Micronized purified flavonoid fraction may prevent formation of intraperitoneal adhesions in rats

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Objective: To evaluate the efficacy of an anti-inflammatory and capillary regulator drug, micronized purified flavonoid fraction (MPFF), in the prevention of postoperative formation of adhesions.

Design: A double-blind, controlled study evaluated the efficacy of MPFF in reducing postoperative adhesion formation in a rat model.

Setting: Animal care facility of an academic research setting.

Animal(s): Thirty Sprague-Dawley female rats randomly divided into three groups.

Intervention(s): Starting on day of surgery, group 2 rats received oral MPFF (100 mg/kg per day for 7 days). Group 3 rats were intraperitoneally injected with 5 mL of saline (containing 200 mg/kg per day of MPFF for 3 days). Control rats received no medication. A standardized surgical trauma was applied in all animals. Three weeks after surgery, the rats were killed, and the adhesions were scored according to macroscopic and microscopic scales.

Main Outcome Measure(s): Postoperative adhesions.

Result(s): Both oral and intraperitoneal administration of MPFF reduced the scores of adhesions according to macroscopic and microscopic scales. There was no difference between the routes of administration.

Conclusion(s): A statistically significant reduction in postoperative formation of adhesions was observed after oral and intraperitoneal administration of MPFF in our experimental animal model. However, further studies are required to reveal its mechanism of action. (Fertil Steril® 2005;84(Suppl 2):1083–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: Intrabdominal adhesion, micronized purified flavonoid fraction, anti-inflammatory, capillary regulator

Despite the improvement in surgical techniques and instruments, intraabdominal adhesions are still a major source of postoperative morbidity and mortality after abdominal surgery. It has been reported that 93%–100% of the patients who have undergone abdominal surgery develop adhesions (1), some of whom might later develop bowel obstruction, infarction, fistulization, and erosion (2). Recurrent and chronic abdominal complaints, pain, secondary female infertility, and difficulty during subsequent surgical procedures are the other problems encountered (3–7).

Approximately, 3% of laparotomies, 29% of all admissions for obstruction (3), and 15% to 20% of female infertility (4) are caused by adhesions. With increasing numbers of surgical interventions, the clinical workload and the economic costs of adhesion-related diseases are rising constantly (3). Therefore, reduction of postoperative adhesion formation would be of clinical benefit. Various substances have been used to prevent adhesion formation both in animal studies and clinical trials. However, there is no ideal method of preventing formation of adhesions currently.

In the classic pathway of adhesion formation, peritoneal injury from trauma, infection, or ischemia cause secretion of inflammatory mediators. These mediators result in increased vascular permeability and subsequent inflammatory reaction, which are followed by release of fibrin-rich exudates. Fibrin deposition within these inflammatory exudates, organization of fibrin with fibroblast invasion, and finally, formation of collagen produce mature fibrous adhesions (8–10). Any substances interfering with either of these steps may prevent formation of adhesions.

Micronized purified flavonoid fraction (MPFF) is a well-recognized phlebotonic drug that has been used for the treatment of venous insufficiency (11). It has demonstrated phlebotonic activities, lymphokinetic abilities, and modulatory effects on inflammatory mediators and hemorheological parameters in preclinical studies, as has been extensively reported (12). Considering these data, we planned to investigate the effect of this anti-inflammatory drug on postoperative formation of adhesions. Because it has modulating effects on inflammatory mediators and vascular permeability, we hypothesized that postoperative adhesions might be decreased with MPFF.

MATERIALS AND METHODS

This study was performed in the laboratories of the Research Unit of Department of Health Sciences at Dicle University, Diyarbakir, Turkey. Approval from the Research Committee (Institutional Review Board) of Dicle University was ob-
tained before the study. The standards of the National Institutes of Health, as described in the Guide for the Care and Use of Laboratory Animals, were followed.

A total of 30 female Sprague-Dawley rats, each weighing between 200 and 250 g, were used. After overnight fasting, all animals were anesthetized with 60 mg/kg of ketamine hydrochloride (Ketalar, 50 mg/mL; Parke-Davis, Istanbul, Turkey). After shaving and disinfection of the skin, a 3-cm midline incision was made. The cecum was grasped and scraped with the side of a size-15 scalpel until the serosa began to bleed slightly. Then it was sutured with 5–0 silk and replaced in the abdominal cavity in its natural position. A 1-cm² defect in the parietal peritoneum (peritoneum covers the interior surface of the abdominal wall), was created on the right lateral side of the wall. The midline incision was closed in one layer with continuous 3–0 silk sutures.

Animals were divided into three groups, each consisting of 10 rats. Group 1 (control) rats received no medication. Starting on the day of surgery, group 2 rats received by mouth (by an esophageal canula) MPFF (Daflon, 500 mg; Servier, France; 100 mg/kg per day for 7 days). Group 3 rats were intraperitoneally injected with 5 mL of saline containing 200 mg/kg of MPFF per day, for 3 days.

All rats underwent a second laparotomy on the 21st postoperative day for evaluation of adhesion formation. Adhesions were examined macroscopically and graded blindly according to the scale of Blauer and Collins (13) (Table 1). Adhesion-carrying tissues were excised en bloc and fixed in 10% neutral buffered formaldehyde solution for 24 hours. Each sample was embedded in paraffin block by a standard method. Then sections with a thickness of 5 μm were cut and stained with hematoxylin and eosin for light microscopy. The samples were evaluated blindly by the same pathologist for the general structure and the amount of fibroblastic activity and fibrosis. Thus, according to the criteria mentioned, the fibrotic score of each rat was calculated (14) (Table 2).

The Kruskal-Wallis test was used to test for differences in the grades of adhesions observed in the three groups of rats. Individual comparisons among the means were made by the post hoc least-significant differences method. Differences with a P value of ≤.05 were considered statistically significant.

RESULTS

All operations were uneventful, and all rats achieved a full recovery after the first laparotomy. No animal was excluded from the study. The rats in the control group had extensive adhesions between the abdominal wall and the organs (Fig. 1). However, the rats in groups 2 and 3 had lesser adhesive attachments to intraabdominal structures (Fig. 2). The median scores of adhesions according to the scale of Blauer and Collins (13) for each group are presented in Table 3. Kruskal-Wallis analysis showed a significant difference between groups on median adhesion scores (P=.009). Rats in groups 2 (P=.004) and 3 (P=.001) had significantly lower

<table>
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<th>TABLE 1</th>
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<td><strong>Adhesion description for each grade (scale of Blauer and Collins for macroscopic assessment).</strong></td>
</tr>
<tr>
<td>Grade</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
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<td>3</td>
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<td>4</td>
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**TABLE 2**

<table>
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<th>Grade</th>
<th>Histopathological signs</th>
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<tr>
<td>0</td>
<td>No fibrosis.</td>
</tr>
<tr>
<td>1</td>
<td>Thin bunches of cellular fibrosis.</td>
</tr>
<tr>
<td>2</td>
<td>Wide areas of fibrosis with reduced vascularization.</td>
</tr>
<tr>
<td>3</td>
<td>Areas of fibrosis formed by thick bunch of collagen.</td>
</tr>
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**FIGURE 1**

The adhesions between the organs and abdominal wall in the control group.
scores than did control rats. There was no significant difference between the scores of rats in groups 2 and 3 ($P=.652$; Table 3).

There were lymphocytes, plasma cells, polymorphonuclear leucocytes, and areas of fibrosis consisting of fibroblasts on histological examination of control rats (Fig. 3). The rats in groups 2 and 3 showed lesser infiltration of inflammatory cells, fibroblasts, and areas of fibrosis (Fig. 4). The median fibrosis scores for each group are presented in Table 3. There was a significant difference between groups in median fibrosis score ($P=.000$), which was significantly lower in rats in groups 2 ($P=.000$) and 3 ($P=.000$) than in control rats. There was no significant difference between the scores of rats in groups 2 and 3 ($P=.398$).

**DISCUSSION**

Adhesion is an abnormal fibrous tissue resulting from the fibroproliferative inflammatory response that occurs after injury from ischemia, infection, or trauma to the organs in the abdomen (2, 6, 14). Degranulation of stromal mast cells and release of a large number of inflammatory mediators, including histamine, serotonin, lysosomal enzymes, cytokines, oxygen free radicals, and vasoactive kinins occurs after any inflammatory event (8, 9, 15, 16).

Extravasation of proteinaceous exudates consisting of monocytes, histiocytes, polymorphs, and plasma cells follows the increased permeability of the blood vessels, especially the venules (8). Fibrin polymerization, which is an autonomic accompaniment of intraperitoneal inflammation, occurs when the extravasating platelets and fibrin come into contact with the exposed basal membrane. These fibrin clots resolve through fibrinolysis in the presence of adequate amounts of plasminogen activator activity. However, after peritoneal injury (caused by thermal injury, infection, foreign-body reaction, poor surgical technique, or ischemia), plasmin-
Histological section of a rat from MPFF-received group: fewer amounts of inflammatory cells and fibroblasts, and lesser fibrosis (stain, hematoxylin-eosin; original magnification, ×400).


...ogen activator activity of the mesothelium is reduced, fibrinolytic activity is suppressed, and fibrin products persist. Eventually, the fibrous adhesions are invaded by fibroblasts. Stimulation of angiogenesis and synthesis of collagen follow it (2, 4, 5, 8–10, 16–18). Fully developed fibrous adhesions are seen at 10 days and become maximal at 2–3 weeks after peritoneal injury (9).

Attempts to reduce adhesion formation have used a variety of techniques directed at each of the steps in adhesion formation, including prevention of the initial inflammatory response by blocking eicosanoids (11, 19) or oxygen free radicals (19–22); dissolution of fibrinous exudate using fibrinolytic agents (4, 18, 19, 23–25); inhibition of collagen formation (4, 5, 14); interference with the apposition of damaged peritoneal surfaces (2, 7, 8, 15, 19, 26); and stimulation of intestinal motility by using prokinetic agents (11).

It has been known that serious damage occurs after reperfusion of the ischemic tissue in the case of peritoneal injury caused by vascular trauma. Inflammatory mediators such as arachidonic acid and its metabolites, prostaglandin E2 and its derivatives, nitric oxide, oxygen free radicals, and cytokines are secreted, and new vascular structures appear (8, 20–22, 25, 27, 28). These mediators reduce the strength of the collagen on the basal membrane of the vessels and of the tunica media of the venules. They increase the permeability of the blood vessels, stimulate the chemotaxis of the polymorphs and the reticuloendothelial cells, and inhibit the activity of plasminogen activators (1, 17, 28). Oxygen free radicals that are produced by phagocytes in the extracellular area affect this process by starting cytolysis, increasing the permeability of blood vessels, and assuring the continuation of the inflammatory process. Attempts to block that cascade directly or indirectly at any step prevent adhesions by reducing inflammation, which is the first step in adhesion formation. Drugs such as anti-inflammatory agents (5, 29, 30), oxygen free-radical scavengers (19–22), mast cell stabilizers (7, 8, 20), and capillary regulators have been used for this purpose. To our knowledge, MPFF, an anti-inflammatory drug that also regulates capillary permeability, has not been previously examined. However, it appears as a good choice for this purpose because MPFF exhibits a number of anti-inflammatory actions, including inhibition of inflammatory mediator release and attenuation of reactive oxygen metabolite formation (31–34). It reduces mast cells, prevents synthesis of oxygen free radicals, and acts as their scavenger, thus improving the permeability of capillaries increased by bradykinins and oxygen free radicals and thereby the capillary resistance. Micronized purified flavonoid fraction also inhibits the synthesis of PGE2, PGF2α, and thromboxane B2 by the enzymes phospholipase A2, cyclooxygenase, and lipooxygenase (27, 28, 35, 36). Indeed, we observed that administration of MPFF either by the oral or intraperitoneal route statistically significantly reduced postoperative formation of adhesions.

The use of substances intraperitoneally may prevent the apposition of traumatized ischemic tissues during initial healing (5, 26, 37, 38). They also may affect adhesions by preventing binding of macrophages and infiltration of fibroblasts (37). Calcium channel blockers, recombinant tissue plasminogen activators, oxidized regenerated cellulose plus heparin, aprotinin, octreotide, carboxymethylcellulose, saline, and hyperosmolar peritoneal dialysis solutions have been used by this route (4, 10, 14, 16, 18, 26, 37, 38).

We intraperitoneally applied MPFF dissolving in saline and used a daily dosage of twice as much when compared with the case of the oral route, because we wanted to limit the duration of intraperitoneal application for 3 days, which is the mean duration of drains after abdominal surgery in clinical settings. Essentially, animals in both of the groups were given nearly the same dosage of the drug at the end of the study (100 mg/kg by mouth every 7 days vs. 200 mg/kg intraperitoneally every 3 days). When the solution was prepared, we saw that the solubility of the drug was limited and that there were residual granules. Despite the probability that granules themselves might cause adhesion formation, we planned to administer the solution intraperitoneally. Because the scene at which the events occur is the intraperitoneal cavity, it appeared feasible to apply the drug by this route. We assumed that the local and systemic effects of MPFF together would give better results on preventing formation of adhesions.

We did not use a group receiving intraperitoneal saline alone to control the probable effects of saline itself when it was applied with MPFF. Although it has been used to prevent adhesion formation, it is widely accepted that saline instilled to the abdominal cavity is reabsorbed into the vascular space within 24 hours in humans and probably even
more quickly in the rat (7, 16, 26, 29, 39). Therefore, from a theoretical point of view, saline instilled intraperitoneally is not expected to prevent formation of adhesions (7, 16, 39). Furthermore, the amount of saline that is used for prevention of adhesions is more than the amount that we have used for dissolution of MPFF (20 mL vs. 5 mL). Moreover, according to the widely held concept that such an amount of saline does not prevent formation of adhesions, many experimental studies have been designed by applying intraperitoneal saline in rats in control groups. At the same time, these studies have no additional groups to control the effects of saline itself that is applied in these groups (4, 10, 18). Acting from the point of view that the effect of saline itself on preventing formation of adhesions is negligible, we did not add a control group receiving saline alone. We observed that intraperitoneal application of MPFF also attenuated adhesion formation and had no superiority on oral administration.

The exact mechanism by which MPFF prevents adhesion formation is unclear. However, when its effects reported in clinical series are considered, the potential roles of MPFF on the pathway of adhesion formation may be listed as follows: reducing the mast cells and their products such as histamine and other vasoactive substances; improving the increased permeability of mesothelial venules; preventing fibrous exudation; inhibiting thromboxane and subsequently formation of thrombus; serving as scavenger of free radicals and protecting the tissues from their deleterious effects; and inhibiting the synthesis of 
PGE$_2$, PGF$_{2a}$, thus preventing inflammatory cytokines to inhibit the plasminogen activators. Indeed, when compared with the control group, the histological examination of MPFF groups revealed lesser infiltration of inflammatory cells, probably caused by the vaso- tonic effect of the drug. In addition, the reduction in inflammatory reaction might be caused by the modulating effects of MPFF on inflammatory mediators. Thus, extravasation of fibrin-rich exudates, invasion of fibroblasts, and deposition of collagen might decrease, as we observed in MPFF groups.

The drugs that have been widely used for prevention of adhesion formation have many side effects that threaten life. For instance, fibrinolytic agents, nonsteroidal anti-inflammatory drugs, and corticosteroids carry the risk of hemorrhage, ulcers with subsequent bleeding, immunosuppression, and healing disorders (3, 17, 25). Liquids, such as dextran, hyaluronic acid phosphatase–buffered saline, polyethylene glycol, lactated Ringers, and hyperosmolar dialysates are absorbed rapidly. Hydroflotation by these macromolecular solutions shows significant adverse effects, such as volume overload, impairment of liver function, and even disseminated intravascular coagulation and anaphylactic shock (3, 5). However, MPFF has an excellent safety profile, as documented by both toxicological animal studies and by clinical studies (11, 40).

When serious adverse effects of alternative drugs that have been reported are taken into consideration, the idea of evaluating the utility of this tolerable agent appears logical. However, further studies are required to determine how MPFF reduces adhesion formation, because all these explanations are hypothetical.

REFERENCES