Nazarchuk Serhii, Sukhodolia Anatolii, Nazarchuk Oleksandr. Clinical and immunological research of qualities of antimicrobial disepidermic cryolyophylized xenoderm grafts. Journal of Education, Health and Sport. 2015;5(8):434-442. ISSN 2391-8306. DOI 10.5281/zenodo.31322
http://dx.doi.org/10.5281/zenodo.31322
http://ojs.ukw.edu.pl/index.php/johs/article/view/2015%3B5%288%29%3A434-442
https://pbn.nauka.gov.pl/works/632549
Formerly Journal of Health Sciences. ISSN 1429-9623 / 2300-665X. Archives 2011–2014 http://journal.rsw.edu.pl/index.php/JHS/issue/archive

Deklaracja.
Specyfika i zawartość merytoryczna czasopisma nie ulega zmianie.
Zgodnie z informacją MNiSW z dnia 2 czerwca 2014 r., że w roku 2014 nie będzie przeprowadzana ocena czasopism naukowych; czasopismo o zmienionym tytule otrzymuje tyle samo punktów co na wykazie czasopism naukowych z dnia 31 grudnia 2014 r.
The journal has had 5 points in Ministry of Science and Higher Education of Poland parametric evaluation. Part B item 1089. (31.12.2014).

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 26.08.2015. Revised 05.09.2015. Accepted: 24.09.2015.

UDC: 616+615.47.014.47-616.5-089-74

CLINICAL AND IMMUNOLOGICAL RESEARCH OF QUALITIES OF ANTIMICROBIAL DISEPIDERMIC CRYOLYOPHYLIZED XENODERM GRAFTS

Serhii Nazarchuk[1], Anatolii Sukhodolia[2], Oleksandr Nazarchuk[3]

[1]Khmelnytskyi Regional Clinical Oncological Dispensary, Department of Abdominal Surgery
[2]Vinnytsya National Pirogov Memorial Medical University, Department of Surgery of the Faculty of Postgraduate Study
[3]Vinnytsya National Pirogov Memorial Medical University, Department of Microbiology, Virology and Immunology

Abstract

In the article the results of the research of immunological changes after the using of antimicrobial disepidermic cryolyophilized xenoderm grafts in the model of intestinal anastomosis forming; clinical effectiveness of their use for prevention intestinal sutures' and anastomosis' insolvency in patients with oncological pathology are presented. In experiment, it is shown, that while using DCXG the essential changes of local immune reactivity have place. It is proved that the tendency of unevenly and not proportionally increasing of cells with main classes Ig A, Ig M, Ig G, Ig E shows highly active and non-stable local immunologic reactions of intestinal mucosa on using DCXG; the intensity of local immune reactions significantly decreases by the 25th day and is nearby to normal ones.

DCXG were used in 50 patients, who underwent surgery in Khmelnytskyi Regional Clinical Oncological Dispensary and Khmelnytskyi Regional Clinical Hospital in 2009–2013 years. The incidence of purulent-inflammatory complications of intestinal anastomosis' insolvency in the main group of patients was above 4,3 % and 16 % in control one.

Key words: sutures' insolvency, anastomosis, xenoderm grafts, antimicrobial materials.

Background. The problem of intestinal sutures' insolvency in surgery is among the most dramatic one. The majority of postoperative purulent-inflammatory complications (peritonitis, abscesses of abdominal cavity, intestinal fistula, etc.), complications, colligated with re-laparotomy, mortality outcomes in abdominal surgery are previously specified by sutures' insolvency. Infectious complications, happening in abdomen after surgery, are considered to be a result of disturbing of intestinal tightness. In the case of non-effective anastomosis' conducting, it happens, that inner intestinal microflora dislocates into the abdomen and causes purulent-inflammatory complications. That's why supplementary strengthening of anastomosis with additive advises, obtaining such bioactive qualities as antimicrobial ones, is of great importance for providing homeostasis in intestinal repair [1–5].

Supplementary strengthening of anastomosis by means of biological and synthetic materials plays positive role in decreasing the accidence of mechanical sutures' insolvency. Using antimicrobial materials is considered to provide much more effective prophylaxis of postoperative sutures' insolvency and purulent-inflammatory complications due to prolonged antimicrobial regimen in site. For prevention of anastomosis' insolvency there were used such biological adhesive materials as collagen films, fibrin materials and fibrin-collagen lamella, Thahocomb and others. All of them have high effectiveness for providing mechanical strength, impermeability, stimulating regeneration of the intestinal anastomosis and protecting its lysis, providing collagen equilibrium. But no one is ideal [6– 9].

Scientists consider that creation of optimal conditions for regeneration of intestinal sutures and anastomosis is one of the main reserves to optimize results of abdominal surgery. Nowadays xenografts got from pig skin, which were experimentally and clinically proved to contain biologically active components, growth factors which positively influence on regeneration are of great interest. Additional donation of antimicrobial agents into their structure can provide high antimicrobial activity. For effective use in surgery to prevent postoperative sutures' and anastomosis' insolvency, the study of immune influence of antimicrobial disepidermic cryolyophilized xenoderm grafts should be carried out [10 – 13].

The aim. Study of immunological changes after the using of antimicrobial disepidermic cryolyophilized xenoderm grafts in the model of intestinal anastomosis and research of their clinical effectiveness in prevention of intestinal sutures' and anastomosis' insolvency in patients with oncological pathology.

Materials and methods. Experimental research was carried out in 54 adult white lineal rats "Wistar" in conditions of vivarium, according to the principals of bioethics regulated by European Convention for the protection of vertebrate animals, implicated in experiments and other scientists (Strasbourg, 1986), European Council Directive 86/609/EEC (1986), The Law of Ukraine №3447-IV "On protection of animals from cruelty" and general ethic principals of experiments, regulated by The First National Congress of Bioethics in Ukraine (2001).

All animals necessarily underwent quarantine (10-15 days) in vivarium. There were used both, male and female, rats with medium age and weight 250 g they had free water regimen. 24 hours feed caloric intake was 5,6-6,3 calories per Kg of their weight. Before the experimental surgery they were starving.

For immunological studying of jejunum wall tissue, animals were divided into control group (n=24), which had intact small intestine and experimental one (n=30), in which

disepidermic cryolyophilized xenoderm grafts (DCXG) were aplicated on the intestine serosa and fixed to the surface, using knotted sero-serous sutures (atraumatic needle 6,0).

Premedication of experimental rats was carried according to standard recommendations by the way of intramuscular injection of 0,1 % atropine sulfate (0,05 ml/kg), 1 % diphenhydramine (0,25 ml), ketorolac (0,5 ml/kg). Anesthesia was carried out by the way of ip injection of ketamine (2 mg/kg) and its potentiating by ether insuflation.

Euthanasia of experimental animals was done on the 7th, 15th, and 25th day of postoperative period (n=10) according to ethics standards and well known recommendations applying deep narcosis (overdose of thiopental sodium) and further decapitation.

For examining of plasmatic cells with IgA, IgM, IgG, IgE we used microtome cuts of jejunum, which further were handled by monospecific antiserum to each antibody, coppied with izocin fluorescein (direct method by Coons) and specific controllers. Fluorescent microscope "Luman R-8" was used. Fluorecent plasmatic cells were determined and calculated in 1 square mm of jejunum mucosa. Ig A was defined in intestinal mucosa by means of radial immunodiffusion in agar with specific SIgA antiserum [14].

Accordingly to the data of experimental research work disepidermic cryolyophilized xenoderm grafts were used in patients, who underwent surgery in Khmelnytskyi Regional Clinical Oncological Dispensary and Khmelnytskyi Regional Clinical Hospital in 2009 – 2013 years. 97 patients were enlisted in this clinical research. Pressurization and strengthening of intestinal sutures using disepidermic cryoliophylized xenodermoiplants was carried out in 47 patients of main group. Other 50 patients from comparison group underwent traditional surgery. In main group 52,0 % of patients were younger then 60 years. Among all patients there were male 43,7% (n 45) and female 56,3 % (n 52). Surgery was carried in patients with cancer of ascending colon (40,0 %), sigmoid colon (24,0 %), descending colon (22,0 %), stomach cancer (2,0 %).

Classification by Saveliev was used in clinical analysis of intestine sutures' conditions [13]. The analysis of the effectiveness of DCXG use in prevention of intestinal sutures' and anastomosis' insolvency was based on comparative estimation of accidence of purulent-inflammatory complications (peritonitis, abscess, infiltration) in patients of both groups. We took into account clinical status of operated patients; data of sonography examining of formed intestinal anastomosis' status (Philips HDI 4000 with convex sensor – frequency higher than 2 MHz in regimen of real time); laparoscopy data; results of laboratory study of extravasate in abdominal cavity. During sonography the status of tissues nearby to anastomosis, thickness of driving and diverter bowel loops, characteristics of peristalsis in this zone, presence or

absence of some liquid in abdomen were estimated. Obtained quantitative data were statistically analyzed.

Results and discussion. In the experimental part of the research while using DCXG there were found essential changes of local immune reactivity in rats (table 1).

Table 1

Characteristics of local immune changes in jejunum after using antimicrobial disepidermic cryolyophilized xenoderm grafts in rats

Characteristics	Groups			
	Control	Experimental group		
		7th day	15th day	25th day
Plasmatic cells with Ig A, cell/mm^2	240,60±4,50	368,90±9,30***	283,70±4,80***	250,50±5,10
Plasmatic cells with Ig M, cell/mm^2	115,80±2,70	310,80±8,10***	173,70±3,00***	120,3±4,2
Plasmatic cells with Ig G, cell/mm^2	59,90±1,80	191,70±4,80***	77,70±2,10***	64,20±2,40
Plasmatic cells with Ig E, cell/mm^2	19,20±0,18	60,30±1,50***	24,90±0,21***	20,30±0,51*
SIg A, g/l	0,710±0,015	0,920±0,024***	0,795±0,018**	0,725±0,024
Cells in jejunum mucosa, cell/mm^2	8760,0 ±105,0	19272,0 ±450,6***	13140,0 ±330,9***	8890,0 ±117,0

* - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$

After the analysis of obtained immunological characteristics, density of plasmatic cells with Ig A per square mm, was determined to increase on 53,3 % by the 7th day of the experiment in comparison to the control (240,60±4,50 cell/mm^2; $p<0,001$). Alike dynamics was appropriate to plasmatic cells, containing Ig M. In control group number of plasmatic cells with Ig M was about 115,80±2,70 cell/mm^2, but when antimicrobial DCXG had been used there level increased in 2,7 times on the 7th day after surgery ($p<0,001$).

In the research numerosity of plasmatic cells with Ig G also increased in jejunum mucosa on the 7th day in 3,2 times, than in control group, where is amount was only 59,90±1,80 cell/mm^2 ($p<0,001$).

It should be mentioned that density of plasmocytes with Ig E in mucosal layer of jejunum had the same tendency to rise up in 3,14 times after application of DCXG in the site of anastomosis ($p<0,001$).

Among immunological parameters there were found changes of secretory Ig A in experimental group. When DCXG had been used increasing of sIg A in mucosa was found to be higher more than 29 % comparably to control and became 0,920±0,024 g/L on the 7th day. Cell density in mucosal layer of jejunum came up to 19272,0±450,6 cell/mm^2, being higher than control in 2,2 times.

Received data have shown that jejunum mucosa expressively reacted on the application of cryoliophylized xenoderm grafts. Amount and density of plasmatic cells (containing Ig A, Ig M, Ig G, Ig E) increased unevenly and not proportionally. Concentration of sIgA in mucosa of jejunum also increased. Such tendency of unevenly and not proportionally increasing of cells with main classes Ig A, Ig M, Ig G, Ig E showed highly active and non-stable local immunologic reactions of intestinal mucosa.

On the 15th day we'd got some different meaning. Density of plasmatic cells with Ig A decreased on 23 % and was 283,70±4,80 cell/mm^2 in comparison then it was on the 7th day.

While studying the density of plasmatic cells with Ig M, we found similar dynamics. The number of plasmatic cells with Ig M decreased from 310,80±8,10 cells/mm^2 (the 7th day) to 173,70±3,00 cells/mm^2 (the 15th day) that was above 44 %. It was found that value of plasmocytes' density, containing Ig G, decreased on 59,4 % in jejunum mucosa on the 2nd week of the study. When DCXG were used, plasmatic cells, containing Ig E decreased from the level of 60,30±1,50 cells/mm^2 (the 7th day) to 24,90±0,21 cells/mm^2 (the 15th day). This difference seemed that intestinal anastomosis strengthening with DCXG leads to decreasing the plasmocytes secretion of IgA on 58,7 % after two weeks of their using. Concentration's of IgA decreasing on 13,6 % by the 15th day (0,795±0,018 g/l) also prove this regularity.

On the 15th day it was found that the density of cell infiltration (13140,0±330,9 cells/mm^2) in jejunum mucosa was 31,8 % lower, than on the 7th day (19272,0±450,6 cells/mm^2).

Analyzed data proved that local immunological characteristics were appropriate to normalize by the 15th day when DCXG were used.

On the 25th day of the after using DCXG, immunological characteristics did not differ comparatively to control ones. For example, on the 25th day number of plasmatic cells in jejunum mucosa, containing IgA, was higher (250,50±5,10 cells/mm^2) only on 4,1 % ($p>0,05$); quantity of those with Ig M (120,30±4,20 cells/mm^2) was 3,7 % higher then control ones (115,80±2,70 cells/mm^2; $p>0,05$).

The amount of plasmatic cells, containing IgG, reached 64,20±2,40 cells/mm^2 in jejunum mucosa that was 7,1 % higher than in control group (59,90±1,80 cells/mm^2; $p>0,05$). After using DCXG, there was determined the normalization of plasmatic cells' number by the 25th day (20,30±0,51 cells/mm^2), which difference was no more than 5,7 % ($p<0,05$).

Statistically, on the 25th day there was proved no difference in jejunum mucosa between concentrations of secretory IgA after application DCXG (0,725±0,024 g/l), and control group ($p<0,05$). Density of cells in jejunum mucosa of control rats was found to be 8760,0±105,0 cells/mm^2 and 8890,0±117,0 cells/mm^2 on the 25th day later intestinal anastomosis' strengthening by means of xenoderm grafts ($p>0,05$).

Accordingly to results, obtained in the research, we may confirm, that significant changes of local immune reactions in the jejunum mucosa after implantations of xenoderm grafts, happened on the 7th day. The amount of plasmatic cells, which contain main immunoglobulins (IgA, IgM, IgG, Ig E), increased unevenly and disproportionally. The level of secretory IgA was also increased. Such local changes seemed about intension and instability of local immune reactions in jejunum by the 15th day of postoperative period. The intensity of local immune reactions significantly decreased by the 25th day and was nearby to normal ones.

It is known, that immunoglobulins have their protective function while decreasing antigenic activation of microbial pathogens which may get into gastro-intestinal tract. However, increasing of plasmatic cells, containing, IgA play prominent role in protection of mucosa. According to this, the use of antimicrobial DCXG additionally may supplementary optimize regeneration stimulating local immune response.

Due to local immunological activity of DCXG in jejunum mucosa, that was proved in experimental part of the research, it was logical to continue clinical study of their effectiveness in anastomosis' protection. In the majority of patients, who underwent surgery with DCXG using, there were conducted colonic anastomosis (90,0 %), and only 10 % of patients had ezofaho-entero anastomosis and gastro-entero anastomosis, (fig. 1).

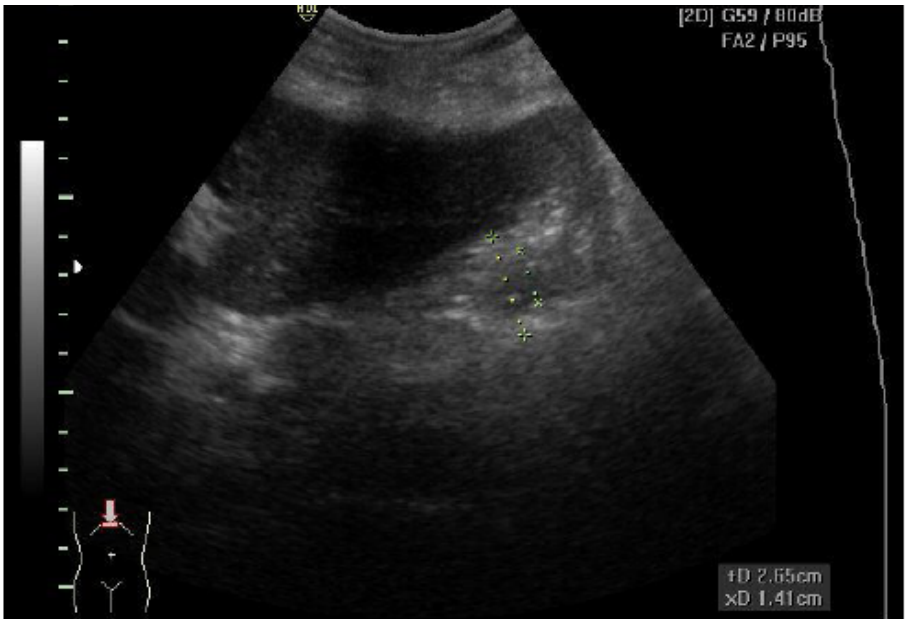

Fig. 1. Sonography of ezofaho-jejuno anastomosis and surrounding tissues in patient P. 58 years (main group). Disorders of reparation in zone of anastomosis are absent.

The analysis of the frequency of postoperative complications in both groups have shown, that in the main group only 4,3 % of cases of inflammatory infiltration happened nearby colonic anastomosis, protected with DCXG.

At same time, an intense increasing of purulent-inflammatory complication in patients of comparative group had been determined. All complications were found when colonic anastomosis had been imposed. Once (2,0 %) we observed acute form of anastomosis' and sutures' insolvency, such as peritonitis.

Also, by the concentration rate of ammonia (470 та 510 mkmol NH_3/l), there had been diagnosed subacute form of intestine sutures' insolvence in two cases (4,0 %). By means of sonography the inflammatory infiltration in 3 patients (6,0 %) and abscess in 2 patients had been found under anastomosis' projection (4,0 %; fig. 2).

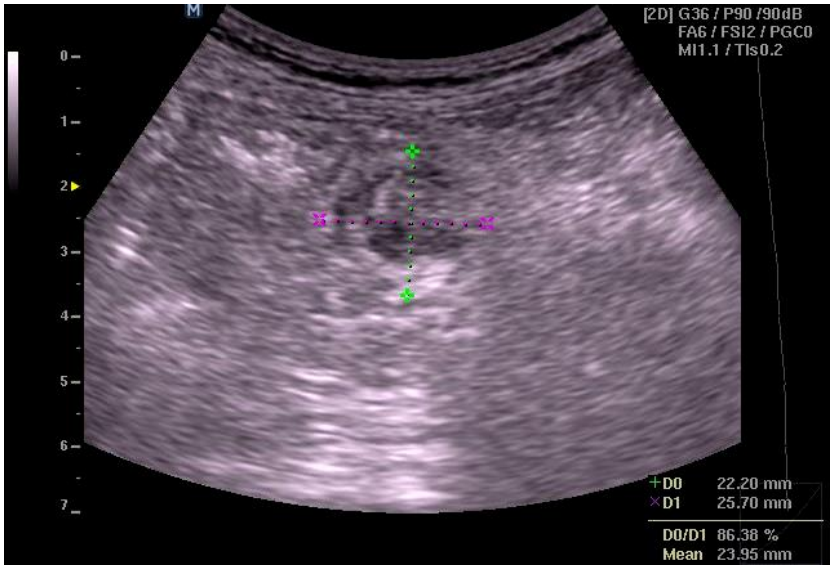

Fig. 2. Patient B., 44 years (comparative group). Sonography of postoperative abscess in zone of colonic anastomosis

Thereby, the incidence of purulent-inflammatory complications of intestinal anastomosis' insolvency in the main group of patients was above 4,3 % and 16 % in control one.

Conclusion

Antimicrobial disepidermic cryolyophilized xenoderm grafts are capable to stimulate tissue immune response, by the way of activating of immunoglobulins' increase and there facilitating in intestinal anastomosis' and sutures' regeneration. High local immunological activity of antimicrobial xenoderm grafts in the area of formed sutures and anastomosis explain great interest of their further clinical use.

In conditions of high risk of intestinal sutures' insolvency the use of disepidermic cryolyophilized xenoderm grafts for strengthening of anastomosis' zones, especially in exhausted, immunedifficiant oncological patients decreases in 3,7 times purulent-inflammatory complications, concerned with intestinal anastomosis' insolvency in patients, who underwent surgery on gastrointestinal tract, because of xenoderm grafts' ability to activate local immune system.

Bibliography

1. Bogdan C. P. Postoperative Complications Following Surgery for Rectal Cancer / C. P. Bogdan, S. Cassie, A. R. MacLean [et al.] // Annals of Surgery. – 2010. – Vol. 251, No 5. – P. 807–818.

2. Egorov V. I. Intestinal anastomosis. Physical and mechanical aspects / V. I. Egorov. M: Vidar, 2004. – 304 p.

3. Dietz D.W. Postoperative complications. In: ASCRS Textbook of Colon and Rectal Surgery / J. M. Church, D. E. Beck, B. G. Wolff, J. W. Fleshman, J. H. Pemberton, (Eds) // Springer-Verlag New York, LLC, New York, 2006.– 141 p.

4. Shurkalin B. K. Postoperative complications in surgery of colon / B. K. Shurkalin, A. V. Volenko, B. E. Titkov // Bulleting of Pirogov National and Medical Surgical Sentre. – 2009. – Vol. 4. – No 1. – P.60–62.

5. Aliev F. Sh. The view on the mechanical strength of intestinal anastomosis / F. Sh. Aliev, I. A. Chernov, O. A. Molokova // Bulleting of Siberian medicine. – 2003. – No 2. – P. 81–83.

6. Gorskyi V. A. About the increase of reliability of intestinal suture / V. A. Gorskyi, A. V. Volenko, I. V. Leonenko [et al.] // Surgery named after Pirogov. – 2006. – No 2. – P. 47–51.

7. Ger R. Prevention and treatment of intestinal dehiscence by an intraluminal bypass graft / R. Ger, B. Ravo // British Journal of Surgery. – 2005. – Vol. 71. – N. 9. – P. 726 – 729.

8. Possibilities, results and perspectives for sthrengthening of intestinal sutures by means of fiber and collagen substntion TahoKomb / S. S. Angreev// Surgery. – 2004. –No 2. – P. 53–56.

9. The use of combined genetic non-similar substrate in surgical dermo-platelet / V. V. Bigunyak, V. V. Demjyanenko, N. O. Starikova // Hospital Surgery. – 2006. – No 2. – C.52-54.

10. Dudar L. V. The estimation of local immune status of colon mucosa in patients with non-specific ulcerative colitis / L.V. Dudar, N.G. Bichkova // Vrachebnoe delo. – 1994. – No 1. – P. 81–83.

11. Goshchinskiy A. B. About possibility of application of lyophilized xenodermoimplantate for warning of insolvency of intestinal guy-sutures / V.B. Goshchinskiy, S.A. Nazarchuk // Ukrainian Journal of Surgery. – 2011. – № 5 (14). – P. 162 – 166.

12. P'yatnytskyi Yu.S Gut microbiota structure of rats under introduction of the diet shredded xenograft substrate / Yu.S. P'yatnytskyi, O.V. Pokryshko // Annals of Mechnikov Institute. – 2014. – No 2. – P. 58 – 63.

13. Bush L. W. Pig Skin as Test Substrate for Evaluating Topical Antimicrobial Activity / L. W. Bush, L. M. Benson, J. H. // White Journal of Clinical Microbiology. – Sept. 1986. – P. 343 – 348.

14. Gnatjuk C. S. Local immune reactions in the case of cholecystitis / C. S. Gnatjuk // Bulleting of Surgery. - 1997. - Vol. 156, No 6. - C.19-23.