INTRAPERITONEAL ADMINISTRATION OF ROSUVASTATIN PREVENTS POSTOPERATIVE PERITONEAL ADHESIONS BY DECREASING THE RELEASE OF TUMOR NECROSIS FACTOR

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Abstract

Objectives. The purpose of this experimental study was to demonstrate the reduction of peritoneal adhesions formation in rats after intraperitoneal administration of rosuvastatin, due to its anti-inflammatory effect.

Method. Peritoneal adhesions were induced in 120 Wistar-Bratislava rats divided into 4 groups (n=30), using a parietal and visceral (cecal) abrasion model. Group I was designated as control group; in group II, a saline solution was administered intraperitoneally; in groups III and IV, a single dose of rosuvastatin solution, 10 mg/kg and 5 mg/kg respectively, was injected intraperitoneally. The serum values of tumor necrosis factor (TNF-α) and interleukin-1 (IL-1α) were determined on day 1 and day 7 postoperatively (ELISA). Macroscopic assessment of the peritoneal adhesions was conducted on day 14.

Results. Rosuvastatin therapy induced a significant decrease of tumor necrosis factor serum levels in groups III and IV, on day 1 and day 7 (p<0.01). Intraperitoneal administration of rosuvastatin correlated with a decrease of mean interleukin-1α levels on postoperative day 1 in groups III (p=0.0013) and IV (p=0.00011), but not on day 7, where the differences were no longer statistically significant (p=0.8) The reduction of postoperative peritoneal adhesions in the experimental rat model is supported by the anti-inflammatory effect of rosuvastatin, mediated mainly by the tumor necrosis factor.

Conclusions. Rosuvastatin prevents the formation of postoperative peritoneal adhesions in rats. This effect may be linked to the inhibition of proinflammatory cytokines release in the early stages of adhesions formation. The present study suggests that rosuvastatin may be an efficient pharmacological agent in the prevention of postoperative peritoneal adhesions development, and requires further studies as it has a promising application value.

Keywords: rosuvastatin, peritoneal adhesions, inflammation, TNF-α, IL-1α

Introduction

The development of postoperative peritoneal adhesions remains an almost inevitable consequence of most abdominal surgical procedures and represents a major cause of morbidity and mortality. They are not always symptomatic, but may cause intestinal occlusion, chronic abdominal and pelvic pain, infertility, and difficult access in case of recurrent abdominal surgery [1].

Peritoneal adhesions represent pathological "connections" usually formed between the epiploon, intestinal loops, viscera, and the abdominal wall. Their appearance varies from fine, avascular films to actual connective tissue bridges that may contain vascular and nervous structures or may lead to direct contact between adjacent organs [2].

Current strategies for the prevention of postoperative adhesions may be grouped into 4 categories:

general principles, surgical technique elements, mechanical barriers, and pharmacological agents. Acting via inflammation suppression, coagulation manipulation, and an increase in the fibrinolytic activity, the pharmacological agents represent potentially efficient therapies.

Statins (inhibitors of HMG-CoA reductase, an enzyme playing a central role in cholesterol synthesis) are widely used in the treatment of hyperlipidemia and prevention of cardiovascular diseases, but they have also proven to have anti-inflammatory, anti-thrombotic, antioxidant, antifibrotic, and fibrinolysis-modulating effects [3].

In the process of peritoneal adhesions development, inflammation represents the initial response to peritoneal aggression [4]. The modulation of the inflammatory response triggered by the surgical trauma may prevent the development of adhesions [5]. Rosuvastatin is a member of the statin class, with a strong hypolipidemic effect. We set out from the hypothesis that rosuvastatin, through its anti-inflammatory effect, reduces the formation of peritoneal adhesions in an experimental rat model. The anti-inflammatory effect mechanism is linked with a decrease in the release of proinflammatory cytokines.

Material and method

A number of 120 white male Wistar-Bratislava rats provided by the Center for Practical Skills and Experimental Medicine of the Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, weighing 200 ± 20 g each, were randomly divided into 4 equal groups (n=30). A model for the induction of peritoneal adhesions was applied, by abrasion of the parietal and visceral peritoneum at the level of the cecum, with a Cytobrush (a spiral-shaped brush for endocervical sampling), over an area of approximately 2.0 x 2.0 cm. Abrasion was performed until punctiform bleeding occurred, as evidence of peritoneal trauma. Group I was designated as control group; in group II (n=30), 1 ml saline solution was administered intraperitoneally before the closure of the abdominal wall; in groups III and IV (n=30), a therapeutic procedure was initiated, by injecting intraperitoneally a rosuvastatin solution, with different concentrations: 2 mg rosuvastatin calcium/1 ml (10 mg/kg) in a single dose in group III, and 1 mg rosuvastatin calcium/1 ml (5 mg/kg) in a single dose in group IV. Surgical gloves without powder were used and the intervention lasted less than 20 minutes. All interventions were carried out by the same surgeon. The experimental study was carried out in strict compliance with the Helsinki Declaration.

On postoperative day 1 and day 7, blood samples were taken from the retro-orbital plexus. Blood samples were centrifuged at 3000 rpm for 30 minutes. Serum was stored at -20°C. The serum concentrations of tumor necrosis factor (TNF-α), interleukin-1α (IL-1α) were measured using ELISA kits (BioVendor – Laboratorni medicina a.s., Brno, Czech Republic), and RAF130R (TNF-α) and RAF047R (IL-1α), according to the manufacturer's instructions. The values were expressed in pg/ml. The detection limit for TNF-α was 11 pg/ml, and for IL-1α, 4 pg/ml.

On the 14th postoperative day the peritoneal adhesions formed in the 4 groups were documented and assessed from a quantitative point of view – adhesions formed at the site of the trauma and percentage of area coverage. For the qualitative assessment, the severity score according to the method of Evans et al was used [6], as follows: 0 degree – absence of adhesions; first degree – fine, avascular, and easily separable adhesions; second degree – adhesions separable by traction, with limited vascularization; and third degree – adhesions separable by dissection, with a rich vascularization.

Statistical analysis

The collected data was expressed as ± standard deviation of the selection mean value corresponding to the groups. In order to assess the differences among groups, the simple analysis of variance among groups (one-way ANOVA) was used, a confidence level of $p < 0.05$ being considered statistically significant, in which the null hypothesis may be rejected.

Results
TNF-α serum values

On day 1 postoperatively, the average of the TNF-α serum values in the control group (group I) was 134.26 pg/ml (SD: 11.90); in the group where saline solution was administered intraperitoneally (group II), it was 134.6 pg/ml (SD: 11.98); in the group where rosuvastatin with a concentration of 10mg/kg was administered (group III), it was 77.46 pg/ml (SD: 15.34); and finally, in the rats in whose case rosuvastatin with a concentration of 5 mg/kg was administered (group IV), it was 82.43 pg/ml (SD: 11.43).

On day 7 postoperatively, the average of the TNF-α serum values was 126.90 pg/ml (SD: 9.30) in group I, 127.43 pg/ml (SD: 11.06) in group II, 79.56 pg/ml (SD: 15.19) in group III, and 83.83 pg/ml (SD: 12.37) in group IV (Figure 1a).
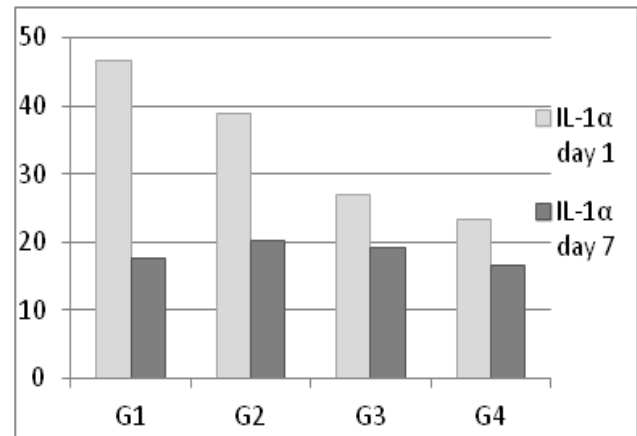
IL-1α serum values

On day 1 postoperatively, the average of the serum values of IL-1α in group I was 46.56 pg/ml (SD: 27.09), with 4 unmeasurable values. In group II the average was 38.86 pg/ml (SD: 22.80), with 3 unmeasurable values. In group III the average was 27.03 pg/ml (SD: 16.52), with 6 unmeasurable values. The average of the values in group IV was 23.23 pg/ml (SD: 14.63) with 6 unmeasurable values.

On day 7 postoperatively, the average for the serum values of IL-1α in group I was 17.53 pg/ml (SD: 15.20) with 8 unmeasurable values. In group II the average was 20.26 pg/ml (SD: 17.53), with 9 unmeasurable values. In group III the average was 19.1 pg/ml (SD: 16.68), with 11 unmeasurable values. The average of the values in group IV was 16.6 pg/ml (SD: 14.71), and 11 unmeasurable values (Figure 1b).
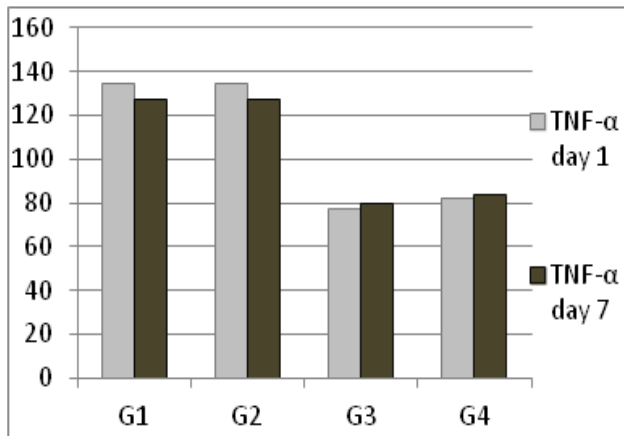
a. Average of TNF-α serum values (pg/ml) in the 4 groups, day 1 and day 7.

b. Average of IL-1α serum values (pg/ml) in the 4 groups, day 1 and day 7.

**Figure 1.** Average of TNF-α and IL-1α serum values.

Macroscopic assessment

In the control group (group I), peritoneal adhesions (with different degrees of severity) developed in 25 rats (83.33%), in group II – in 24 rats (80%), in group III adhesions were induced in 16 rats (53.33%), and in group IV – in 15 rats (50%) (Figure 2).
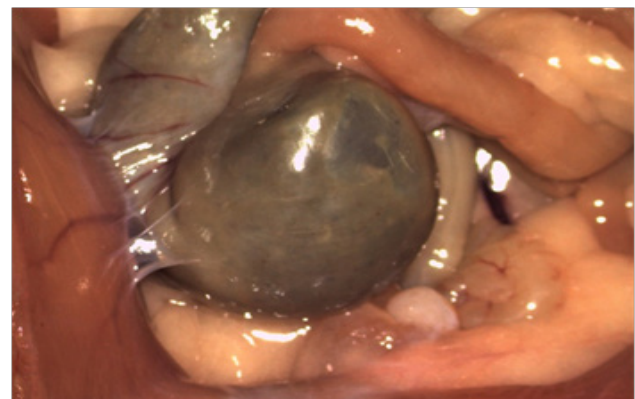
The area covered by adhesions (expressed as a percentage of coverage of the traumatized area) was 51.6% in group I, 50% in group II, and 26.66% and 27.5% in groups III and IV, respectively.

The mean macroscopic severity score was 2.03 (SD: 0.67) in group I (control group), 1.86 (SD: 0.74) in group II, 0.9 (SD: 0.56) in group III, and 0.9 (SD: 0.63) in group IV.
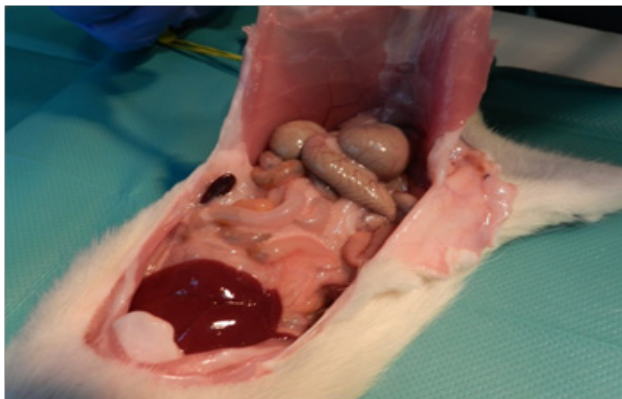
No adhesions

First degree adhesion

Second degree adhesion

Third degree adhesion

Figure 2. Macroscopic appearance of peritoneal adhesions.

The TNF-α serum values measured in the 4 groups were proportional to the degree of severity and the area covered by adhesions (Figure 3).
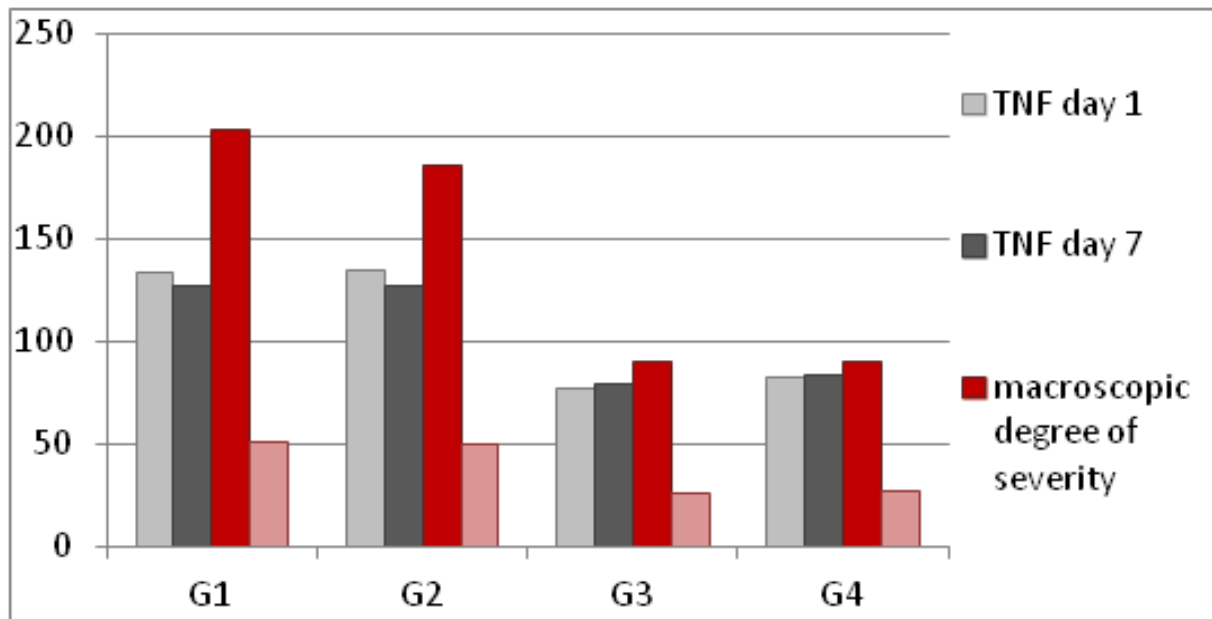

Figure 3. The evolution of the TNF-α serum values in the 4 groups, on postoperative day 1 and day 7 as compared to the macroscopic degree of severity and the area covered by adhesions.

Discussion

The selected experimental model for inducing adhesions, by abrasion of the parietal and visceral cecal peritoneum, replicates the surgical mechanical trauma and provides good results in terms of the number and quality of the adhesions formed [7].

Although the study of the pathogenetic mechanisms involved in the formation of adhesions and the process of finding efficient treatment methods have been ongoing for decades, postoperative peritoneal adhesions continue to represent a major cause of morbidity and mortality.

Pharmacological strategies for preventing the development of adhesions take into account the pathogenetic factors involved through the suppression of inflammation, manipulation of coagulation, and an increase in fibrinolytic activity. Statins potentially combine these effects. Although this class of drugs was introduced in clinical practice and used on a wide scale as hypolipidemic medication (through the inhibition of HMG-CoA reductase, an enzyme playing a central role in the synthesis of cholesterol), studies have demonstrated the existence of a series of other effects as well, such as anti-inflammatory, antifibrotic, antioxidant, and fibrinolysis-modulating effects [3].

Rosuvastatin is a long-acting statin which is efficient in the treatment of hyperlipidemia [8,9]. A series of experimental studies (on rats) have demonstrated the following effects:

prevention of postoperative peridural fibrosis [10], reduction of fibrosis in cyclosporine-induced nephropathy [11], prevention of postoperative knee intra-articular adhesions [12], improvement of the hepatopulmonary syndrome (through the inhibition of inflammatory angiogenesis) [13], and an increase in the anti-inflammatory activity and the inhibition of the proinflammatory functions (in cultured microglial cells) [14].

Lovastatin, simvastatin, and atorvastatin have proven to be efficient in preventing the formation of postoperative adhesions, when administered intraperitoneally as single therapy or in association with mechanical barriers [15,16,17,18]. Their action mechanism is not fully known, but anti-inflammatory and fibrinolysis-modulating effects - by increasing the tissue plasminogen activator (tPA) level and decreasing plasminogen activator inhibitor (PAI-1) - have been demonstrated [19].

Surgical peritoneal trauma induces an inflammatory response. Studies have proven that during the acute stage of the inflammatory response, the mesothelial cells and the peritoneal macrophages produce a multitude of proinflammatory cytokines (TNF-α, IL-1, IL-6, TGF-β1) which, either individually or synergistically, reduce the tPA synthesis and increase the release of PAI-1 [4,20].

Experimental studies have proven the existence of a correlation between the increase in the IL-1 and

TNF-α levels (postoperatively) and the formation of peritoneal adhesions [21,22]. They also suggest that the administration of anti-TNF-α antibodies reduces the formation of peritoneal adhesions, [23] however, there are certain studies that do not support these results [24].

The average of the TNF-α serum values for groups I and II did not show any statistically significant differences, either on day 1 ($p=0.914$), or on day 7 postoperatively ($p=0.84$).

Rosuvastatin therapy significantly decreased TNF-α serum levels in groups III and IV, on day 1 and day 7 postoperatively ($p<0.01$).

The IL-1α serum levels measured in group I were similar to those measured in group II, in that there were no statistically significant differences, either on day 1 ($p=0.23$) or on day 7 postoperatively ($p=0.52$).

Intraperitoneal administration of rosuvastatin correlated with a decrease of the mean IL-1α levels on postoperative day 1 in groups III ($p=0.0013$) and IV ($p=0.00011$), but not on day 7, where the differences were no longer statistically significant ($p=0.8$). These data were interpreted taking into account the large number of unmeasurable determinations.

Between group I (control group) and group II (in whose case a saline solution was administered intraperitoneally) there were no statistically significant differences in terms of the adhesions formed, from the point of view of the macroscopic assessment ($p=0.59$).

Rosuvastatin therapy (in groups III and IV) was correlated with the reduction of postoperative peritoneal adhesions (assessed in terms of number, area coverage, macroscopic degree of severity). Between group I (control group) and group III, as well as between the control group and group IV, the difference was statistically significant ($p<0.01$). Between groups III and IV there were no statistically significant differences.

Conclusions

Our study has demonstrated that rosuvastatin administered intraperitoneally prevents the development of peritoneal adhesions in an experimental rat model. This effect is associated with the inhibition of the inflammatory reactions in the early stages of adherence formation, through a decrease in the release of TNF-α and IL-1α cytokines.

The TNF-α serum values were closely correlated with the degree of adhesion formation, correlation which was not maintained for the IL-1α serum levels, a large part of these levels being unmeasurable.

There were no side effects associated with rosuvastatin therapy in the experimental model for inducing adhesions in rats. A clinical study with the purpose of continuing this experimental research will be able to support the efficacy and safety of the therapy with intraperitoneally administered rosuvastatin in preventing the formation of postoperative adhesions.

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