REASEARCH ARTICLE

Cyclooxygenase-2 and level cryoglobulin significance for small bowel anastomotic leak healing

Abstract

It is known that the leading pathophysiological aspect in patients with Intestinal Anastomoses (IA) is volemic and hemodynamic disorders, which are caused by the reduction of arterial inflow and impaired venous outflow due to compression of intracellular vessels, and sequestration of fluid. The aim study was to examine role cyclooxygenase-2 and level cryoglobulin significance of anastomotic leakage.

Material and Methods: From 96 consecutive patients that were selected by the method of random sampling, which performed resections of the segments of the small intestine with the formation of intestinal anastomoses, in the SI "ZIGUS NAMSU" in the period from 2016 to 2018, paraffin-embedded colonic or rectal tissue sections were collected from the pathology department's database for DNA extraction.

Cryoglobulin is serum immunoglobulins that re-precipitate at a temperature below 37°C. Separation of Cryoglobulin from serum was performed by the A.E. Kalovidoris method with modifications.

Results and Discussion: The 96 patients were examined, Cryoglobulinemia (CGE) was detected in the majority -59 (62.5%) patients, 4 of which was subsequently revealed inactive hepatitis C; the remaining 37 patients (38.5%) did not have CGE. In our study, 95% of patients had the first type of CGE that we associate with self-aggregation via the IgFc fragment and 4.2% had the third type of CGE that correlated with the latent form of hepatitis C in these patients. Humans with genetically impaired COX-2 expression show high anastomotic leakage incidence of 59 patients have is cryoglobulinemia-undergoing intestinal resection, 11 were homozygous for the PTGS2-765G>C polymorphism. Three of 11 (27%) developed anastomotic leakage compared with 14 of 59 (23.7%) patients with the -765GC or -765GG genotype.

Conclusion: For decades, Cryoglobulin has fascinated investigators and clinicians. The research into this intriguing disorder (anastomotic leakage) weaves together the history of medicine during the last century. Finally, the striking association between COX-2 and cryoglobulinemia along with the multifaceted clinical expression of this anastomotic leakage re-launch the challenge.

Keywords: cyclooxygenase-2, cryoglobulin, anastomotic healing

Introduction

The enzyme cyclooxygenase-2 (COX-2), also known as prostaglandin-end peroxide synthase 2 (Ptgs2), plays an important role in gut homeostasis. In general, cyclooxygenase regulates the conversion of arachidonic acid into prostaglandins, of which prostaglandin E2 (PGE2) is reported to restore intestinal integrity in experimental models of intestinal inflammation and damage [1-8].

It is known that the leading pathophysiological aspect in patients with Intestinal Anastomoses (IA) is volemic and hemodynamic disorders, which are caused by the reduction of arterial inflow and impaired venous outflow due to compression of intracellular vessels, fluid sequestration, and sequestration of fluid [9-13].

COX-2-induced production of prostaglandins in mesenchymal stem cells in the colon has an immune-modulatory role2, and these mesenchymal stem cells may thereby act as monitors of the colonic environment. Also, they might have important functions in colonic wound healing [5]. Furthermore, COX-2 expression is induced in macrophages

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and myofibroblasts upon exposure to pro-inflammatory cytokines and bacterial products, leading to proliferation and protection against apoptosis [2,3,14-17].

In the further development of pathological changes the following factors are crucial: The gradual exhaustion of the detoxification potential of the liver; translocation of intestinal microflora and acquisition of pathogenic properties; growth of the total mass of toxic products in internal environments; development of systemic microcirculatory disorders in organs and tissues, impaired cellular metabolism; development and progression of peritonitis, the second source of intoxication [3,18-21].

In our opinion (and this is confirmed by studies in related pathologies [9,10,18]), it is relevant to study the incidence of Cryoglobulinemia (CGE) in patients with cancer insolvency, which is appropriate in the aspect of prevention of postoperative complications, rapid recovery of functions bowel and further effective rehabilitation of operated patients.

Material and Methods

■ Human tissue collection

From 96 consecutive patients that were selected by the method of random sampling, which performed resections of the segments of the small intestine with the formation of intestinal anastomoses, in the SI "ZIGUS NAMSU" in the period from 2016 to 2018, paraffin-embedded colonic or rectal tissue sections were collected from the pathology department's database for DNA extraction.

■ DNA isolation from human tissue

Genomic DNA was obtained from formalin-fixed paraffin-embedded tissue using either a QIAamp DNA FFPE Tissue Kit (Qiagen) or an in-house protocol. In short, 4 tissue sections of 8 mm per sample were deparaffinized, and samples were lysed under denaturing conditions with proteinase K (at least 3 hrs. at 55°C). After centrifuging multiple times, pure genomic DNA was obtained and concentration was determined using Nanodrop. To obtain more purified DNA, all samples were twice precipitated with sodium acetate, both after the amplification and the restriction step.

■ PTGS2-765G>C Genotyping

A common promoter variant in the COX-2 gene, -765G>C, leads to lower expression of COX-2 as the -765C allele, compared with the -765G allele, reduces promoter activity21. In this study, it was also demonstrated that the -765C allele correlates with lower C reactive protein levels. Therefore, the presence of -765G>C may have significant clinical implications. We hypothesized that the PTGS2-765G>C polymorphism would be associated with an increased risk of anastomotic leakage through lower promoter activity of the COX-2 gene. Amplification of this region was performed using specific forward and reverse primers (CCGCTTCCTTTGTCCATCAG and GGCTGTATATCTGCTCTATATGC, respectively). Subsequently, ASCII restriction enzyme (New England Biolabs, Ipswich, MA) was applicated (60 min incubation at 37°C, 20 min inactivation at 65°C) resulting in both a 188 base pair product and a 118 base pair product in case of a -765G allele, or an uncut 306 base pair product in case a -765C allele. The presence of homozygous PTGS2-765G>C polymorphism (-765CC) was then correlated to anastomotic leakage.

Cryoglobulins are serum immunoglobulins that re-precipitate at temperatures below 37°C. Separation of cryoglobulins from serum was performed by the A.E. Kalovidoris method with modifications. The concentration of cryoglobulins was evaluated on the SF-46 spectrophotometer in dynamics on the 1st, 2nd, 3rd, 7th day. Control values for cryoglobulins in serum were tested in 15 healthy subjects and ranged from 60 μg/ml-80 μg/ml, which corresponds to the norms obtained in the works of Ferri C., Zignego A.L. et al.

Statistical analysis was performed using Statistica 6.0 software (StatSoft, Inc. 2001) and SPSS 7.5 on a PC. The data are presented as means ± standard deviations (SD). Statistical differences in the data were evaluated by Student's t-test or one-way analysis of variance as appropriate and were considered significant at $p<0.05$ [22].

Results and Discussion

The 96 patients were examined, Cryoglobulinemia (CGE) was detected in the majority -59 (62.5%) patients, 4 of which was subsequently revealed inactive hepatitis C; the remaining 37 patients (38.5%) did not have CGE. Because CGE is heterogeneous sign, we used the classification

of J. S. Brouet 1974 in our study, according to this classification cryoglobulins were divided into three types, depending on the components included in the cryoprecipitate. The first type includes immunoglobulins of the same class with one type of light chain (IgG, IgM, and IgA). The monoclonal component of this class has rheumatoid factor activity and is IgM. The monoclonal component of this class is IgM and it has rheumatoid factor activity. The third type is mixed cryoglobulins containing complexes of polyclonal immunoglobulins IgG+IgM, IgG+IgM+IgA, as well as low molecular weight IgM.

In our study, 95% of patients had the first type of CGE that we associate with self-aggregation via the IgFc fragment and 4.2% had the third type of CGE that correlated with the latent form of hepatitis C in these patients. The Fc fusion proteins introduce the option of modulating effector functions. The approved Fc fusions have an IgG1-Fc, which can elicit Antibody-Dependent Cellular Cytotoxicity (ADCC) and Antibody-Dependent Cellular Phagocytosis (ADCP) via the activating FcγRs, but cannot trigger complement as they lack the Fab domain [23-25].

We conducted a new study of the quantification of cryoglobulins in patients operated on the intestine; patients underwent resection of segments of the small intestine with intestinal anastomosis formation. Most patients who underwent resection of segments of the small intestine had I type of CGE and the content of cryoglobulins was low and medium (53 patients); patients with questionable CGE did not have low cryoglobulin content; however, this may be due to the low number of observations (2 patients). The study found that all examined patients had a significant increase in cryoglobulin levels by an average of 106.5% on the first day after surgery. It should be noted that high and medium degree CGE was observed in patients whose postoperative period was further complicated by the development of postoperative complications.

We provided a new investigation of the quantification of cryoprecipitate in patients with anastomotic leakage in parallel with the concentration of immunoglobulins of classes G and M, as well as immunoglobulins of class A, it is associated with fixation of cryoglobulins in the microvasculature with manifestations of autoimmune aggression against the intestinal epithelium. On the 7th day of the disease, an increase in IgG quantification of 4.3% and a significant ($p \leq 0.05$) increase in IgM by 19.4% relative to the initial level were established. Increasing the quantification of antibodies at day 7 may be associated with the development of a secondary immune response. In some cases, the increase in IgG and IgM concentration relative to their initial level occurred as early as the 3rd or 5th day of observation.

Both groups of patients a decrease in the concentration of cryoglobulins was found on the 2nd day after surgery and a significant increase on the 3rd day. On the 5th day, there was a decrease in the concentration of cry proteins in the blood of patients. In the study of the initial parameters of the immune status in patients of the studied groups, before surgery and on the first day after surgery revealed ($p \leq 0.05$), significant differences. It was found that in patients with cryoglobulinemia the immunogram values were significantly ($p \leq 0.05$) lower.

For a correct evaluation of serum cryoglobulins, the first steps (blood sampling, clotting, and serum separation by centrifugation) should always be carried out at 37°C and the cryocrit determination and cryoglobulin characterization at 4°C (after seven days). Moreover, cryocrit determinations should be done on blood samples without anticoagulation to avoid false-positive results caused by cryofibrinogen. Unfortunately, there are no standardized methodologies for cryoglobulin measurements that are accepted worldwide. Serum cryoglobulin values usually do not correlate with the severity and prognosis of the disease. Very low cryocrit values, often difficult to quantify, can be associated with severe, active cryoglobulinemia syndrome; on the contrary, high serum cryoglobulin concentrations may characterize an oligo symptomatic or asymptomatic disease course. In these subjects, particularly in the presence of a cryogel phenomenon, rheological alterations caused by blood hyperviscosity can be observed. Finally, a sudden decrease or disappearance of serum mixed cryoglobulins, sometimes associated with abnormally high C4 values [23-25.

Humans with genetically impaired COX-2 expression show high anastomotic leakage incidence of 59 patients have is cryoglobulinemia-undergoing intestinal resection, 11 were homozygous for the PTGS2-765G>C polymorphism. Three of 11 (27, 27%)

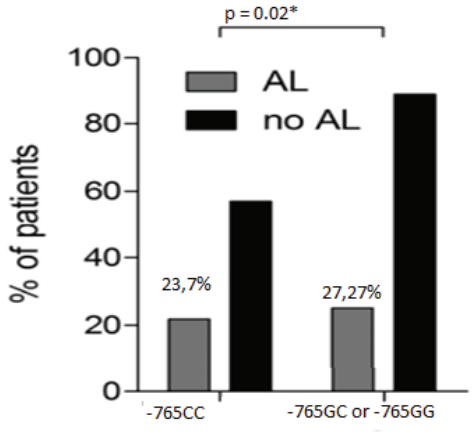

FIGURE 1. Effect of human genotype at common promoter variant in the COX-2 gene on anastomotic leakage incidence.

developed anastomotic leakage compared with 14 of 59 (23,7%) patients with the -765GC or -765GG genotype (p=0.02, **FIGURE 1**).

This study shows that COX-2 is of critical importance in preventing anastomotic leakage after colonic surgery. Intriguingly, PTGS2-765G>C polymorphism in humans, associated with reduced COX-2 expression, was associated with higher anastomotic leakage rates.

Conclusion

This research topic showed that COX-2 and cryoglobulinemia are critically involved in the mucosal repair. Furthermore, abundant evidence exists showing that COX-2 and its derived prostaglandins stimulate intestinal cancer progression through enhanced angiogenesis and proliferation and by decreasing apoptosis. Although these mechanisms should be counteracted to reduce cancer progression, they are indispensable in adequate wound healing. The presence of cryoglobulins in the course of anastomotic leakage.

The presence cryoglobulins in the course of anastomotic leakage can be regarded as transient insufficiency of circulating immune complex clearance; in these conditions, tocsins particles or antigens can be directly involved in immune complex-mediated systemic manifestations. This is immune complex-mediated systemic vacuity caused by the deposition of cryoprecipitate circulating immune complexes.

For decades, cryoglobulins have fascinated investigators and clinicians. The research into this intriguing disorder (anastomotic leakage) weaves together the history of medicine during the last century. Finally, the striking association between COX-2 and cryoglobulinemia along with the multifaceted clinical expression of this anastomotic leakage re-launch the challenge.

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