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ORIGINAL PAPER

PLATELET-RICH AUTOPLASMA EFFECT ON INTESTINAL ANASTOMOSIS REGENERATION IN RABBITS

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Abstract

Introduction. Wound regeneration is an important process in surgical interventions. Failure of an intestinal anastomosis is an important intestinal surgery problem.
The objective of the study. To evaluate the effect of platelet-rich plasma (PRP) and compare the effectiveness of various application methods in intestinal anastomosis regeneration in rabbits.
Methods. Platelet-rich auto-plasma was obtained from 81 rabbits, which was used to study two methods of applying PRP (injection into the muscle layer / soaking in the intestinal wall) in comparison with the control group, on the end-to-end small-intestinal anastomosis.
Results. The analysis of the histological and morphometric data showed that the regeneration between the samples of intestinal anastomosis treated with PRP soaking and injections into the intestinal wall is more favorable in comparison with the control group, which, in turn, was determined by the quantitative ratio of inflammatory infiltrate, fibroblast proliferation, neo-angiogenesis, and collagen deposition. PRP

Résumé

L'effet de l'auto-plasma riche en plaquettes sur la régénération de l'anastomose intestinale chez le lapin

Introduction. La régénération de la plaie est un processus important dans les pratiques chirurgicales. Cependant, l'échec d'une anastomose intestinale reste un problème de chirurgie intestinale.
L'objectif de l'étude. Évaluer l'effet du plasma riche en plaquettes (PRP) et comparer l'efficacité de différentes méthodes d'application dans la régénération de l'anastomose intestinale chez le lapin.
Méthodes. Un auto-plasma riche en plaquettes a été obtenu chez 81 lapins, qui ont été utilisés pour étudier deux méthodes d'application de PRP (injection dans la couche musculaire / immersion dans la paroi intestinale) par rapport au groupe témoin sur l'anastomose de bout en bout de l'intestin grêle. .
Résultats. L'analyse des données histologiques et morphométriques de l'étude a montré que la régénération entre les échantillons d'anastomose intestinale traités avec du trempage au PRP et les injections dans la

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soaking in the intestinal wall significantly increased the number of adhesions battles. The analysis of the deformation and strength characteristics of the formed anastomoses also showed significantly high values of the rupture strength of the anastomoses treated with PRP, in contrast with the control group.

Conclusion. PRP injection into the muscular layer of the intestinal wall significantly reduces the development of adhesions in comparison with the PRP-saturated group of intestinal anastomoses. Further research is needed to clarify the optimal method of PRP application, which will improve the regeneration of the intestinal anastomosis.

Keywords: intestinal anastomosis, platelet-rich plasma, PRP–therapy, wound healing, rabbit.

List of abbreviations:
FGF – fibroblast growth factor
IGF – insulin-like growth factor
Lm – lymphocyte count
Mph – macrophage count
Np – neutrophil count
Pc – plasma cell count
PDGF – platelet-driven growth factor
PRGF – plasma-rich in growth factor
PRP – platelet-rich plasma
TGF-β – transforming growth factor

paroi intestinale est plus favorable que celle du groupe témoin, laquelle a été déterminée par la méthode quantitative, le rapport de l'infiltrat inflammatoire, la prolifération des fibroblastes, la néo-angiogenèse et le dépôt de collagène. Le trempage de PRP dans la paroi intestinale a considérablement augmenté le nombre de procès d'adhésions. L'analyse des caractéristiques de déformation et de résistance des anastomoses formées a également révélé des valeurs significativement élevées de la résistance à la rupture des anastomoses traitées avec du PRP, contrairement au groupe témoin.

Conclusion. L'injection de PRP dans la couche musculaire de la paroi intestinale réduit considérablement le développement d'adhérences par rapport au groupe d'anastomoses intestinales saturé en PRP. Des recherches supplémentaires sont nécessaires pour clarifier la méthode optimale d'application du PRP, ce qui permettra d'améliorer la régénération de l'anastomose intestinale.

Mots-clés: anastomose intestinale, plasma riche en plaquettes, PRP-thérapie, cicatrisation des plaies, lapin.

Introduction

Failure of an intestinal anastomosis is an intestinal surgery problem which has not been solved yet. However, there are a lot of studies aimed to reduce the potential life-threatening complications[1].

Today, the global practices concentrate on studying the wound regeneration at the cellular level. It is obvious that the platelets are the first blood cells responding to a wound, it is also known that they promote tissue regeneration by growth factor and other active agents[2]. Growth factors secreted by the platelets are polypeptide molecules with different structures and functions. Platelet-driven growth factor (PDGF), transforming growth factor (TGF-β), vascular endothelial growth factor, fibroblast growth factor (FGF), insulin-like growth factor (IGF) are among the most critical growth factors[3]. Therefore, these factors are known for their mutagenic and chemotactic properties which promote wound healing.

Nowadays, platelet-rich plasma is widely used in clinical practices, because high concentration of growth factors should promote better tissue regeneration[4-6]. Despite a large number of studies devoted to PRP effect on intestinal anastomosis regeneration, still the impact of auto-plasma on adhesion and different methods of PRP application are underdeveloped[7,8]. As a rule, soaking the intestinal walls with PRP is followed by anastomosis[5,9], although injected PRP, which proved to be highly effective in other areas of medicine[5,10-12], has not been applied to intestinal anastomosis.

Regeneration of intestinal anastomosis is surely an important process in surgical practices, because a breach in the continuity of the intestinal anastomosis causes wound disruption and, as a result, a high morbidity and mortality rate. The first three days are the crucial period to form intestinal anastomosis[13], when the wound is unstable, and the suture suffers from wound-bursting pressure, and it would be helpful to stimulate the regeneration of intestinal anastomosis.

It is in this period when the growth factors secreted by the platelets could play the key role in regulating the migration of the intestinal epithelial cells and anastomosis regeneration.

It should be noted that many experimental studies dealing with the strength of the performed intestinal anastomoses define wound-bursting strength at an increased intestinal pressure[5-9], however, we believe the anastomosis regeneration indicators should

be extended by measuring the intestinal anastomosis strength with morphometric indicators[14,15].

The objective of the study

To evaluate the effect of platelet-rich plasma (PRP) and compare the effectiveness of various application methods in intestinal anastomosis regeneration in rabbits.

Materials and Methods

Laboratory animals: 81 laboratory rabbits, chinchillas used in the research, were of different genders, as intestinal anastomosis regeneration is not driven by this factor[16], which enables the usage of this microsurgical suturing technique for intestinal anastomosis.

PRP preparation and activation

All procedures were performed aseptically at the room temperature. Before the anesthesia injection and experiment, we took 8 ml of blood from a peripheral vein in the ear of laboratory animals to PRGF EndoRet (Plasma Rich in Growth Factors from Biotechnology Institute, Spain) test tubes with *ACD-A anti-coagulant* recommended for PRP-therapy. PRP was obtained using Labofuge 200 centrifuge (Germany) under the test tube instructions from a manufacturer.

To evidence the validity of the experiment, flow cytometry (*ADVIA 120 Hematology System*, Bayer) was used to count the platelets in every PRP sample.

In the 1st group of the laboratory animals, before applying the anastomosis, the resected intestinal edges were submerged into activated PRP in a Petri dish for 10 min until PRP solution was completely jellified.

In the 2nd group of laboratory animals, before applying the anastomosis, the muscular layer of the intestinal wall was injected with platelet-rich autologous plasma in the amount of 0.2 ml per 1 cm^2. A pre-anastomosis and post-anastomosis bowel were circularly injected.

In the 3rd group of laboratory animals, before applying the intestinal anastomosis, the muscular layer of the intestinal wall was injected with psychological saline solution in the amount of 0.2 ml per 1 cm^2. A pre-anastomosis and post-anastomosis bowel were circularly injected.

The animals recovered from the anesthesia when the anesthetic drugs were not injected any longer. Extubation was performed with the steady spontaneous breathing, then an animal was transferred to a clean prepared cage.

Ketonal 20 mg/kg was provided in the first 24 hours for postoperative analgesia. During the experiment, the animals were housed in kept a vivarium in Atchabarov Research Center in accordance with Standard operational procedures for treatment and care of the animals in the postoperative period. The Standard is developed together with the Laboratory of Experimental Medicine.

The laboratory animals were euthanized with the Almabaev method by administering lethal doses of thiopental sodium and saturated potassium chloride solution IM (Euthanasia methods for laboratory animals in experiments // Patent RK „ : 11756// 15.07.2002).

The laboratory animals were euthanized randomly depending on the observation period, by 6 heads in every group.

Twenty and one laboratory animals were euthanized in 7 days after surgery by 7 heads in every group when the wound-bursting pressure was evaluated. Once the laboratory animals were euthanized, relaparotomy was performed, anastomosis was identified and carefully isolated from the adjoining adhesions. A segment of approximately 4 cm in length with the anastomosis in the middle was resected. The obtained samples were washed with saline solution from feces.

Evaluation of deformation and breaking strength indicators for anastomosis

The resected segments of the anastomosis were placed in a testing device (Tinius Olsen 1 ST, Redhill, Surrey, UK) with HORIZON app at the distance of 10 mm between the clamps. The extension rate (Ve) was 5 mm/min and was constantly applied. The width of the intestinal wall was 975±95mkm on average. The minimal force necessary to induce the anastomosis rupture was taken as the anastomosis breaking strength. This strength was defined from a force-deformation curve prepared by a special app (HORIZON). Before the experiment, the segments were stretched at 0.1 H to apply the force evenly over the anastomosis site, which provides more accurate attachment of the samples to the clamps of the testing device.

Evaluation of adhesions

Severity and area of the adhesion between the small-small intestine and abdominal wall were evaluated in accordance with a widely system of parameters[17] (Table 1).

It should be noted that the adhesions were evaluated by three independent researchers who had not been informed about the surgical procedures. The

Table 1. Macroscopic evaluation of adhesion intensity in the abdominal cavity[17]

Scores	*Number of adhesions*	*Adhesion structure*	*Adhesion area*	*Endodermal canal deformation*
0	no adhesion	no	no	no
1	one adhesion between the organs or between organs and abdominal wall	membranous	1 anatomic site (in our case blind intestine)	Slight deformation without narrowing of lumen
2	2 adhesions between the organs or with abdominal cavity	soft, avascularized	1 floor of abdominal cavity (blind intestine + other organs)	Moderate deformation without narrowing of lumen
3	More than 2 adhesions between organs or with abdominal cavity	solid, avascularized	3 floors of abdominal cavity	deformation, narrowing of ½ lumen
4	Adhesion conglomerate	solid, vascularized	More than 2 floors	Expressed deformation, narrowing of more than ½ lumen

obtained data were summarized and averaged for each animal.

Histological and morphological evaluation

Histological examination requires the anastomosis samples, which were taken and immediately fixed in 4% formaldehyde solution. The samples were dehydrated, embedded into paraffin and cut into 4-μ m sections, stained with hemotoxylin and eosin (H & E), and examined by two blinded expert pathologists. The main purpose of the histological examination was to evaluate the regenerative process along the intestinal suture in three groups in 24, 48, 72 hours, and 7 days after surgery. The evaluation parameters were inflammation intensity (leucocytic infiltration, vascular stasis, clotting, edema), connective tissue formation and structure, formation of new vessels, joining of intestinal wound edges, presence and time of epithelization.

Morphological method involves the usage of an application (Image-Pro plus 6.0; Media Cybernetics).

Morphological method examines the following indicators: fiber – crypt index (f/c index), lymphocyte count (Lm), neutrophil count (Np), plasma cell count (Pc), macrophage count (Mph), intraepithelial lymphocyte count (I/e lm), epithelial stromal coefficient (e/s), mitosis count in 1 mm^2 outside lymphoid follicles[18].

Animal biological material was disposed of under the procedure for biological waste disposal (these instructions for biological waste disposal are developed under the subclause 46-11 of the Article 8 of the Law of the Republic of Kazakhstan as of 10 July 2002 „On Veterinary Medicine").

Statistical evaluation

A package of statistical software R 3.4.4. for Windows was used for statistical analysis. Arithmetic mean value (M), a standard deviation (SD) were derived from the quantitative indicators. The results are presented as M±SD. Qualitative features were described in absolute (n) and relative values (%). Variability indexes were calculated. The differences between the parameters under questions were considered to be statistically important at $p<0.05$.

Unidimensional variance analysis ANOVA was chosen to be a statistical test. This test assumes that the samples from the groups are independent, and F-distribution is used to test the hypothesis in case of variance analysis. Mann-Whitney U-test (Wilcoxon test) was performed to evaluate the deformation and breaking strength indicators for the anastomoses.

Results

PRP analysis

Blood samples taken from the laboratory animals showed a mean hematocrit of 38 ± 5.4%, a mean leukocyte count of 7.9 ± 4.1 10^3/μL, a mean platelet count of 580 ± 190 10^3/μL.

Obtained 8 ml of whole blood yielded a harvest of 0.7-0.8 mL of PRP, with a mean platelet concentration of 1302 ± 480 10^3/μL. Thus, we managed to achieve a three-fold increase in platelet concentration in comparison with the initial count, leucocyte count in PRP samples was 1.1 ± 0.6 10^3/μL.

Analysis of histological and morphological data

Analysis of the morphological indicators for the 1st, 2nd groups of the laboratory animals (soaking) revealed the maximum infiltration with the neutrophil leukocytes on the 2nd and 3rd days, by 7th day their concentration decreased by 32% in comparison with that in the control group (Table 2). A decline in the specific protective reactions manifested in a decrease of plasma cell count[19] in all groups of the laboratory animals could be observed on the 3rd day after surgery,

Table 2. Dynamics in morphological indicators

Morphological indicators	*1st day*			*2nd day*		
	1st group	*2nd group*	*3rd group*	*1st group*	*2nd group*	*3rd group*
f/c index	0.5	0.6#&	0.4	0.6	0.6	0.5
Lm	10±0.1†	10±0.7#	14±0.4	12±0.2†	11±0.6#	15±0.8
Np	2.1±0.3†	2,0±0.6#	6±0.1	2.7±0.2	2.7±0.4#	6.4±0.5
Pc	5.2±0.2†	4.9±0.3#	6.9±0.4	5.6±0.6†	5.1±0.2	7.1±0.3
Mph	2.4±0.3†	2.5±0.4#	2.1±0.6	2.1±0.1†	2.0±0.4#	1.7±0.1
l/e lm	2.9±0.1	2.8±0.3	2±0.5	2.8±0.6	2.7±0.4	2.6±0.4
e/s	72±1†	70±2#	62±2	73±2†	72±1#	65±3
Mitosis	0.6±0.1†	0.6±0.3#	–	1.1±0.2†	1.0±0.3	1.3±0.2

Morphological indicators	*3rd day*			*7th day*		
	1st group	*2nd group*	*3rd group*	*1st group*	*2nd group*	*3rd group*
f/c index	0.7†	0.7#	0.6	0.9	1.0#&	0.7
Lm	12±0.4†	11±0.1#	15±0.2	10±0.6†	9±7.8#	11±1.2
Np	2.4±1†	2.3±0.6#	7.2±0.3	1.9±0.7†	1.7±0.8#	6.9±0.1
Pc	4.4±0.8	4.3±0.9#	5.1±0.4	2.9±0.7†	2.8±0.3#	6.0±0.6
Mph	2.0±0.3	2.4±0.1#	2.0±0.2	2.3±0.2†	2.6±0.4# &	2.1±0.1
l/e lm	2.8±0.7	2.9±0.1	3.2±1.4	2.9±0.6	3.0±0.4	4.0±0.2
e/s	74±1†	73±2	66±1	75±1†	74±1#	67±2
Mitosis	1.6±0.3	1.9±0.2#&	0.7±0.2	2.0±0.4†	2.5±0.2#&	1.2±0.3.x

Notes: e/s – epithelial stromal coefficient, f/c index – fiber-crypt index, I/e lm – intraepithelial lymphocyte count, Lm – lymphocyte count, Mitosis – mitosis count in 1 mm^2 lymphoid follicles, Mph – macrophage count, Np – neutrophil count, Pc – plasma cell count;
† –1st group from 3rd, # – 2nd group from 3rd, & – 1st group from 2nd confidence indicators (p <0.05).

Table 3. Adhesion evaluation

Criteria	*1st day*			*2nd day*		
	1st group	*2nd group*	*3rd group*	*1st group*	*2nd group*	*3rd group*
Number of adhesions	2±1	0±1	0±1	4±2	1±1	1±1
Adhesion structure	2±1	0±1	0±1	3±1	0±1	0±1
Adhesion extension	2±1	0±1	0±1	2±1	0±1	0±1
Deformation of endodermal canal	2±1	0±1	0±1	3±1	0±1	0±1
Result	8±3	0±1	0±1	12±4	1±1	1±1

Criteria	*3rd day*			*7th day*		
	1st group	*2nd group*	*3rd group*	*1st group*	*2nd group*	*3rd group*
Number of adhesions	4±2	1±1	1±1	4±2	2±1	2±1
Adhesion structure	3±2	1±1	1±1	4±1	3±1	2±1
Adhesion extension	3±2	1±1	1±1	4±1	1±1	1±1
Deformation of endodermal canal	3±1	1±1	1±1	3±2	1±1	1±1
Result	13±4	1±1	1±1	16±4	4	4

although a plasma cell count in the 1st and 2nd groups was reliably less than that in the control group.

A mitosis count in crypt area is reliably higher in the 2nd group of the laboratory animals on the 3rd and 7th days than that in the 1st and 3rd groups, which means their higher capacity for regeneration. Macrophage concentration is reliably higher in the 1st and 2nd groups in comparison with the control

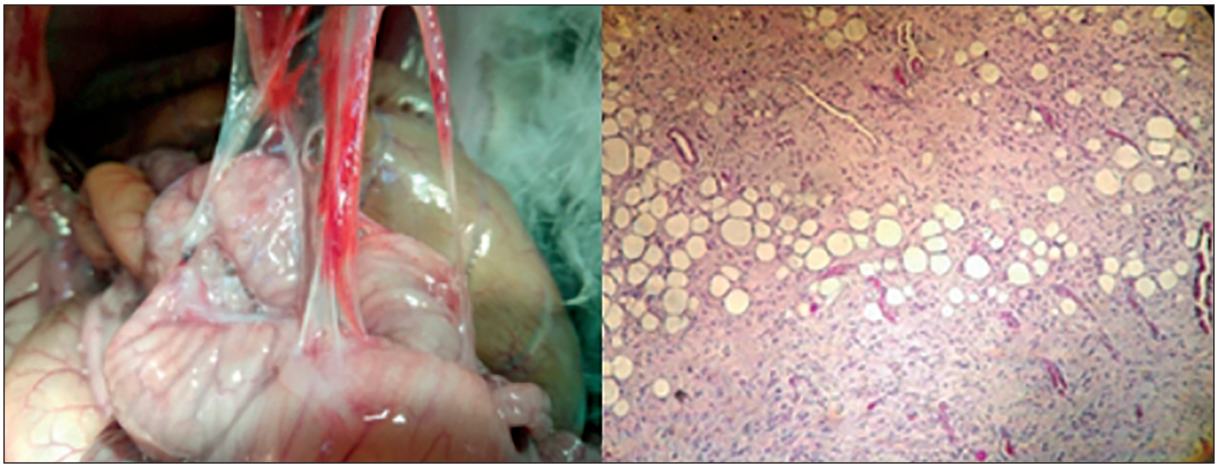

Figure 1. 1st group of the laboratory animals (PRP soaking, 7th day). Extensive adhesive process, vascularized solid adhesions (left). A histological picture of an adhesion (right): Hematoxylin and eosin staining ×20.

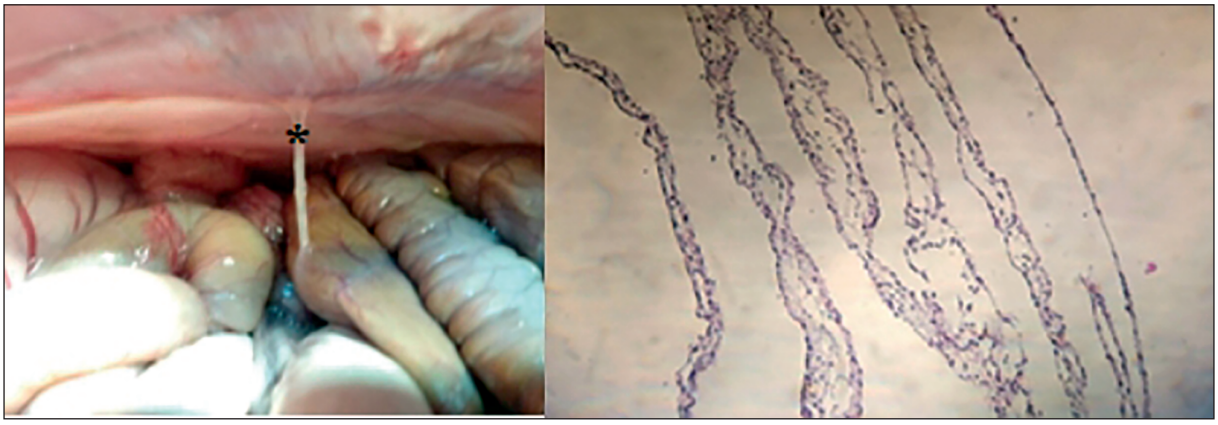

Figure 2. 2nd group of the laboratory animals (PRP injection, 7th day). Separate, soft adhesions (left). Neo angiogenesis is not found. A histological picture of an adhesion (right): Hematoxylin and eosin staining ×20.

group during all observation period, although the 7th day showed significantly higher macrophage concentration in the 2nd group than in the 1st group, which surely illustrates an extension of tissue growth area.

Notably, an epithelial stromal coefficient (e/s) fell in the 3rd group of laboratory animals, which evidences for the reduction in the content of epithelial cell count and more amount of the connecting tissue.

Lymphocyte count fell on the 3rd day in the 2nd group in comparison with the control group, the 1st group showed less considerable fall in the lymphocyte count on the 7th day.

Macroscopic examination and histology

All laboratory animals were euthanized at the agreed time, no serious complications or diseases were observed during the experiment. A macroscopic examination of the abdominal cavity in all laboratory animals did not find any failure with the intestinal anastomosis, peritonitis or any other infection.

The criteria of the adhesion evaluation were applied to evaluate the examination results (Table 3) by three degrees of adhesion intensity:

- 0–4 scores – 1st degree (minor adhesive process);
- 5–10 scores – 2nd degree (moderate adhesive process);
- 10 scores and more – 3rd degree (intense adhesive process).

Adhesion evaluation illustrated that the formed anastomoses treated by PRP (soaking method) in the 1st group of laboratory animals showed the highest degree of adhesion intensity (Figure 1) ($p = 0.01$) in comparison with the anastomoses treated by PRP (injection) in the 2nd group of the laboratory animals (Figure 2) and in the 3rd group of the laboratory animals (control).

The microscopic evaluation (Table 4) showed significant epithelization of mucosa for the 1st group ($p = 0.032$), 2nd group ($p = 0.041$) in comparison with the control group. Neo angiogenesis driven reorganization of the first capillary network is reliably higher

Table 4. A microscopic evaluation of the formed anastomoses

Indicators	*PRP soaking, 1st group*	*PRP injection, 2nd group*	*Control, 3rd group*
Epithelization	2.4±0.4	2.3±0.5	1±0.2
Inflammation	4±0.7	3.1±0.5	3.2±0.5
Collagen	2.3±0.8	2.2±0.6	1.9±0.3
Fibrosis	2.2±0.3	2.5±0.5	2.0±0.4
Neo angiogenesis	3±0.7	2.9±0.8	1.7±0.2

Table 5. Deformation and breaking strength indicators

Parameters	*1st group*	*2nd group*	*3rd group*
Mean	1.76	1.81	1.54
Mean square deviation	0.28	0.17	0.23
Max	2.49	2.51	2.30
Min	1.32	1.30	1.17

in the 2nd group (p = 0.029) than in the control group. It should be also noted than no valid differences in an inflammatory infiltrating reaction, collagen and fibrosis formation in comparison with the control group were found.

Analysis of deformation and breaking strength indicators

The mean value of the deformation and breaking strength indicators (Table 5) for anastomosis rupture in the 1st group was 1.76 ± 0.28 H, in the 2nd group was 1.81 ± 0.17 H, no statistical differences between the two groups were observed (p = 0.69) in comparison with the 3rd group (a control group) 1.54 ± 0.23 H (p = 0.04) and (p = 0.05) (Mann-Whitney *U-test* (Wilcoxon test)). A rupture was always within the formed anastomosis sites.

Discussion

Undoubtedly, platelets as the first responders for different traumatic injuries of the body integrity serve a therapeutic purpose which, in its turn, should be further examined[20].

PRP is a blood plasma with a great amount of platelets which release the growth factors for wound healing and tissue regeneration. PRP preparation is a procedure with the minimal technical costs, while its efficiency in tissue regeneration is supported by many randomized clinical trials in different areas of medicine[8].

Activated platelets release more than 300 active agents from their intracellular granules, contain a lot of cytokines, mitogens, anti-inflammatory factors, and other bioactive molecules which are important regulators in a complicated microenvironment of the regenerative process, which, in its turn, enhance the healing process. Thus, all components necessary for tissue regeneration are concentrated in a wound area.

However, PRP preparation methodology requires special attention, which will definitely be illustrated by further studies.

Despite the benefits from PRP therapy, it has some drawbacks at the preparation and activation stages, as well as at a PRP application stage. In our research, infectious contamination of the anastomosis when the intestinal edges are submerged into PRP solution is one of the process difficult to control, which undoubtedly caused additional difficulties during the surgery.

It is important to understand that our knowledge in the area of secretion molecular mechanisms, genetic regulation and PRP-therapy effects on the indirect processes remains to be incomplete[21].

Literature review shows that there have been several studies into PRP effect on the intestinal anastomosis regeneration[5,9], although these studies compared PRP soaking in the intestinal walls with PRP injection in anastomosis walls. The analysis of the histological and morphometric data illustrated that regeneration between the intestinal anastomosis sites treated by PRP soaking and PRP injection into the intestinal wall (Table 2) was more favorable in comparison with the control group, which was determined by the quantitative correlation between the inflammatory infiltrate, proliferation of fibroblasts, neo angiogenesis, and collagen deposition (Table 4).

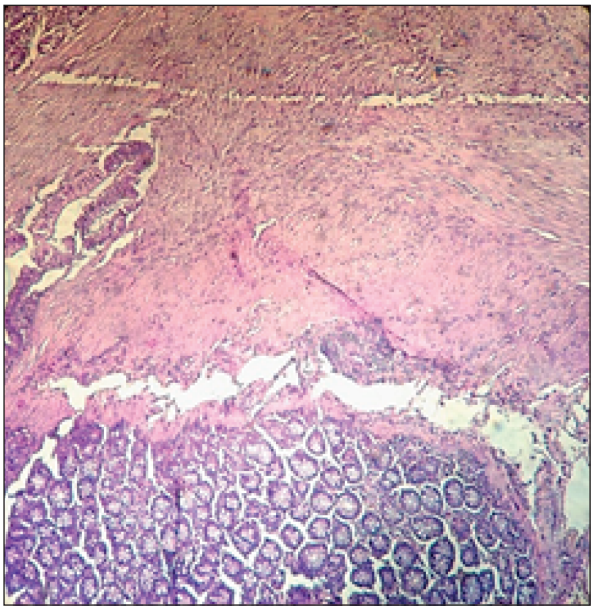

Figure 3. Histological picture of a formed anastomosis on the 7th day. 1st group (PRP soaking). Hematoxylin and eosin staining

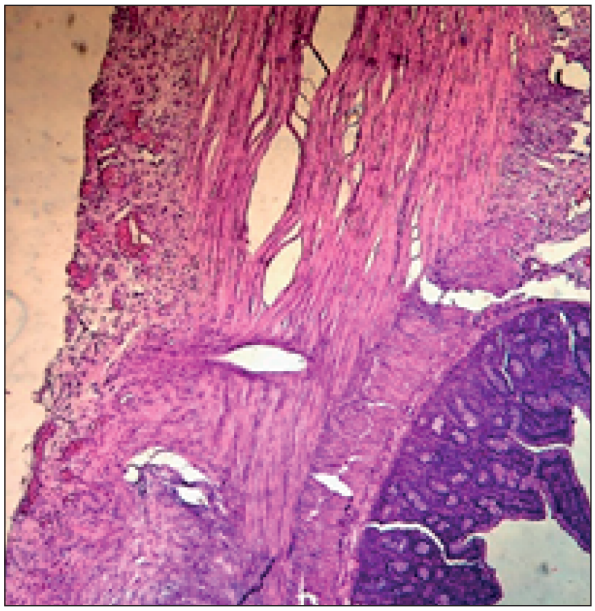

Figure 4. Histological picture of a formed anastomosis on the 7th day. 2nd group (PRP injection). Hematoxylin and eosin staining

The obtained data prove that PRP accelerates the rate and degree of adhesion in a wound[22].

PRP injection[23] in the intestinal wall is more advantageous in comparison with the soaking method. A histological picture did not reveal any fibrosis growth in injection area, any micro angiopathy, and inflammatory process in the 2nd group of the laboratory animals.

Thus, PRP causes cell proliferation and contributes to the synthesis of angiogenic factors during the intestinal anastomosis regeneration, which enhances the healing process with no regard to PRP application.

The present study evaluates the effects from different PRP application methods on adhesion process. The obtained data (Table 2) showed that the adhesion process among a group of the laboratory animals, where PRP soaking was applied (Figure 3), was immediate, a histological picture of an adhesion showed a solid vascularized adhesion on the 7th day, in comparison with the control group and PRP injected group (Figure 4), where membranous adhesions with no signs of neo angiogenesis were found on the 7th day.

PRP soaking in the intestinal wall reliably increased the number of the adhesive battles. The formed conglomerate of the adhesions contributed to the expressed deformation and narrowing of more than ½ lumen in the intestine. Such a complication occurred due to many platelets around the formed anastomosis[24,25].

PRP injection in the intestinal wall helped to prevent these complications as no residues were found in the abdominal cavity.

The analysis of the deformation and breaking strength indicators for the formed anastomoses also showed reliably high values of the breaking strength for the anastomoses treated by PRP in comparison with the control group.

This research illustrated that further studies in different PRP application methods for the intestinal anastomosis are needed.

Conclusions

PRP application by soaking and injections in the intestinal wall can positively affect the intestinal anastomosis regeneration. PRP injection in the muscular layer of the intestinal wall reduces the risk of adhesion development in comparison with the PRP soaked group with the intestinal anastomoses. Further studies are needed to identify the best possible PRP application method which improves the intestinal anastomosis regeneration.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law."

„All institutional and national guidelines for the care and use of laboratory animals were followed"

„No funding for this study"

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