Grades	Adhesion size	Adhesion severity
0	No adhesion	No adhesion
1	Presence of adhesion in 25% of the model area	Spontaneously separating adhesion
2	Presence of adhesion in 50% of the model area	Separation of adhesion with traction
3	Whole model area covered with adhesion	Separation of adhesion with a sharp dissection

Table 2 Microscopic histopathological fibrosis scoring

Grades	Definition
Grade 0	No fibrosis (no fibroblasts and/or collagen fibers)
Grade 1	Slight fibrosis (few fibroblasts and/or collagen fibers)
Grade 2	Median fibrosis (more fibroblasts and/or collagen fibers)
Grade 3	Severe fibrosis (lots of fibroblasts and/or collagen fibers)

significantly among groups 1, 3 and 4 ($P > 0.05$, Table 3). Adhesion scores of the groups were obtained by calculating the arithmetical means of adhesion size and severity. Differences in mean adhesion scores among the four groups were significant ($P = 0.0001$). The mean adhesion score in group 2 was significantly higher than those of groups 1 ($P < 0.001$), 3 ($P < 0.01$) and 4 ($P < 0.001$). In contrast, the differences among groups 1, 3 and 4 were not significant ($P > 0.05$, Table 4).

Microscopic fibrosis scores and their statistical analysis were revealed in Table 5. Group 3 and 4 scores were significantly different than group 2 ($P < 0.0001$) and group 3 was also significantly different than group 4 ($P < 0.001$).

Discussion

PPA occurs as a result of damage (mechanical, ischemic, chemical, infectious and/or inflammatory) to the monolayer mesothelial cells of the peritoneum. There is a fibrin-rich exudation to the damaged area. Fibrins establish bands among the other peritoneal surfaces that are in contact with this area. These bands and the hyaluronic acid-rich matrix filling the gaps between them provide an environment conducive to collagen synthesis. Collagen synthesis, in turn, leads to actual adhesions.[1,2,12–14]

Several techniques, substances and agents have been investigated to prevent PPA. These include various surgical methods, minimal invasive and laparoscopic techniques, pharmacological agents targeting the inflammatory response and/or fibrin formation induced by mesothelial cell trauma, liquids to form a mechanical barrier between mesothelial surfaces, gels and solids. Although some techniques or agents have proven useful, none showed complete success.[1,12,15]

The most commonly investigated approach in recent years has been the use of mechanical barriers between peritoneal surfaces. These barriers can be formed by using unabsorbable solids (such as amnion), solids that are absorbable through liquidification at body temperature (such as hyaluronic acid derivatives), or liquids (such as methylene blue).[16–18] This approach is considered practical, relatively cheap and suited to peritoneal physiology.[19]

Mechanical barriers using gelatinous liquids with high viscosity have yielded relatively high success rates. These liquids are thought to prevent the formation of PPA by providing a protective layer between the surfaces and preventing contact between deperitonized surfaces and surrounding tissues.[20,21] *In vitro* studies have shown that cells or groups of cells located on the two sides of a high viscosity environment moved towards one another in a delayed fashion, preventing or delaying adhesion.[22]

Various models have been developed to experimentally induce PPA abrasion,[23] including local peritoneum excision,[24] ischemic damage,[25] introduction of foreign objects (such as talcum powder) into the peritoneal cavity,[25] thermal damage[26] or bacterial contamination.[27] We used an abrasion model because it mimicked the mechanical trauma occurring during laparotomy. All types of manipulation during laparotomy, either by hand or using surgical instruments, constitute a mechanical trauma. Moreover, the most common cause of PPA is mechanical trauma owing to laparotomy.[1,12] The experimental abrasion model uses several approaches on different locations in the peritoneal cavity, including direct abrasion on the wall of the cecum,[28] abrasion of the cecum wall with gauze until subserosal petechial hemorrhages can be observed,[23] abrasion of the cecum wall with a scalpel[29] or needle,[29] uterine horn abrasion[26] and abrasion on the peritoneal

Table 3 Adhesion size and severity grades of the groups

	Grade	Group 1		Group 2		Group 3		Group 4		
		n	%	*n*	%	*n*	%	*n*	%	
Adhesion size	0	10	100	0	0	9	90	10	100	
	1	0	0	0	0	1	10	0	0	
	2	0	0	0	0	0	0	0	0	χ^2: 42.75
	3	0	0	10	100	0	0	0	0	$P = 0.0001$
Adhesion severity	0	10	100	0	0	9	90	10	100	
	1	0	0	0	0	0	0	0	0	
	2	0	0	2	20	1	10	0	0	χ^2: 37.42
	3	0	0	8	80	0	0	0	0	$P = 0.0001$

Table 4 Macroscopic adhesion scores of the groups and their statistical analysis with Kruskal–Wallis test

Groups	Mean ± Standard deviation	Median
Group 1	0 ± 0	0
Group 2	5.8 ± 0.42	6
Group 3	0.30 ± 0.95	0
Group 4	0 ± 0	0
Kruskal–Wallis test	36.10	
P value	0.0001	

Table 5 Microscopic histopathologic fibrosis scores of the groups

Fibrosis score	Mean ± Standard deviation	Median
Group 1	0 ± 0	0
Group 2	2.8 ± 0.32	3
Group 3	1.60 ± 0.70	1.5
Group 4	0.60 ± 0.51	1
Kruskal–Wallis test	31.80	
P value	0.0001	

surfaces of the abdominal wall.[20] Traumas generated by these techniques, however, may be difficult to standardize. Standardization problems can include the number of sites with petechial hemorrhage, the area involved, and the pressure applied.

The apparatus used in this present study to create abrasion was designed and applied for the first time by our team. We were able to standardize each component of this abrasion model with this apparatus. For example, the surface area of trauma was standardized by fixing the size of the surface in contact with the peritoneum 2×1 cm. The trauma location was standardized by making the apparatus fixed and stable, such that the pendular movement always affected the same point, thus, creating abrasion at the same location. The number of traumas was standardized by using the same number of pendular movements and the pressure was standardized by using a standard force derived from 500 g weight.

There are two fundamental stages of PPA management: preventing the occurrence of peritoneal trauma and preventing adhesion of the traumatized peritoneal surface to any other surface. Intervening in the first stage is simpler, because it requires only the development of a barrier, and more effective, because inflammation and subsequent wound-healing processes are rather complicated and comprise many unclear stages. The rate of success of approaches for which the physiopathologies are not completely understood will be relatively low and even incidental. Furthermore, regardless of the agent and/or approach selected, it is essential to ensure that there is no toxic effect on the peritoneal mesothelial cells while accelerating the wound-healing process and/or preventing adhesion of mesothelial surfaces until the healing process is complete. An agent and/or approach that meets all of these needs is difficult and, to date, no agent and/or method has been found that achieves all of these objectives.

Glycerol is a common molecule in living organisms. As a result of its biocompatibility, it is used in many pharmaceutical and cosmetic products.[6–8] Certain types of meshes used in surgery for the treatment of hernias are produced or coated with composites containing glycerol as it is absorbable and has a low incidence of irritation and toxicity on live tissues.[30,31] We applied glycerol to reduce the probability of PPA occurrence following intraperitoneal administration of the meshes. Furthermore, a reduced incidence of PPA has been reported in ventral hernia patients in whom polyester with collagen–polyethylene glycol–glycerol coating meshes was used.[32]

Glycerol is frequently added to peritoneum dialysis solutions. The inclusion of glycerol has been reported

to augment the efficacy of peritoneal dialysis and to provide a protective effect on peritoneal surfaces.[33] Little is known, however, about the wound-healing qualities of glycerol, although glycerol trinitrate (also known as nitroglycerine), has been shown to accelerate the healing process in patients with chronic anal fissures.[34,35]

There were two reasons to test glycerol: its biocompatibility and its ability to permanently coat all surfaces with which it is in contact. We injected 0.1 mL glycerol into the peritoneal cavity of ten rats without creating abrasions to assess its potential toxic effects prior to abrasion. We did not administer glycerol to this group by means of laparotomy because incision may have led to adhesions, yielding false-positive results. Re-laparotomies performed on rats in this group showed no incidence of adhesions and we observed no abnormalities during the histopathological examination of the biopsies collected from the anterior surfaces of cecum walls. Similarly, we did not find any indication of a toxic reaction in the peritoneal cavity.

We observed that the number of PPAs were significantly reduced when the area where abrasions were created were later covered with glycerol. Only one of the ten rats developed PPA (adhesion size: grade 1, adhesion severity: grade 2, microscopic histopathological fibrosis: grade 2). In contrast, none of the rats treated with glycerol prior to creating abrasions showed evidence of PPAs. Although their macroscopic adhesion scores did not differ significantly, microscopic fibrosis scores were lower in the latter group. Our results therefore indicate that glycerol administration, before or after peritoneal trauma, decreases PPA occurrence, and that glycerol is more effective when administered before peritoneal trauma.

When we performed same material and method research with octyl methoxycinnamate revealed the same results.[9] In that research, before peritoneal trauma macroscopic adhesion score was 1.8 ± 2.39, after peritoneal trauma was 0.4 ± 0.84.

The mechanism behind these effects of glycerol may be due to the benefits of glycerol on the wound-healing process and/or by its viscosity, causing it to form a layer between surfaces, thereby effectively preventing contact between peritoneal surfaces and surfaces that are exposed to trauma. Glycerol was more effective when administered before than after trauma, so it is likely that the effects of glycerol in preventing trauma are due to its ability to cover peritoneal surfaces more than its effect on the wound-healing process.

In conclusion, we have shown here that glycerol administration into the abdominal cavity successfully decreased PPA formation. Although it was effective regardless of whether it was administered before or after trauma, the effect was greater when administered before trauma.

Performing the same experimental protocol using octyl methoxycinnamate resulted in similar results.[9] In that work, pre-administration of octyl methoxycinnamate gave an adhesion score of 1.8 ± 2.39 whereas post-administration gave an adhesion score of 0.4 ± 0.84.

Thus, PPA formation can be decreased by administering a suitable viscous fluid into the entire peritoneal cavity or only into the area that is to be manipulated before the start of laparotomy or laparoscopy. Further studies using high viscosity fluids that are not toxic to vital tissues are required to confirm our results.

Acknowledgments

No support was taken from any person and/or institution for this research.

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