EXPERIMENTAL STUDY

Effects of pioglitazone and metformin on abdominal adhesion formation in an experimental model

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ABSTRACT

BACKGROUND: This study evaluated the use of metformin or pioglitazone in preventing or reducing the development of post-operative intra-abdominal adhesion (PIAA) by employing histopathological, immunohistochemical, and biochemical analyses in an experimental adhesion model.

METHODS: Fifty Wistar-Albino rats were divided into five groups: Group I (Control), Group II (Sham Treatment), Group III (Hyaluronic Acid), Group IV (Metformin), and Group V (Pioglitazone). Adhesions were induced in the experimental groups, except for the sham group, using the scraping method. After 10 days, rats were euthanized for evaluation. Macroscopic adhesion degrees were assessed using Nair's scoring system. Immunohistochemical and enzyme-linked immunosorbent assay (ELISA) methods were utilized to assess serum, peritoneal lavage, and intestinal tissue samples. Fructosamine, interleukin-6 (IL-6), transforming growth factor-beta (TGF-β), and fibronectin levels were measured in serum and peritoneal lavage samples.

RESULTS: The groups exhibited similar Nair scores and Type I or Type III Collagen staining scores (all, $p>0.05$). Pioglitazone significantly reduced serum IL-6 and TGF-β levels compared to controls ($p=0.002$ and $p=0.008$, respectively). Both metformin and pioglitazone groups showed elevated IL-6 in peritoneal lavage relative to controls, while fibronectin levels in the lavage were lower in pioglitazone-treated rats compared to the sham group (all, $p<0.005$).

CONCLUSION: Pioglitazone, but not metformin, demonstrated a positive biochemical impact on preventing PIAA formation in an experimental rat model, although histological impacts were not observed. Further experimental studies employing different dose/duration regimens of pioglitazone are needed to enhance our understanding of its effect on PIAA formation.

Keywords: Pioglitazone; adhesion; adhesion model; metformin; peroxisome proliferator-activated receptor (PPAR).

INTRODUCTION

Postoperative intra-abdominal adhesion (PIAA) formation has long been considered an inevitable outcome of abdominal or pelvic surgery. Despite advances in surgical techniques, PIAA is still a significant source of morbidity and mortality.[1] The likelihood of PIAA formation in patients undergoing abdominal surgery is reported to be approximately 54%.[2] PIAAs can lead to complications such as ileus, infarction, fistulas, chronic pelvic pain, infertility, and may present technical challenges in subsequent surgeries.[3,4]

Adhesion formation is a complex process initiated by various factors including injury to peritoneal structures, tissue isch-

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emia, and the presence of foreign materials.[5] It is well-established that PIAA formation involves oxidative damage and an inflammatory response, characterized by cytokine production and the induction of transforming growth factor-β (TGF-β), which disrupts the balance between fibrinogenesis and fibrinolysis (6). Despite trials of various surgical techniques and adjuvants, such as barrier agents or drugs, to prevent PIAA, no ideal method has emerged that both protects mesothelial cells and minimizes surgical damage while ensuring hemostasis.[3,7] Although some adjuvants have shown potential in experimental studies due to their anti-inflammatory or fibrinolytic effects, consensus on their effectiveness and safety has not been reached, and their application is limited to the specific expertise of the practitioner.[5]

Metformin is a safe, inexpensive, and reliable molecule, established as a first-line drug for type 2 diabetes.[8] In vivo and in vitro studies have demonstrated that metformin possesses potent anti-fibrotic properties, enhanced by its anti-oncogenic, anti-inflammatory, and antioxidant effects.[9,10] Peroxisome proliferator-activated receptors (PPARs) α, β/δ, and γ are key regulators of adipogenesis, glucose metabolism, insulin resistance, inflammation, and fibrogenesis.[11] Pioglitazone, a PPAR-γ agonist, is used as an insulin-sensitizing drug in type 2 diabetes and offers cardioprotective effects—primarily through its regulation of glucose and lipid metabolism in muscle, adipose tissue, and liver.[12] Furthermore, pioglitazone has demonstrated anti-fibrotic properties in experimental models and human trials. This property is linked to its anti-inflammatory, antioxidant, and anti-apoptotic effects, which contribute to an endothelium-sparing impact.[12,13] Although metformin and/or pioglitazone may potentially prevent or reduce PIAA, very few studies have examined this potential. We aimed to address this gap in knowledge by investigating the effects of pioglitazone and metformin on PIAA development in an experimental rat model.

MATERIALS AND METHODS

Study Design and Setting

This experimental animal study was conducted at the Istanbul Universty Experimental Medicine Research Laboratory, following approval from the Istanbul Universty-Cerrahpaşa Animal Experiments Local Ethics Committee (November 01, 2022 / E-74555795-050.01.04-524571). Fifty male Wistar-Albino rats, aged 8-10 weeks and weighing 200 to 250 g, were used in the study. Animals were individually housed in separate cages within air-conditioned rooms, subject to 12-hour light and dark cycles. Automated systems maintained stable temperature (24±2 °C) and relative humidity (45-50%). Throughout the study, all rats had unrestricted access to standard laboratory chow and tap water. No special medication or diet was administered to the rats before the surgical procedure. Blood samples were collected from the tail vein to assess serum fructosamine levels of each animal prior to the intervention.

Animal Care and Experimental Groups

All experimental procedures and animal care adhered to the Guide for the Care and Use of Laboratory Animals. On the day of the intervention, all rats were anesthetized using 60 mg/kg ketamine and 8 mg/kg 2% Xylazine hydrochloride administered intraperitoneally. Surgical operations to create an adhesion model were performed by the same surgeon under sterile conditions. The scraping model was used to induce the PIAA, involving two stages of damage: direct mechanical damage to the bowel wall caused by scraping with gauze and ischemic damage resulting from vascular clamping (Fig. 1) (14). After thoroughly cleaning the surgical area with copious povidone-iodine, a laparotomy was performed using a 2-3 cm midline incision. Following the laparotomy, the scraping model was applied to all experimental groups except the sham group. Subsequently, all experimental groups were sutured in two continuous layers using 3.0 prolene. The rats were randomly divided into five groups as follows:

Group 1 (Control): The scraping model was applied during laparotomy. Each rat received 1 ml of tap water daily by oral gavage for 10 days.

Group 2 (Sham): After laparotomy without any intervention-related injury, each rate received 1 ml of tap water daily by oral gavage for 10 days.

Group 3 (Hyaluronic acid): The scraping model was applied during laparotomy, followed by treatment with 2 ml of hyaluronic acid gel without closing the abdomen. Each rate also received 1 ml of tap water daily by oral gavage for 10 days.

Group 4 (Metformin): After establishing the scraping model at laparotomy, each rat was administered 1 ml of 40 mg/kg metformin solution daily by oral gavage for 10 days.

Group 5 (Pioglitazone): Following the scraping model to initi-

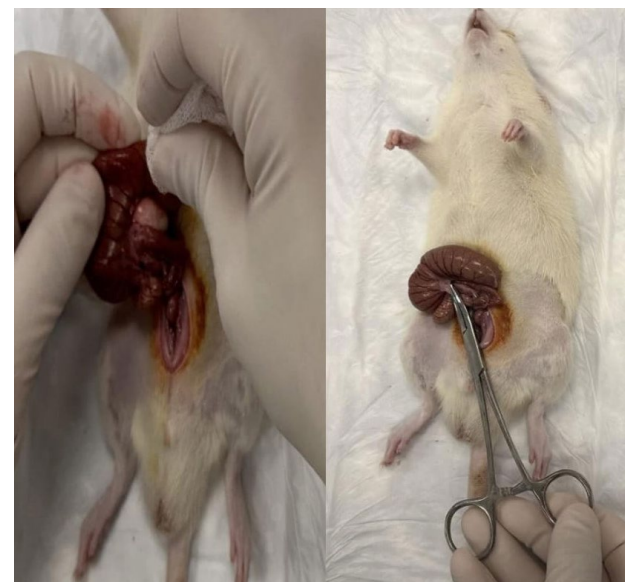

Figure 1. Model demonstrating direct mechanical damage to the bowel wall via gauze scraping and ischemic damage through vascular clamping.

ate adhesion formation at laparotomy, each rat was given 1 ml of 3 mg/kg pioglitazone daily by oral gavage for 10 days.

Post-intervention Care and Sampling

Rats were maintained on their normal diets and housed in standard cages throughout the postoperative period, with daily examinations for surgical complications. General anesthesia was administered to all rats on the 10th post-intervention day. Maximum visibility was achieved by making a U-incision and retracting the abdominal walls downward, followed by the collection of peritoneal lavage and intestinal tissue samples. Sacrification was accomplished by collecting intracardiac blood.

Microscopic, Macroscopic, and Immunohistochemical Analyses

All tissue samples were fixed in 10% neutral buffered formalin for at least 24 hours. After embedding in paraffin, 5 µm-thick sections were stained with hematoxylin and eosin and examined under a fluorescence microscope (Olympus BX51, Olympus, Tokyo, Japan), with particular attention to adhesion and inflammation scores. For immunohistochemical staining, sections from the areas with the highest adhesion were evaluated using Collagen Type I and Collagen Type III antibody kits (Fine Test, Wuhan, China).

An independent surgeon, who was blinded to the group assignments, assessed the degrees of adhesion macroscopically after the U-incision using Nair's scoring system.[15] The Nair scoring system was applied as follows: a score of 0 indicated no evidence of adhesion between internal organs or between internal organs and the abdominal wall; the presence of a single band was scored as 1 point; the presence of two bands was 2 points; more than two bands, or multiple adhesions involving the intestines with abdominal wall adhesions, was scored as 3 points; direct adhesion of internal organs to the abdominal wall, regardless of the number of bands, was scored 4 points. Histopathological inflammation scores were determined microscopically as follows: absence of inflammation scored 0 points, presence of macrophages, lymphocytes, and plasma cells scored as 1 point, presence of macrophages, eosinophils, neutrophils, and plasma cells as 2 points, and the presence of inflammatory cell infiltration and micro abscess formation scored as 3 points. All paraffin sections were evaluated for immunohistochemical staining with Collagen Type I and Collagen Type III antibodies using a fluorescence microscope and imaging system (Nikon, Japan). Scoring was as follows: less than 3% staining or no staining scored as 0 points, 3-33% stained area as 1 point, 34-66% stained area as 2 points, and more than 66% stained area as 3 points. An experienced pathologist, who was blinded to the groups and experimental material, examined the sections.

Biochemical Analyses

Blood and peritoneal lavage samples for biochemical analysis were centrifuged at 3000 rpm for 5 minutes. The resulting serums were transferred to Eppendorf tubes and stored at -80 °C until analysis. Analyses were conducted at the end of the study. Serum fructosamine levels were measured from tail vein blood prior to laparotomy and from intracardiac blood at the end of the experiment using a Rat Frutosamine enzyme-linked immunosorbent assay (ELISA) kit (SRB Technology, Shanghai, China). The measurement range of the fructosamine kit was 5-1500 nmol/ml, with an intra-assay coefficient of variation (CV) of less than 10%, an inter-assay CV of less than 12%, and a sensitivity of 4.678 nmol/mL. Fibronectin levels in serum and peritoneal lavage were determined using an ELISA kit (ELK Biotechnology, Wuhan, China), with a detection limit of 6.25-400 ng/ml and a sensitivity of 2.81 ng/ml. Interleukin-6 (IL-6) and TGF-β levels in serum and peritoneal lavage samples were measured using ELISA kits (ELK Biotechnology, Wuhan, China). The detection limit was set at 7.82-500 pg/ml for IL-6 and 15.63-1000 pg/ml for TGF-β. The sensitivities were 3.3 pg/ml for IL-6 and 6.4 pg/ml for TGF-β. Both intra-assay and inter-assay CVs were less than 10% for IL-6 and TGF-β.

Statistical Analyses

IBM SPSS (Statistical Package for the Social Sciences) for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. All assessments adhered to a significance threshold of 0.05 (two-tailed p-value). Histograms and Q-Q plots were employed to assess the normal distribution of the variables. Descriptive statistics were presented using the median (25th percentile - 75th percentile) for continuous variables due to non-normal distribution, and frequency (percentage) for categorical variables. Between-groups analyses were performed using the Kruskal-Wallis test. Pairwise comparisons were adjusted using the Bonferroni correction method. Repeated measurements of fructosamine were analyzed using the Wilcoxon signed-rank test.

RESULTS

During the study, none of the animals died or developed intestinal obstruction due to adhesion. The study groups displayed similar median values according to the Nair Scoring System (p=0.129). The histopathological inflammation scores showed a median score of 0 (0-1) for the Sham group, significantly lower than those of the control, metformin, and pioglitazone groups (p=0.004) (Fig. 2). No significant differences

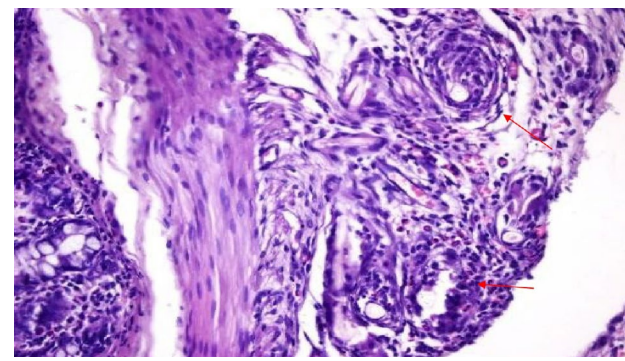

Figure 2. Inflammatory cell infiltration and micro abscess formation, H&E stained (400x magnification).

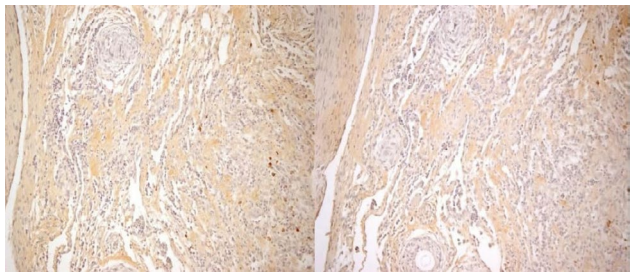

Figure 3. Over 66% area stained for collagen type I and III, IHC staining at 200x magnification.

were detected between the other groups (all, p>0.05). Type I and Type III Collagen staining scores were similar across all groups (p=0.374 and p=0.255, respectively) (Fig. 3). Histopathological results are summarized in Table 1.

Serum fructosamine levels significantly increased at the end of the intervention, except in the pioglitazone group (all, p<0.05) (Fig. 4). The serum IL-6 value for the pioglitazone group was 10.89 (8.65-15.46) pg/ml, significantly lower compared to the sham group [20.3 (14.4-25.68) pg/ml] and the hyaluronic acid group [23.34 (15.89-26.31) pg/ml] (p=0.002) (Fig. 5). Serum fibronectin levels were similar across groups (p=0.349) (Fig. 6). Serum TGF-β levels were lowest in the pioglitazone group [25.88 (22.5-34.28) pg/ml], showing a significant difference from the control group (p=0.008) (Fig. 7).

The peritoneal lavage IL-6 concentration in the pioglitazone group was 64.3 (55.26-74.62) pg/ml, significantly higher than in the control and sham groups (p<0.001) (Fig. 8). The lavage IL-6 value for the metformin group was 59.1 (50.57-67.38) pg/ml, also significantly higher than the control group (p<0.001). The peritoneal lavage fibronectin value of the sham group [24.66 (22.61-27.58) ng/ml] was significantly higher compared

Table 1. Median values by group based on histopathological parameters

	Groups					
	Control (n=10)	**Sham (n=10)**	**Hyaluronic Acid (n=10)**	**Metformin (n=10)**	**Pioglitazone**	**p (1)**
Nair's Scoring	0 (0-1)	0 (0-1)	1 (0-2)	1 (0-1)	1 (1-2)	0.129
Score 0	6 (60.0%)	7 (70.0%)	3 (30.0%)	4 (40.0%)	2 (20.0%)	0.563
Score 1	2 (20.0%)	2 (20.0%)	3 (30.0%)	4 (40.0%)	4 (40.0%)	
Score 2	2 (20.0%)	1 (10.0%)	2 (20.0%)	2 (20.0%)	4 (40.0%)	
Score 3	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	
Score 4	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	
Histopathological Inflammatory Score	2 (2-3)	0 (0-1)†	2 (1-2)	2 (2-3)#	2 (2-2)#	0.004
Score 0	0 (0.0%)	7 (70.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0.003
Score 1	1 (10.0%)	1 (10.0%)	2 (20.0%)	1 (10.0%)	0 (0.0%)	
Score 2	5 (50.0%)	1 (10.0%)	5 (50.0%)	6 (60.0%)	8 (80.0%)	
Score 3	4 (40.0%)	1 (10.0%)	2 (20.0%)	3 (30.0%)	2 (20.0%)	
Type I Collagen Immunostaining	1.5 (0-2)	0 (0-1)	1.5 (1-3)	2 (0-3)	1 (0-2)	0.374
Score 0	4 (40.0%)	6 (60.0%)	2 (20.0%)	3 (30.0%)	3 (30.0%)	0.864
Score 1	1 (10.0%)	2 (20.0%)	3 (30.0%)	1 (10.0%)	3 (30.0%)	
Score 2	3 (30.0%)	1 (10.0%)	2 (20.0%)	2 (20.0%)	2 (20.0%)	
Score 3	2 (20.0%)	1 (10.0%)	3 (30.0%)	4 (40.0%)	2 (20.0%)	
Type III Collagen Immunostaining	1.5 (0-2)	0 (0-1)	1.5 (1-3)	1 (1-3)	1 (0-2)	0.255
Score 0	4 (40.0%)	7 (70.0%)	2 (20.0%)	2 (20.0%)	3 (30.0%)	0.400
Score 1	1 (10.0%)	1 (10.0%)	3 (30.0%)	4 (40.0%)	3 (30.0%)	
Score 2	3 (30.0%)	1 (10.0%)	2 (20.0%)	0 (0.0%)	2 (20.0%)	
Score 3	2 (20.0%)	1 (10.0%)	3 (30.0%)	4 (40.0%)	2 (20.0%)	

Descriptive statistics are presented using the median (25th - 75th percentile) for continuous variables due to their non-normal distribution, and frequency (percentage) for categorical variables. (1) Between-group analysis was performed using the Kruskal-Wallis test. (2) Within-group analysis was performed utilizing the Wilcoxon signed-rank test. †: Significant difference from the Control group. #: Significant difference from the Sham group.

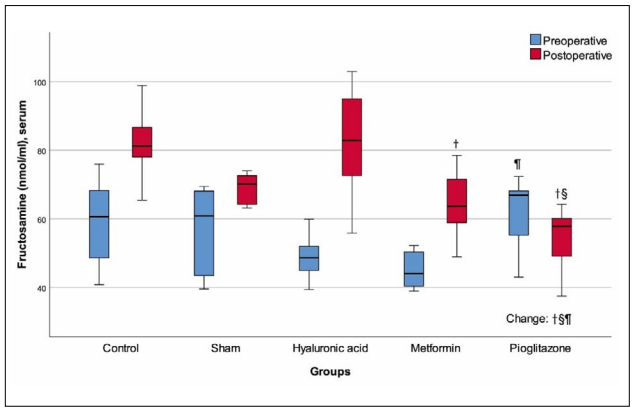

Figure 4. Serum fructosamine levels across different study groups.

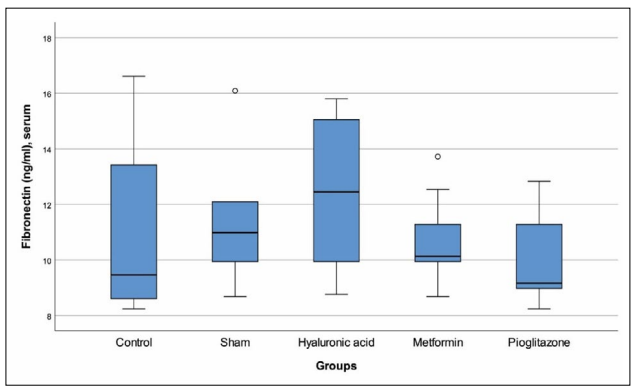

Figure 6. Serum fibronectin levels categorized by group.

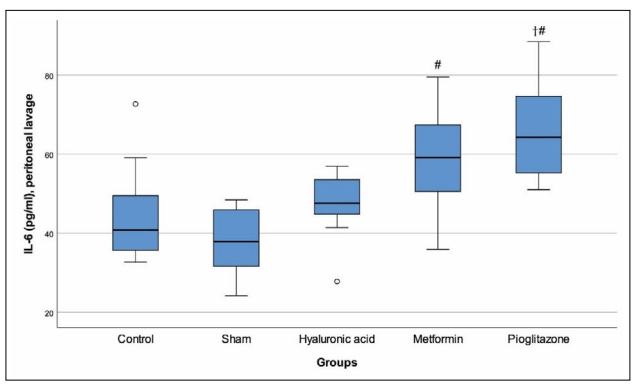

Figure 8. Peritoneal lavage IL-6 levels measured across groups.

to the pioglitazone and metformin groups (p=0.006) (Fig. 9). Additionally, TGF-β levels measured in the peritoneal lavage were similar across all groups (p=0.141) (Fig. 10, Table 2).

DISCUSSION

This study aimed to evaluate the potential role of metformin and pioglitazone in preventing or reducing the development of PIAA both histopathologically and biochemically in an experimental adhesion model. No significant differences were observed between groups in terms of macroscopic or immunohistochemical staining evaluations. Serum fructosamine levels remained unchanged with pioglitazone administration,

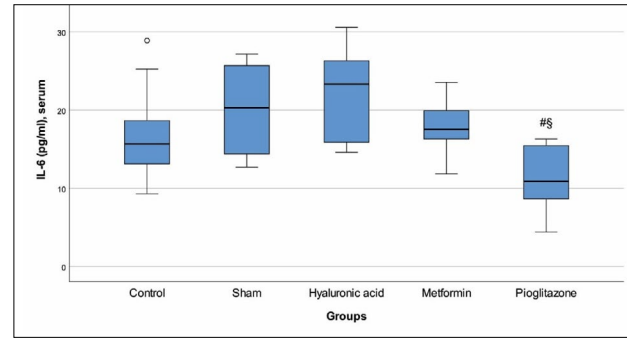

Figure 5. Serum IL-6 levels analyzed across various groups.

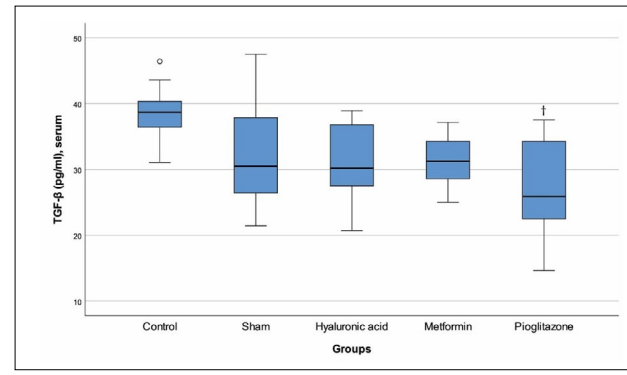

Figure 7. Serum TGF-β levels across various groups.

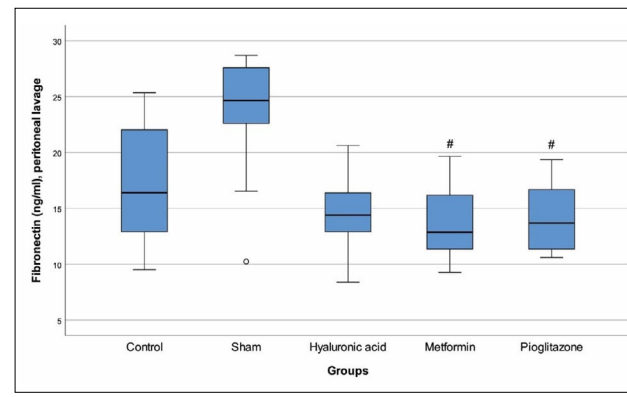

Figure 9. Peritoneal lavage fibronectin levels across different groups.

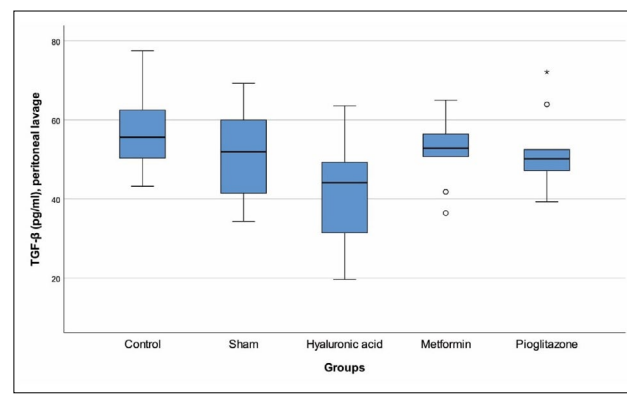

Figure 10. TGF-β levels in peritoneal lavage analyzed by group.

Table 2. Median levels by group for biochemical parameters

	Control (n=10)	Sham (n=10)	Hyaluronic Acid (n=10)	Metformin (n=10)	Pioglitazone (n=10)	p (1)
Fructosamine (nmol/ml), Serum						
Preoperative	60.68 (48.60-68.24)	60.88 (43.49-68.08)	48.60 (44.96-52.00)	44.00 (40.37-50.34)	66.85 (55.24-68.13)¶	0.009
Postoperative	81.24 (78.08-86.65)	70.12 (64.20-72.57)	82.87 (72.65-95.02)	63.69 (58.89-71.55)†	57.88 (49.10-60.12)†§	<0.001
p(2)	0.005	0.005	0.007	0.005	0.074	
Change(3)	22.93 (16.82-28.15)	10.05 (4.49-20.30)	36.10 (23.96-46.52)	19.72 (14.39-30.18)	-7.04 (-12.71- -6.14)†§¶	<0.001
Serum Measurements						
IL-6 (pg/ml)	15.68 (13.12-18.66)	20.30 (14.40-25.68)	23.34 (15.89-26.31)	17.53 (16.31-19.93)	10.89 (8.65-15.46)#§	0.002
Fibronectin (ng/ml)	9.46 (8.61-13.42)	10.98 (9.94-12.09)	12.45 (9.94-15.05)	10.13 (9.94-11.28)	9.17 (8.98-11.28)	0.349
TGF-β (pg/ml)	38.70 (36.42-40.35)	30.48 (26.42-37.85)	30.20 (27.50-36.78)	31.27 (28.57-34.28)	25.88 (22.50-34.28)†	0.008
Peritoneal Lavage Measurements						
IL-6 (pg/ml)	40.81 (35.68-49.51)	37.84 (31.64-45.89)	47.60 (44.83-53.55)	59.10 (50.57-67.38)#	64.30 (55.26-74.62)†#	<0.001
Fibronectin (ng/ml)	16.39 (12.91-22.02)	24.66 (22.61-27.58)	14.40 (12.91-16.39)	12.88 (11.36-16.17)#	13.69 (11.36-16.69)#	0.006
TGF-β (pg/ml)	55.64 (50.36-62.50)	51.89 (41.43-60.00)	44.11 (31.43-49.29)	52.86 (50.71-56.43)	50.18 (47.14-52.50)	0.141

Descriptive statistics are presented using the median (25th - 75th percentile) for continuous variables due to their non-normal distribution, and frequency (percentage) for categorical variables. (1) Analysis between groups was conducted using the Kruskal-Wallis test. (2) Within-group comparisons were assessed using the Wilcoxon signed-rank test. (3) Changes between preoperative and postoperative measures are reported, with positive values indicating an increase and negative values a decrease. †: Significant difference from the Control group. #: Significant difference from the Sham group. §: Significant difference from the Hyaluronic Acid group. ¶: Significant difference from the Metformin group.

indicating its role in postoperative glycemic control. Additionally, serum levels of IL-6 and TGF-β were significantly lower in the pioglitazone group, while both the metformin and pioglitazone groups showed increased IL-6 and decreased fibronectin levels in peritoneal lavage.

PIAA represents a significant health issue in abdominal surgery, negatively impacting quality of life and increasing healthcare costs. The mechanisms underlying their formation have not been fully elucidated. Although PIAA may develop in 55-90% of intra-abdominal surgeries, the time interval for symptom development, along with clinical presentation and intensity, varies widely and cannot be predicted reliably.[16] Secondary surgical interventions due to adhesions are required in nearly 3% of all laparotomies and 1% of all surgical operations. These interventions incur additional costs and increase the risks of prolonged operation times, bleeding, and luminal organ perforation.[17] Therefore, preventing PIAA is crucially more important than treating them.

To prevent adhesion formation, research has focused on blocking fibrin accumulation, removing resultant fibrin, and suppressing fibroblastic proliferation, which are critical initial steps in the pathogenesis of PIAA.[4] Despite the development of various pharmacological agents and physical barriers, alongside advances in surgical techniques, an ideal method has yet to be established. Treatments such as systemic or intraperitoneal corticosteroids, recombinant tissue plasminogen activators, antihistamine agents, high molecular weight dextran, saline, anti-cytokine agents, aprotinin, octreotide, and heparin have been explored for their anti-adhesion properties through various mechanisms affecting the coagulation cascade and inflammatory pathways. However, none have proven to be sufficiently effective in humans.[18] Currently, the most commonly used and relatively successful method involves physical barriers, such as membranes or gels, which reduce adhesion formation by physically separating the damaged peritoneal tissue from intra-abdominal organs. Materials such as a bioresorbable membrane composed of sodium hyaluronate and carboxymethylcellulose (Seprafilm), or a gel containing sodium hyaluronate (Sepracoat), are used to prevent adhesions.[19] Diamond et al. demonstrated that Sepracoat provided a safety profile comparable to a placebo and significantly reduced the incidence, severity, and extent of postoperative adhesion development compared to a placebo.[20] However, this efficacy has not been confirmed in some clinical studies, and not all physical barriers effectively protect against PIAA as they fail to address critical biological issues, including protection against bacterial infections and issues in managing biocompatibility and biodegradability.[18] Our study examined these inconsistencies in the results of such studies in our adhesion model, showing that applying hyaluronic acid gel did not significantly prevent adhesion formation and increased cytokine levels that contribute to adhesion. To overcome these difficulties, which we also observed, drug-

releasing anti-adhesion barriers have gained scientific interest as a potential strategy. Moon et al. used metformin-loaded agar films, chemically cross-linked with 20% concentrations of citric acid, as anti-adhesive barriers in their study. They demonstrated that the administration of this barrier for 14 days significantly reduced the clinical adhesion score and the thickness of the adhesion interface, suggesting the dual role of the physical barrier and metformin-based pharmaceuticals.[18] Metformin, a potent adenosine monophosphate-activated receptor activator used as an anti-diabetic medication, also has anti-fibrotic effects through the TGF-β-induced signaling pathway, cell metabolism, inflammation, and oxidative stress.[21] Rangarajan et al. reported that in a bleomycin-induced murine lung fibrosis model study, the administration of metformin was effective in preventing and slowing the progression of fibrosis, promoting the resolution of fibrosis, and reversing established fibrosis.[22] Experimental studies have also found decreased fibrosis induced by metformin in various models including folic acid-induced renal fibrosis, adenine-induced chronic kidney disease rat model, dehydroepiandrosterone-induced polycystic ovarian syndrome, radiation-induced pulmonary fibrosis, peritoneal tissue of a peritoneal dialysis animal model, skin of a bleomycin-induced scleroderma model, and peritendinous tissue of an injury-induced peritendinous adhesion rat model.[23] However, there is insufficient evidence from clinical studies regarding the anti-fibrotic effect of metformin. Our findings indicated that despite administering treatment at the top of the therapeutic index in the metformin group, postoperative glycemic control was not achieved, and the cytokines that contribute to adhesion formation increased significantly, suggesting that metformin negatively impacts PIAA formation. The inconsistency between our study data and some publications in the literature may be due to differences in doses or different species used in the studies affecting clinical outcomes. Since only a single dose of metformin was used according to our study design, a dose-related effect could not be determined, and it may have shifted from a therapeutic dose to a toxic dose, considering the genetic factors of the rats.

Pioglitazone is a member of the thiazolidinedione family and acts as a PPAR-γ agonist, which enhancing insulin sensitivity and exerting anti-inflammatory and antiangiogenic effects by influencing pathways in glucose and lipid metabolism, immune regulation, and endothelial function.[24] Besides pioglitazone, other synthetic PPAR-γ agonists include rosiglitazone, ciglitazone, and troglitazone. These agonists have been shown to block the secretion of inflammatory cytokines such as TNF-α, IL-1β, IL-6, inducible nitric oxide synthase, gelatinase B, matrix metalloproteinase-9 (MMP-9), and scavenger receptor A.[25] Postoperative macrophages, which are completely different from resident macrophages, secrete various substances including IL-1, IL-6, and TNF, and the induction of these cytokines is known to alter the course of the inflammatory process and adhesion formation.[4] Ongoing research since the discovery of PPAR-γ agonists has revealed additional functions, including anti-fibrotic and antioxidant properties. Deng et al. reported in an in vitro study that pioglitazone inhibits fibrosis and inflammation in non-alcoholic fatty liver disease by suppressing the expression levels of Tissue Inhibitor of Metalloproteinases 2 (TIMP2) and Platelet-Derived Growth Factor (PDGF) (26). Herrington et al. demonstrated a decrease in adhesive conditions with targeted treatment using pioglitazone both before and for five days after surgery in a chimeric mouse model of experimental endometriosis-associated postsurgical adhesions.[27] A 1 mg/kg/day dose of rosiglitazone has been shown to prevent or reduce adhesions both clinically and histopathologically in a rat uterine horn adhesion model, likely by decreasing the initial inflammatory response and subsequent exudation.[28] Similarly, Aksakal et al. reported that treatment with rosiglitazone, but not melatonin, was effective in preventing adhesion formation in a rat uterine horn model.[25] Hong et al. revealed in an experimental study that perioperative administration of pioglitazone improved arginase activity and reduced PIAA formation in wild-type, but not in CD11b-Cre/PPAR fl/fl mice, without compromising anastomotic healing.[29] Consistent with the literature, our findings indicate that postoperative glycemic control was achieved in the pioglitazone group, and inflammatory cytokines, which may contribute to adhesion formation, were significantly reduced. According to the results of our study, although it was not histopathologically confirmed that pioglitazone clearly prevented PIAA formation, it is reasonable to suggest that pioglitazone had a positive effect on its prevention. We recommend conducting further experimental and clinical studies with larger sample sizes and exploring different doses and treatment intervals to confirm the current data.

Limitations

The study has certain limitations that must be acknowledged. Firstly, as an experimental rat study, its conclusions need to be validated by additional experimental studies that explore different concentrations and application methods, and subsequently, by clinical trials, which are feasible given the well-established safety profiles of the drugs used in this study. The follow-up period of the study was limited to 10 days, which did not allow for evaluation of the long-term effects of pioglitazone and metformin on PIAA formation. Additionally, many factors that could influence the formation of PIAA were not assessed in our study, which could introduce bias. Therefore, evaluating various parameters throughout the study may prove valuable. While several scoring systems have been reported to assess PIAA during clinical and experimental trials, none have achieved universal validation. Consequently, researchers should interpret our results with caution, considering the limitation of available methodologies and the potential for inter-observer variability. Despite this unavoidable problem, we aimed to minimize variability by employing diverse approaches to examine PIAA formation, including histopathological and biochemical measurements.

CONCLUSION

Our study demonstrates that pioglitazone, unlike metformin, had positive effects on PIAA formation as indicated by biochemical results. Although no histological superiority was demonstrated, it appears that pioglitazone may influence the underlying process of PIAA formation in our experimental model. Further experimental and clinical studies with larger samples, varying doses, and different treatment durations are necessary to confirm and build upon our findings on PIAA formation.

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DENEYSEL ÇALIŞMA - *ÖZ*

Sıçanlarda oluşturulan abdominal adezyon modelinde pioglitazon ve metforminin etkisinin değerlendirilmesi

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AMAÇ: Sıçanlarda oluşturulan deneysel bir adezyon modelinde histopatolojik, immünohistokimyasal ve biyokimyasal analizler kullanılarak postoperatif karın içi adezyon (PIAA) gelişimini önleme veya azaltmada metformin ve pioglitazonun etkisini değerlendirmek.

GEREÇ VE YÖNTEM: Elli Wistar-Albino cinsi sıçan beş gruba ayrıldı: Grup I (kontrol), Grup II (sham), Grup III (Hyaluronik asit), Grup IV (metformin) ve Grup V (pioglitazon). Sham deney grubu dışındaki tüm deney gruplarında yapışıklıklar scraping model oluşturularak yapıldı ve 10 gün sonra değerlendirme için sıçanlara ötenazi uygulandı. Makroskopik adezyon dereceleri Nair skor sistemi kullanılarak değerlendirildi. Serum ve periton lavaj örneklerinde fruktozamin, IL-6, TGF-β ve fibronektin düzeyleri ölçüldü. Bağırsak doku örneklerinin değerlendirilmesinde histopatolojik skorlama sistemi kullanıldı ve immünohistokimyasal kitlerden yararlanıldı.

BULGULAR: Gruplar Nair skorlaması ve Tip I - Tip III Kollajen boyanma skorları açısından benzerdi (hepsi, p>0,05). Pioglitazon grubunda kontrol grubuna kıyasla serum IL-6 ve TGF-β düzeylerini anlamlı düzeyde düşük saptandı (sırasıyla p=0,002 ve 0,008). Metformin ve pioglitazon grupları peritoneal lavajda kontrol grubuna kıyasla daha yüksek IL-6 sergilerken, peritoneal lavajdaki fibronektin seviyeleri pioglitazon ile tedavi edilen sıçanlarda sham grubuyla karşılaştırıldığında daha düşük saptandı (hepsi, p<0.005).

SONUÇ: Deneysel adezyon modelinde PIAA oluşumunun önlenmesinde pioglitazon histolojik olarak olmasa da biyokimyasal olarak olumlu bir etkiye sahipti. PIAA oluşumu üzerindeki etkisini daha iyi anlamak için pioglitazonun farklı doz/süre rejimlerini kullanan ileri deneysel çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Adezyon; adezyon model; metformin; pioglitazon; PPAR.

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