

EFFECT OF THE PLATELET-RICH PLASMA COATING OF IMPLANTED POLYPROPYLENE MESHES ON ABDOMINAL ADHESIONS AND OXIDATIVE STRESS IN RABBITS

Hypothesis / aims of study

The use of meshes in surgeries is a very common practice. The polypropylene mesh is the most used type of mesh, which causes adhesions with the intestine when in contact with it. Adhesion occurs when there is peritoneum surface injury, with intense peritoneal inflammatory response, fibrinous exudate development, pro-inflammatory cytokine activation, and reduced fibrinolysis. Fibrinolysis reduction results in the formation of fibrous bands between organs and the abdominal wall, called adhesions. This study proposes the use of platelet rich plasma (PRP) as a protective material since there have been a few studies on the effect of PRP on meshes, but these studies have demonstrated an improvement in the mesh incorporation, greater neovascularization, and decrease in recurrence and adhesions.

Study design, materials and methods

An experimental study was carried out with 30 New Zealand white rabbits (6 months old). **After induction of anesthesia (ketamine 40mg/kg and xylazine 3mg/kg), 5 mL of blood was collected from the animals by cardiac puncture to obtain the PRP. After plasma collection, a midline abdominal incision was made** and in each animal, two meshes were implanted: the polypropylene mesh without plasma on the left side and the same mesh coated with PRP on the right side. A defect in the abdominal wall was created, such that the mesh could be placed in the interface between the peritoneum and bowel loops. Then, a layered closure was carried out. **Ten rabbits served as controls and underwent median laparotomy and layered closure alone, without mesh insertion (euthanized at 60 days). From the 20 remaining rabbits, 10 rabbits had the mesh for 30 days and 10 rabbits had the mesh for 60 days.** The adhesion tenacity observations were graduated as 0- no adhesions, 1- easy release of adhesions from tissues, 2 - traction required to release adhesions, 3- blunt dissection required to release adhesions, and 4- fine dissection required to release adhesions. The modified Diamond scale (mDs), which divides the adhesions according to their extent, was used. Then, the meshes were removed for analysis. The inflammatory parameters were evaluated by measuring the myeloperoxidase (MPO) activity (indirectly measures neutrophil activity) and the N-acetylglucosaminidase (NAG) activity (indirectly measures macrophages). The oxidative stress parameters included evaluation of ferric reducing antioxidant power (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical reducing ability, reduced glutathione (GSH) endogenous antioxidant levels, specific endogenous antioxidants, and the determination of superoxide anion production. The results of the inflammatory and oxidative stress parameters were analyzed by analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The results were considered significant at $p < 0.05$. The Mann-Whitney-Wilcoxon Test for paired data was used to evaluate the tenacity and extent of adhesions. The comparison analysis of the control group was conducted using the Kruskal-Wallis test.

Results

Evaluation of the tenacity and extent of adhesions, after 30 and 60 days, showed no statistical difference between the two groups (with and without PRP) and of these groups for the control group. There were no differences between the groups, in the inflammatory and oxidative stress parameters. There was an increase in neutrophils (measured indirectly by MPO) in over 60 days in the group without PRP compared to the control group ($p < 0.05$), and a significant reduction of these cells in the group with PRP. The NAG activity (which indirectly evaluates macrophage activity) increased for all time points in the group without PRP, compared to the control. However, it decreased in the group with PRP compared to that in the group without PRP at 60 days. Regarding the effects of oxidative stress, the analysis showed a significant increase in antioxidant levels in the group with PRP at 60 days. This increase was measured by evaluating the FRAP and ABTS radical reducing ability (both tests evaluate nonspecific antioxidants). There was a decrease in FRAP in the group without PRP in 60 days compared to that in the control group, and the group with PRP displayed increased FRAP. There was a decrease in GSH in the group without PRP in 60 days compared to that in the control group, and the group with PRP. Superoxide anion production was also evaluated by nitroblue tetrazolium (NBT) reduction assay. The assay results indicated that the group without PRP had increased superoxide anion levels at all time points and that PRP causes a decrease in these levels at 60 days.

Interpretation of results

There has been no other study on the effect of PRP on the oxidative stress generated by polypropylene mesh implantation. Although the group with PRP did not present differences in adhesions, the evaluation of inflammatory activity and oxidative stress indicated the use of PRP as beneficial. This result is significant since complications such as erosion, extrusion, and adhesions are closely related to inflammatory reactions to the implant. There are a few studies on the use of PRP on meshes for hernia repairs. An *in vitro* study with seven types of meshes with PRP achieved adhesion reduction and improved biocompatibility with its use after 6 weeks {1}. In this study, the possible benefit of the use of PRP occurred at a later stage. This agrees with the findings of Gerullis et al., who studied the *in vivo* and *in vitro* biocompatibility of mesh coated with autologous plasma and observed no effect of the mesh on early inflammatory events of inflammatory infiltration and macrophage invasion {1,2}. The hypoxia generated by surgical trauma is a determining factor for the formation of adhesions, this oxygen deficit generates oxidative stress, which is highly damaging for cellular functions {3}. Immediately after implantation and with the onset of the inflammatory response, neutrophils begin to produce oxidants. Production of reactive oxygen species, proteases, and growth factors, mediated by neutrophils and macrophages, results in tissue destruction, fibroblast proliferation inhibition, aberrant collagen accumulation, and fibrosis {3}. In addition, polypropylene oxidation produces free radicals with subsequent depolymerization, oxidative degradation, leaching, and hydrolysis. These changes result in the loss of mesh structure with changes in molecular weight and mechanical integrity. In this study, the use of PRP reduced the oxidative stress by significantly increasing the FRAP and ABTS radical reducing ability. This reduction is important for the maintenance of implant integrity. There was also an increase in reduced glutathione

(GSH), an important biological antioxidant with a protective role in adhesion formation {3}. A decrease in superoxide anion levels, which increases with surgical trauma, was detected. Fibroblasts, when exposed to superoxide anions, increase the TGF-beta and collagen I levels, both involved in the pathogenesis of the adhesions {3}. The PRP usage results are very interesting because they indicate a decrease in the occurrence of adhesion triggering events. A decreased inflammatory response was observed with decreasing levels of NAG in the lysosome of activated macrophages. This is a significant observation since the concentration of macrophages at the site is high for a few hours after implantation and the real invasion takes place after 7 days. The inflammatory reaction continues for several months, depending on the material implanted and the site of implantation. This process becomes chronic over time, with the formation of small granulomas, and the result ranges from fibrosis to functionally normal tissue. The extensive presence of macrophages on site suggests a determinant role of macrophages in tissue remodeling after mesh implantation {2}.

In addition, the levels of MPO, an important inflammatory marker and an enzyme present in neutrophils, decreased with PRP. MPO is a heme-protein, which uses hydrogen peroxide and chloride ions to generate cytotoxic acids and diffusible radical species {3}. Studies have revealed an important role of this enzyme in the development of fibrous tissue, indicating that MPO inhibition could be a target for therapeutics to reduce post-operative adhesions. Thus, the combination of lower inflammatory response (indicated by decrease in MPO activity) and lower NAG levels (associated with decreased oxidative stress), potentially reduces the risk of fibrosis and other integration complications and maintains the physical integrity of polypropylene.

Concluding message

The use of PRP in polypropylene meshes showed no significant difference in the presence and severity of the adhesions. However, there was a reduction in the inflammatory response and oxidative stress with PRP use.

References

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Disclosures

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