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THE EFFECTS OF TOPICAL FIBRIN GLUE AND EPIDERMAL GROWTH FACTOR (EGF) ON THE COLON ANASTOMOSIS HEALING PROCESS – A COMPARATIVE EXPERIMENTAL STUDY

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Summary. *In this experimental study on small animals (Wistar) we evaluated the effectiveness of local protection methods (application of fibrin glue) and combined fibrin glue and EGF on colon anastomosis healing – prevention of dehiscence. Ninety experimental animals, with segmental left colonic resection in general anesthesia, were divided into 3 groups, each of 30 animals. In the control group, termino-terminal anastomosis with single-layer alternate suture was performed after segmental resection. In the second and third group, anastomosis protection was performed with extraluminal application of fibrin glue (coating) and combined fibrin glue and EGF. In the postoperative course of 21 days, 3 control animals died (10%) due to acute diffuse peritonitis caused by anastomosis dehiscence, in the group with combined fibrin glue and EGF 1 animal died (3.3%), while in the group with extraluminally applied fibrin glue only, all animals survived. The results demonstrate that local protective measures in colonic anastomosis yield better results; fibrin glue application and combined fibrin glue and EGF demonstrated similar results.*

Key words: *Colonic anastomosis, fibrin glue, EGF*

Introduction

Colon surgery is one of the most delicate and most complex in modern abdominal surgery due to well known specific anatomic, physiologic and microbiologic characteristics of the large bowel. Anastomoses are the most significant part of most colonic interventions. Anastomosis dehiscence, not uncommon and very serious postoperative complication in gastrointestinal surgery are more frequent in colon compared to other portions of the digestive tract. In spite of the advances in the last three decades in surgical techniques, suture material, effective preoperative preparation, antibiotic therapy, anesthesia, the rate of colon anastomosis dehiscences is still unacceptably high – up to 17% in elective and even 30% in urgent interventions (1,2). Such a situation has led many surgeons throughout the world to devise and investigate new and more effective methods, techniques and materials to prevent dehiscences. Local protective methods to "protect" colon anastomoses during their complex biologic healing process thus have been the interest of many surgeons. Out of a large number of various protective methods in colorectal surgery, protective endoluminal latex prosthesis, biofragmental ring (mechanical protection) and local application of fibrin bioadhesive are most frequently applied, with the most promising results. Numerous ongoing experimental and prospective clinical studies are expected to offer the answer regarding the real value of these methods. We decided to assess the value of local combined application of EGF and fibrin glue, ie. fibrin glue without growth factor in colon anastomosis protection.

Material and methods

Our experimental investigation was performed at the Faculty of Medicine in Niš, Dept. of Experimental Surgery, Centre of Biochemical Studies and Biochemical Laboratory, Clinical Centre Niš in 2002 and 2003.

As experimental animals Wistar rats were used, weighing 350-490 g. All animals were operated under general anesthesia. Premedication consisted of Atropin and Bensedin. General anesthesia of 30-60 minutes duration was performed with Ketalar at 8 mg/100 g BW dose, intraperitoneally.

All surgical interventions were performed without preoperative colon preparation. Abdominal cavity was opened through superior and inferior medial 4 cm long incision. After the exploration of abdominal cavity, on the left halh of the colon partial resection was done 3-4 cm above the peritoneal reflection, with preceding ligation of paraintestinal arcade with colon tissue removal 1.5-2 cm.

After the removal of colonic segment, bowel continuity was restored with termino-terminal anastomosis in three ways (which was the reason why the animals were divided into three groups).

I. Control group

In this group, after partial colon resection termino-terminal (T-T) anastomosis was performed with single one-layer sutures and slowly resorbing material (6-0, 7-0).

II. Fibrin glue group

After the indentical proceedings as with the previous group, fibrin glue was applied to the created anastomosis (Blood Transfusion Centre, Niš). The first component of this preparation was the cryoprecipitate obtained from blood donors blood (containing 50-60 g/l fibrinogen, factor XIII and fibronectin) and an antifibrinolysis agent (aprotinin, 1000 IU/ml). The second component was $CaCl_2$ (40 mmol/l) and bovine thrombin, 500 units/ml.

Fibrin glue was exposed to the environmental (room) temperature; after reaching that temperature the contents were placed into sterile 2 ml syringes.

Both prepared components of fibrin glue were simultaneously applied with Duploject, an original and by us modified system, first to the posterior wall of the anastomosis (dry surface) in the amount of 0.3-0.5 ml. After that, a period of 2-3 minutes is required for the adhesive jelly-like layer of glue to be formed. Identical proceedings are repeated for the anterior wall.

III. Combined fibrin glue and epidermal growth factor (EGF) group

After the identical proceedings as with the previous group, we locally applied combined recombinant human EGF (RD systems®) to the anastomosis in the concentration of 10 mcg/ml, purity over 97%, with the sterile syringe, and fibrin glue in the amount of 0.3-0.5 ml in already described way.

After the operation, experimental animals were placed each into separate special cage, where they had available food (customary for that species) and water from postoperative day I.

The animals were carefully clinically monitored up to 21 days and planned sacrifice. General status of the animals was monitored, together with their behaviour and possible changes (refusal to take food and water), appearance of the operative wound, bowel peristaltics and stools. Six animals from each of the groups were sacrificed on days III, V, VII and XIII (without pain and after premedication and general anesthesia). Inspection of the abdominal organs and anastomosis was performed through the upside down "U" incision (enabling elevation of the anterior abdominal wall). Special attention was paid to the macroscopic appearance of anastomosis, postoperative adhesions and peritoneal inflammatory events.

Determination of the bursting pressure (PPKA) of the colon wall with anastomosis was done immediately after animals had been sacrificed in all three groups. PPKA measurements were performed at the Dept. of Experimental Surgery, Centre of Biochemical Studies, Faculty of Medicine Niš. A segment (cca 3 cm long) of the descendent part of the colon with anastomosis was sampled. Measurements were performed with tensiometer. The bowel segment was ligated on one end; the other end was attached to the glass cannula connected to the system for manual inflation with air and manometer. The bowel segment was then immersed into a water bath and inflated with air at the rate of 10 mmHg/sec. The moment of anastomose bursting was defined as the appearance of the first gas bubble in the water bath.

Collagen concentration was determined indirectly through quantitative determination of L-hydroxy-proline in mg/g of bowel wall homogenate at the anastomosis site. Sampling was for this purpose done after PPKA measurement, on days III, V, VII and VIII.

Histomorphologic analysis of the anastomosis tissue was performed with light and scanning electron microscopy. Light microscopy was performed with traditional tissue staining: hematoxylin eosin (H&E) and PAS, as well as some special fibrin staining (by Weigert), elastic fibre staining (Weigert's resorcine-fuchsin) and collagen (Masson trichrome stain).

Results

The results of the comparative study were analysed comparing the following parameters:

Postoperative complications

In controls, in 13 (43.3%) animals the following postoperative complications were observed: wound infection in 5 (38.5%); intraabdominal adhesions in 4 (30.1%), partial anastomosis dehiscence with perianastomotic abscessus in 1 (7.8%), total anastomosis dehiscence in 3 (23.1%) animals. In the group with extraluminally combined EGF and fibrin glue there were 8 diseased animals (27%), out of which in 3 (37.5%) wound infection was observed, in 1 (12.5%) partial anastomosis dehiscence and in 4 (50%) intraabdominal adhesions. The lowest incidence of postoperative complications was observed in fibrin glue only group in 4 (13.3%) animals: wound infection in 2 (50%), partial dehiscence with perianastomotic abscessus in 1 (25%) and intraabdominal adhesions in 1 (25%) animal.

Comparation of postoperative complications demonstrated that there was statistically significant difference between control and experimental groups (fibrin glue; combined fibrin glue and EGF); the level of significance was $p<0,1$ by Pearson's χ^2-test.

Mortality

Out of 90 experimental animals 7 died (7.8%). The highest mortality rate was registered in controls – 6 (20%). Causes of mortality were in this group: total anastomosis dehiscence with acute difusse peritonitis and adhesive ileus with 3 deaths each (50%). In the group with combined fibrin glue and EGF there was 1 death (3.3%) caused by total anastomosis dehiscence with acute diffuse peritonitis. In the group with only fibrin glue there were no deaths.

Fischer's test of exact probability of mortality results demonstrated statistically significant difference by $p<0.05$ on the side of experimental groups compared to controls. The same test did not demonstrate statistically significant difference between the fibrin glue plus EGF group and fibrin glue only group ($p<0.05$).

Measurement of the anastomosis mechanical strength

The values of anastomotic colon segment bursting was directly correlated with the passage of time (Graph 1).

Comparative t-test analysis demonstrated statistically significant difference between all experimental animals and controls in the initial healing period (III and V postoperative days, fibrin glue only group) ($p<0.05$ to $p<0.001$ per t-test), as well as in the final healing period (VII and XIII postoperative day, fibrin glue plus EGF group) ($p<0.05$).

There was no statistically significant difference in the median pressures in fibrin glue only and fibrin glue plus EGF groups, regardless of the postoperative day ($p<0.05$).

Biochemical tests

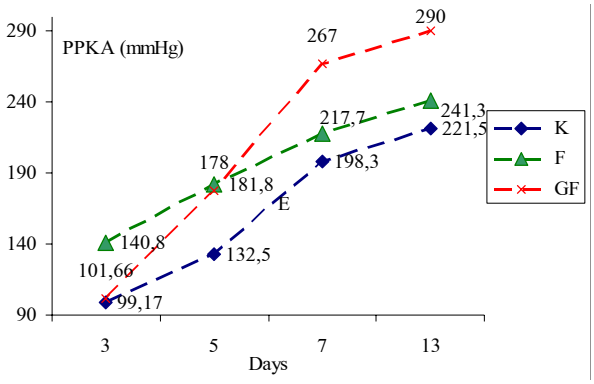
Hydroxyproline concentration results at the anastomosis site per each group/day (as the collagen synthesis indicator) are shown in Table 1.

Statistical processing of the obtained results demonstrates significant differences in hydroxyproline values between controls and experimental groups per each postoperative day. The difference between fibrin glue plus EGF group and fibrin glue only group was statistically significant (to the advantage of the first) only on postoperative day XIII ($p<0.05$; t-test).

Histological investigation of anastomoses

Complex biological anastomosis healing passed through the well known phases – coagulation, inflammation, cell proliferation, remodeling, with certain differences. Cellular proliferation is the essential one for healing and the surgeon, and in controls it started on postoperative day III. Neovascularisation or angiogenesis, and young collagen synthesis play the central part. On postoperative day III, macrophages for the most part replaced neutrophils, which marked the way to fibroblasts passing to the wound centre. After 7 days, structures of the larger blood vessels appeared in the capillary network – arterioles and venules, so the neovascularisation is completed around day XIII. These processes strengthened anastomoses. In the maturation phase, young collagenous tissue was transformed into old connective tissue through fibroblast number and blood vessel network reduction, with remodeling of collagen bundles in the direction of tension forces, which strengthened colon anastomoses.

In experimental groups, cellular proliferation phase occurred earlier – between days II and III – with more young blood vessels formed simultaneously with young collagen formation. As the consequence of the process, already on day V, the maximal new collagen synthesis was reached, which enabled the critical anastomosis healing period to be shorter.



Graph 1. Average PPKA values by days and groups
PPKA – colon bursting pressure at the site of anastomosis
mmHg – milimeter of Hg
K – Control group
F – Fibrin glue only group
GF – Fibrin glue plus EGF group

Table 1. Average hydroxyproline at the anastomosis site by groups and days (in mg/g tissue)

Groups / Days	F $\overline{X} \pm SD$	K $\overline{X} \pm SD$	GF $\overline{X} \pm SD$
III	20.54 ± 0.94	15.85 ± 2.57	21.31±1.0.97
V	27.92 ± 4.15	18.16 ± 1.31	34.2±2.76
VII	23.25 ± 1.95	18.51 ± 0.79	26.9±3.6
XIII	25.04 ± 1.74	19.59 ± 0.80	33.69±0.2
for sample n=24	24.19 ± 3.60	18.03 ± 2.01	29.02±1.91

mg/g tissue – milligram per gram 10% of bowel tissue homogenate
Postoperative day (III, V, VII, XIII)
n – number of experimental animals
K – Control group
F – Fibrin glue only group
GF – Fibrin glue plus EGF group
$\overline{X}$ – hydroxyproline median value
SD – Standard deviation

With scanning electron microscopy in experimental groups various fibers grouped in bundles could be discerned – fibrin, elastic and collagen – filling up completely anastomotic site, contrasted to controls where the defect was not filled up with the tissue infiltration, though in some places small number of fibers bridging anastomotic edges and scarce cellular reaction could be seen. In fibrin glue and EGF group there was more collagenous fibers bundled longitudinally, compared to fibrin glue only group.

Discussion

The problem of safe colon anastomoses is still very pressing. The most significant and most frequent complication is anastomosis dehiscency. It is still the most common cause of postoperative complications and mortality. Local anastomosis protection measures are constantly being tested in the colon surgery. The focus

of interest of modern colon surgery are various local protection approaches – stents and bioadhesives (3,4,5). Many experimental and clinical studies are being performed to compare these methods and evaluate their value, together with intensive search for new and more effective protection measures. Local application of fibrin glue (biologic haemostatic and adhesive agent) was first used by Matras, Spangler, Scheele in 1978 (local application over colorectal anastomoses) (6). Their idea was to reduce the incidence of dehiscence increasing the strength of anastomoses, reducing the leakage and stimulating their healing. Fibrin glue is a bi-component biologic adhesive system comprising fibrinogen and thrombin, the activation of which creates fibrin, the main component of phase II blood coagulation. As it is a high quality bioproduct invented through the advances in transfusiology, it has quickly gained acceptance in surgery as a hemostatic and adhesive agent ("biologic glue"). Nowadays, homologous and autologous fibrin glue are used. Autologous glue eliminates the risk of disease transmission, provides better immunologic resistance and reduces the expenses related to preparation, which are the reasons it is more and more applied in modern surgery. One of the most significant advances in the field of modern molecular biology and biochemistry in the last three decades are certainly growth factors and cytokines (wound hormones, tissue hormones). Growth factors are biologically active polypeptides influencing growth, differentiation and metabolism of target cells through specific receptors. Numerous growth factors have been identified so far, taking part in the complex wound healing process, with new ones being identified constantly. Using these facts, in the last several years a number of authors investigated the use of various growth factors (local or systemic) in order to enhance some of the phases of wound healing process. However, only PDGF (Platelet derived growth factor) has been used in modern medicine so far (Beclapermin®) as a topical aid to ulceration healing on diabetic foot. One of significant growth factors is also EGF, discovered in 1962 in murine salivary glands; EGF stimulates the growth of epithelial cells, fibroblasts and smooth muscle cells (7,8).

Our analysis demonstrated that postoperative complications and dehiscence rate is lowest in animals with extraluminally applied fibrin glue compared to controls and the group with combined fibrin glue and EGF. Kanellos et al. published in 2004 their results of comparative analysis of experimental colon anastomosis protection with fibrin glue and intraperitoneal administration of 5-fluorouracil (5-FU). In their fibrin glue plus 5-FU group there were no dehiscences, compared to controls and 5-FU use, with 37.5% leakage rate (9). Mortality rate was highest in controls, then in fibrin glue plus EGF group, while in fibrin glue only group we did not have any mortality. Anastomosis dehiscence and peritonitis are the most common causes of mortality.

Comparing the results of mechanical strength of anastomoses in fibrin glue group with controls, the value of colon wall bursting was higher and of statistical significance in the initial healing process in the first group; the case was the same with fibrin glue plus EGF group compared with controls in the final healing phase. It was the result of the adhesive power of fibrin glue ie. the amount of fibrinogen, peaking around postoperative day V, the critical period of anastomosis healing. Biological compatibility of the substance and the possibility of direct influence of intraluminal contents on one hand, as well as tension and adhesive power of fibrin glue on the other, increase the force needed for anastomosis bursting in the critical healing phase. Combined fibrin glue and EGF enhance healing in its final stages, stimulating epithelial cells and fibroblasts. Brown et al. published in 1989 their results of wound healing study at the site of skin graft-taking. Local application of EGF (as ointment) speeded up epitelisation and reduced healing time (10,11,12).

Hydroxyproline analyses investigation in the colon wall at the site of anastomosis in experimental groups demonstrated significantly higher values on all postoperative days compared to controls. The highest values were observed on day V. Comparing the results of the group with fibrin glue plus EGF with fibrin glue only group, it was observed that in the final stages of healing hydroxyproline concentration was significantly higher in the first group, which matches well with the measurements of mechanical strength of anastomosis. However, continual increase of colon wall bursting values is not reflected in hydroxyproline values, which was the finding of other authors as well (13,14). It is possible that bursting force of isolated colon depends not only on collagen concentration but also on collagen fiber distribution, collagen fiber type, elastin presence, adhesive glycoproteins and other extracellular matrix components.

Histological analysis of anastomotic colon wall samples demonstrated favorable influence of fibrin glue on the complex process of colon anastomosis healing, reflected in: stimulation of proliferative response, quicker establishing of fibroplastic phase, more abundant young collagen synthesis and reduction of "critical" healing period. Similar results were published by Fini et al. analyzing the healing process of experimental anastomoses with synthetic and fibrin glue application (15). Addition of EGF to fibrin glue stimulates fibroblast number increase in II and III phase of wound healing, which induce larger numbers of interweaved collagen fibers, which additionally strengthens colon anastomosis (16).

Conclusion

From the results obtained in this experimental study we may conclude:

- Local protective methods in the form of fibrin glue application and combined fibrin glue plus EGF reduce the frequency of colon anastomosis dehiscence.

- Fibrin glue and fibrin glue plus EGF enable optimal results since the mechanical strength of anastomosis is increased, its porosity is reduced and wound healing is speeded up.
- Fibrin glue and combined fibrin glue and EGF application yield similar results.
- Local protective methods should be applied only on technically perfectly performed anastomoses, which we should always bear in mind.

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EFEKTI LOKALNE APLIKACIJE FIBRINSKOG LEPKA I EPIDERMALNOG FAKTORA RASTA NA PROCES ZARASTANJA ANASTOMOZA KOLONA – KOMPARATIVNO EKSPERIMENTALNA STUDIJA

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Kratak sadržaj: *U ovoj eksperimentalnoj studiji sa malim životinjama (Wister pacovima) vršena je procena vrednosti primene lokalnih protektivnih metoda u obliku aplikacije fibrinskog lepka i kombinovane primene fibrinskog lepka i EGF na ishod zarastanja anastomoza kolona – prevencija dehiscencije. 90 eksperimentalnih životinja kod kojih je u opštoj anesteziji vršena segmentalna resekcija levog kolona podeljeno je u tri grupe od po 30 životinja. U kontrolnoj grupi posle segmentalne resekcije urađena je termino-terminalna anastomoza jednoslojnim naizmenicnim šavom. U drugoj i trećoj grupi, na isti način operisanih životinja, zaštita anastomoza vršena je ekstraluminalnom primenom (oblaganjem) fibrinskog lepka i kombinovanom primenom fibrinskog lepka i EGF. U postoperativnom toku u kome su životinje praćene 21 dan u kontrolnoj grupi uginulo je 3 (10%) životinja zbog akutnog difuznog peritonitisa, čiji je uzrok bio dehiscencija anastomoze, u grupi sa kombinovanom primenom fibrinskog lepka i EGF 1 (3,3%), dok su u grupi sa ekstraluminalno aplikovanim samo fibrinskim lepkom sve životinje preživele.*
Rezultati ovog eksperimenta pokazali su da se primena lokalnih protektivnih mera kod izvođenja anastomoza na kolonu mogu ostvariti bolji rezultati pri čemu su metode aplikacije fibrinskog lepka i kombinovane primene fibrinskog lepka i EGF pokazale slicne rezultate.

Ključne reči: *Anastomoze kolona, fibrinski lepak, EGF*