

Peritoneal fibrinolytic activity and adhesiogenesis

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Rezumat

Activitatea peritoneală fibrinolitica și adeziogeneza

Introducere: Aderențele postoperatorii după intervenții chirurgicale abdominale sau pelvine rămân o importantă problemă clinică, ce provoacă infertilitate, durere și obstrucție intestinală. Prevenirea și tratamentul lor rămân puțin și neadecvat înțelese. Formarea de adeziuni este datorată organizării unei matrice de fibrină, care are loc în timpul procesului de coagulare atunci când există supresia fibrinolizei.

Metode: În acest studiu, au fost prelevate și analizate țesut peritoneal și lichid peritoneal de la două grupuri de pacienți. Primul grup, format din 12 pacienți, care au fost supuși unei intervenții chirurgicale abdominale pentru un abdomen acut în care factori cunoscuți de agresiune peritoneală (traumatisme, factori chimici, factori bacterieni) au fost prezenți și care sunt cunoscuți pentru a crește incidența formării de aderențe peritoneale. Un al doilea grup format din 6 pacienți supuși unei intervenții chirurgicale, în absența acestor factori de agresiune peritoneală, și care a acționat ca un grup de control de referință. De la fiecare pacient a fost prelevat țesut peritoneal la momentul intervenției chirurgicale, ce a fost analizat pentru nivelurile de expresie genică de activator de plasminogen tisular (tPA) și inhibitor de activator de plasminogen-1 (PAI-1). De la pacienți, de asemenea, a fost colectat lichidul de drenaj peritoneal și analizat postoperator urmărind cantitățile

de produși de degradare ai fibrinei (FDPs) și fibrinogen.

Rezultate: Scopul acestui studiu a fost de a evalua rolul PAI-1 și tPA la nivel tisular peritoneal. Țesutul peritoneal a fost obținut în timpul intervenției chirurgicale și variația de exprimare a PAI-1 și genei tPA au fost cuantificate. Rezultatele obținute evidențiază o creștere de exprimare a genei PAI-1 și o scădere de exprimare a genei tPA la pacienții cu factori de agresiune peritoneală, comparativ cu pacienții fără, indicând un potențial scăzut fibrinolitic la pacienții cu tendință crescută la aderența peritoneală. Factorii de agresiune peritoneală, de asemenea, au stimulat niveluri crescute de fibrinogen și FDP în exudatele peritoneale.

Cuvinte cheie: aderențe, fibrinoliză, tPA, PAI-1.

Abstract

Introduction: Postoperative adhesions after abdominal or pelvic surgery remain an important clinical problem causing infertility, pain and bowel obstruction. Their prevention and treatment remains poorly understood and inadequate. The formation of adhesions is caused by the organization of a fibrin matrix, an organization that takes place during the coagulation process when there is suppression of fibrinolysis.

Methods: In this study peritoneal tissue and peritoneal fluid from two groups of patients were sampled and analysed. The first group comprised of 12 patients undergoing abdominal surgery for an acute abdomen during which known peritoneal factors of aggression (trauma, chemical, bacterial) were present which are known to increase the propensity for peritoneal adhesion formation. A second group consisting of 6 patients undergoing surgery in the absence of these peritoneal aggression factors acted as a reference control group. Each

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patient had peritoneal tissue sampled at the time of surgery and analysed for levels of gene expression of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1). Patients also had peritoneal drain fluid collected post-operatively and analysed for quantities of fibrin degradation products (FDPs) and fibrinogen.

Results: The aim of this study was to evaluate the role of PAI-1 and tPA genes at peritoneal tissue level. Peritoneal tissue was obtained during surgery and the variation of expression of PAI-1 and tPA genes was quantified. The obtained results highlighted an increase of expression in PAI-1 gene and decrease of expression in tPA gene in patients with increased factors of peritoneal aggression compared to patients without, indicating a decreased fibrinolytic potential in patients with increased peritoneal adhesion propensity. Increased factors of peritoneal aggression also resulted in increased levels of FDPs and fibrinogen in peritoneal exudates.

Key words: adhesions, fibrinolysis, tPA, PAI-1

Introduction

The formation of adhesions following abdominal and pelvic operations remains a common source of morbidity, causing infertility, pain and bowel obstruction. The incidence of intraperitoneal adhesions ranges from 67% to 93% after general abdominal operations and up to 97% after gynecological operations in the pelvic region (1,2). Postoperative adhesions are the leading cause of conversion from laparoscopy to laparotomy and the division of adhesions accounts on average for 20% of operating time in patients with a history of an open colorectal surgery (3). Factors associated with the formation of postsurgical adhesions include: trauma, thermal injuries, infection, ischemia, and foreign bodies. Many other factors, including very tight sutures, when the tension in the sutured peritoneum brings about ischemia, abrasion, exposure to aggression factors such as talcum powder in gloves, reactive suture materials, bowel contents, super-heating due to lamps or irrigation fluid, all can contribute to the formation of postsurgical adhesions.

Peritoneal adhesions are pathological bonds between surfaces within the peritoneal cavity. Damage to parietal or visceral peritoneum leads to exposure of the basal membrane of the mesothelial layer to surrounding tissues. This causes a local inflammatory reaction, leading to the formation of a sero-sanguinous exudate, rich in fibrin. Normal tissue repair is facilitated by this fibrin rich exudate, which acts as a matrix for invading fibroblasts and angiogenesis. However, the degradation of fibrin is essential to restore the pre-inflammatory environment.

Degradation of fibrin, known as fibrinolysis is regulated by the plasminogen system. Incomplete fibrinolysis leads to persistent pathological bonds between peritoneal surfaces

resulting in fibrous peritoneal adhesions. The inactive pro-enzyme plasminogen is converted into plasmin by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). Plasmin degrades fibrin, eliminating the matrix structure of fibrous adhesions. The conversion of plasminogen to plasmin is inhibited by plasminogen activator inhibitor-1 (PAI-1). A reduction in plasmin, resulting in reduced fibrinolysis, promotes increased adhesion formation (4). This was demonstrated in an animal surgical model, where PAI-1 knockout mice had significantly reduced adhesion formation compared to wild type mice (5). Administration of recombinant plasminogen activator promoting increased fibrinolysis was shown to be effective in preventing adhesions in a laparoscopic mouse model (6). Imbalance between fibrin deposition and fibrinolysis is the key driver in the development of adhesion formation (7).

The objective of our study is to demonstrate this at a gene expression level in human surgical patients undergoing abdominal surgery with increased risk of adhesion formation.

Materials and Methods

Structure of patient groups

In this study two groups of patients were analysed, a "study group" and a "control group".

The study group was composed of twelve patients operated on for an acute surgical abdomen. A factor of peritoneal aggression (FPA) promoting the formation of postsurgical peritoneal adhesions was particularly prominent during the operation of each of these 12 patients. The factors of peritoneal aggression were divided into three categories: traumatic, chemical, and bacterial (*Table 1*). Within this group, peritoneal fibrinolytic activity was measured by determining the protein levels of fibrinogen and fibrin degradation products (FDP) in the peritoneal liquid and by analysing the gene expression levels of tPA and PAI-1 from the peritoneal tissue.

The control group acted as a reference group and was made up of six patients operated on in the same hospital for defects of the abdominal wall (three patients) and for acute catarrhal appendicitis with peritoneal serous reaction (three patients). None of the factors of peritoneal aggression noticed in the patients of the first group were present in the control group. All patients consented to be included in this study, which was approved by the ethical committee of the University Emergency Hospital of Bucharest.

Processing of the peritoneal tissue to determine tPA and PAI-1 gene expression levels.

In order to extract the tPA and PAI-1 from the peritoneal tissue, fragments of peritoneal tissues freshly collected from patients operated on for surgical acute abdomen were used, in which the presence of one of the peritoneal aggression factors indicated in the table below had been established. For comparison purposes, the control group was made up of patients with uncomplicated hernias of the abdominal wall,

Table 1. List of intra-abdominal pathologies in patients with FPA (factors of peritoneal aggression)

Diagnosis	Traumatic FPA	Chemical FPA	Bacterial FPA
Hemoperitoneum by spleen rupture	blood	0	0
Hemoperitoneum by entero-mesenteric wound	blood	0	0
Perforated duodenal ulcer juice	0	bile+gastric	
Perforated gastric ulcer	0	gastric juice	0
Perforated gangrenous calculus cholecystitis.			
Biliary septic peritonitis	0	infected bile	E.coli
Perforated gangrenous acalculus cholecystitis.			
Biliary chemical peritonitis	0	uninfected bile	0
Perforated gangrenous appendicitis			
Generalized peritonitis.	0	0	E.coli
Caecal perforation due to colon cancer. Generalized peritonitis.	0	0	E.coli
Uroperitoneum by traumatic rupture of bladder.	0	urine+blood	0
Uroperitoneum by PNL perforation of renal pelvis.	0	infected urine	E.coli
Ruptured ovarian endometriotic cyst.			
Endometriotic peritonitis.	0	endometriotic liquid	0
Ruptured tuboovarian abscess. Pelviperitonitis.	0	0	E.coli

where fragments of saccular peritoneum were extracted and the absence of peritoneal aggression factors was noted. The collection of peritoneal tissue from these patients did not involve any supplementary surgical procedures since the resection of the hernia sac constitutes a normal part of the surgical treatment of abdominal parietal defects.

Biopsy procedure

The biopsy was conducted on the parietal peritoneum (punch biopsy with a 6-mm diameter, Stiefel Lab., Woobum Green, UK) as quickly as possible after the opening of the peritoneal cavity. The tissue disk was dissected from the adherent subperitoneal tissue and minced in fragments with dimensions smaller than 1 mm, which were introduced in recipients covered with RNA stabilizer and kept in a refrigerator at a temperature of 4°C until they were processed.

Processing of samples of peritoneal liquid to measure fibrinolytic activity

For the patients in the first group, the peritoneal liquid was collected by aspiration from the drainage tube located as near to the seat of the maximum peritoneal aggression as possible, beginning from the first day after the operation and repeated daily up to the seventh day. For determining the protein concentration of fibrinogen, the peritoneal liquid was processed in the emergency laboratory of the Emergency University Hospital of Bucharest, where it was sent in a vacuum container for analysis. For the quantitative determination of fibrin degradation products, the peritoneal liquid was collected in a syringe containing 0.1 ml of heparin and was processed on a Compact Immuno-analyser Pathfast Sintergy (Mitsubishi Kagaku Iatron. Inc), utilizing Pathfast D-dimer (Mitsubishi Kagaku Iatron.Inc) kits.

For the patients in the control group, the peritoneal liquid was first collected during the operation and afterwards daily – for two or three days – from a thin drainage tube located in the pouch of Douglas, until the drainage diminished and the drainage tube was removed.

Extraction of RNA from the tissue using the RNeasy® LIPID TISSUE KIT

Principle of method

The RNeasy Lipid Tissue Kit combines the method of lysis of the cells on the basis of phenol-guanidine with purification of the total RNA by means of minicolumns of silicon dioxide. The lysis reactive QIAzol is a monophasic solution of phenol and guanidine thiocyanate, ideal for the lysis of various tissues (especially in tissues with a high fat content) and for the inhibition of RNase. In a first stage, the following operations are conducted: homogenization of the tissue in the lysis reactive QIAzol, addition of chloroform and separation – by centrifuging – of the homogenate in the aqueous phase and the organic phase. In the following stage, the superior phase, which is aqueous (and contains the total RNA) it is collected and then mixed with ethanol and the mixture is passed through an RNeasy minicolumn. The total RNA is then tied to the little column, and the phenol – as well as other contaminants – are eliminated. After successive washing by means of various washing swabs, the total RNA is eluted in water without RNase.

Statistical evaluation of results

Statistical analysis was conducted using Microsoft Excel and Epi Info computer programs. The homogeneity of data distribution was determined by the Bartlett test. A value of p lower than 0.05 of the Bartlett test was considered significant

and it shows a non-homogenous distribution of the data to be analysed, while a value of p equal to or higher than 0.05 indicates a similar dispersion of the subsamples. The significance of the differences between the patient groups with peritoneal risk factors and without peritoneal risk factors was determined by utilizing the parametric Student t -test for two groups and ANOVA for more than two groups, as well as the non-parametric tests Mann-Whitney for two groups and Kruskal-Wallis for more than two groups. The difference was considered statistically significant if the probability p of these tests is lower than 0.05.

The Student and ANOVA tests are applicable only in the case of a homogenous data distribution (the Bartlett test has a $p \geq 0.05$). In the case of a non-homogenous dispersion of subsamples (the Bartlett test has a $p < 0.05$), the Bartlett test is not appropriate and a non-parametric statistical test (the Mann-Whitney and Kruskal-Wallis tests) are used.

Results

Patient pathologies

The individual pathologies of the 12 patients with known factors of peritoneal aggression and therefore considered to have increased risk of peritoneal adhesion formation are listed in Table 1.

Analysis of peritoneal-tissue samples

Relative quantification of the expression of PAI-1 gene

In order to study the expression of PAI-1 gene, the Real-Time PCR procedure was used, going through the following stages: reverse transcription of total RNA into cDNA, optimization of amplification conditions (PAI-1 gene and reference gene), achievement of the standard curve and calculation of the efficiency of PCR reaction. Finally, all the samples were run and normalized as a function of the actin reference gene, and in the end the relative expression of PAI-1 was compared between various patients.

The PCR conditions were optimized for the primer by varying the annealing temperature between 50-60°C on a gradient thermocycler IQCycler (BioRad). The PCR products were directly analysed by electrophoresis in 2% agarose gels in 1X TAE buffer, stained with ethidium bromide, and visualized under UV light (Fig. 1, 2). The optimum annealing temperature for all genes was 54°C.

Fig. 3 shows the amplification curves which represent the fluorescent signals obtained during the reaction. In fact, the graph represents the intensity of the fluorochrome interpolated at the level of the PCR products (thus measuring, in the case of the same gene, the quantity of PCR product formed in each cycle) vs the amplification cycle.

To calculate the efficiency of a PCR reaction, a logarithmic graph is obtained in which the values of C_t are represented vs the dilutions of the sample. In Fig. 4 and 5, the efficiencies of the PCR reaction are represented for the case of amplification of fragments of PAI-1 and actin genes.



Figure 1. Temperature gradient for optimizing the amplification conditions of a fragment of PAI-1 gene. 1: 51°C; 2: 51,8°C; 3: 53,1°C; 4: 54,9°C; 5: 57,4°C; 6: 59,3°C; 7: 60°C; 8: 50bp molecular weight marker

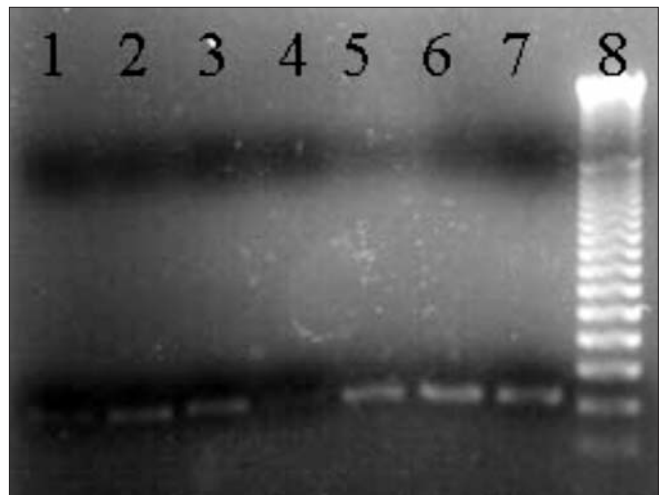


Figure 2. Temperature gradient for optimizing the amplification conditions of a fragment of actin gene. 1: 51°C; 2: 51,8°C; 3: 53,1°C; 4: 54,9°C; 5: 57,4°C; 6: 59,3°C; 7: 60°C; 8: molecular-mass marker 50bp

The efficiency of the PCR reaction is considered to be good if it is 90-110%, which corresponds to a slope value between -3.58 and -3.10. As shown in Fig. 4 and 5, the efficiencies obtained in the case of amplification of fragments of PAI-1 genes and actin were 106.6% (slope -3.173; $R^2=1$) and 105% (slope -3.208; $R^2=0.998$).

After the calculation of efficiencies, samples from various patients were run in order to quantify the relative expression of the PAI-1 gene as a function of the reference gene – actin. In Fig. 6, the amplification curves for all samples are presented.

The obtained melting curves have shown the existence of a single peak (89°C – PAI-1; 84°C – actin), which means that there were no non-specific amplifications, dimers of primers or contaminations with genomic DNA.

The amplification equations were determined for both

Figure 3. Standard curve was obtained using 5 serial dilutions of a cDNA sample: $10 \times$ dilution (10 ng/reaction), $10^2 \times$ dilution (1 ng/reaction), $10^3 \times$ dilution (0.1 ng/reaction), $10^4 \times$ dilution (0.01 ng/reaction), $10^5 \times$ dilution (0.00ng/reaction)

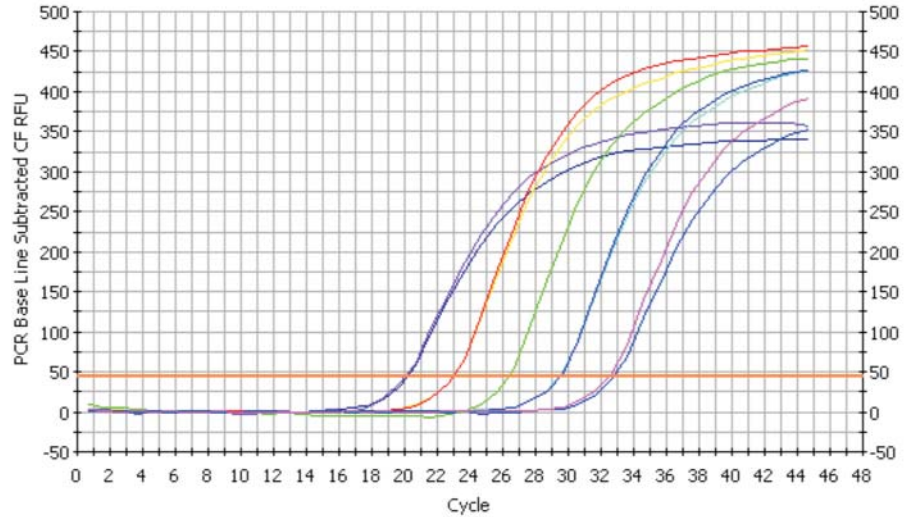


Figure 4. Efficiency of the amplification reaction of a fragment of PAI-1 gene

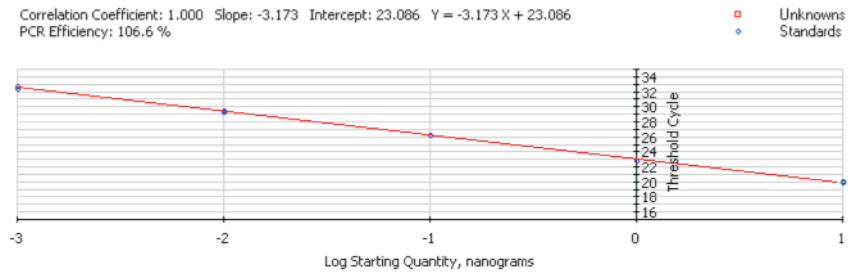


Figure 5. Efficiency of the amplification reaction of a fragment of actin gene

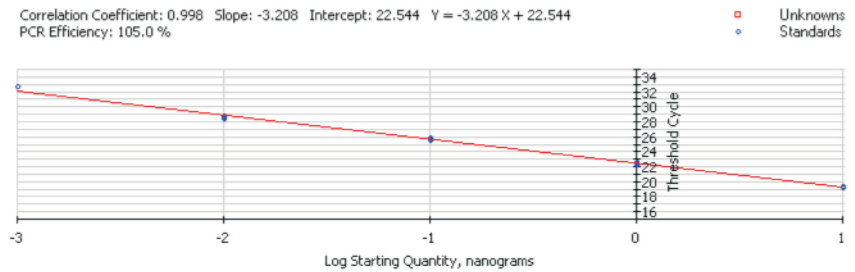
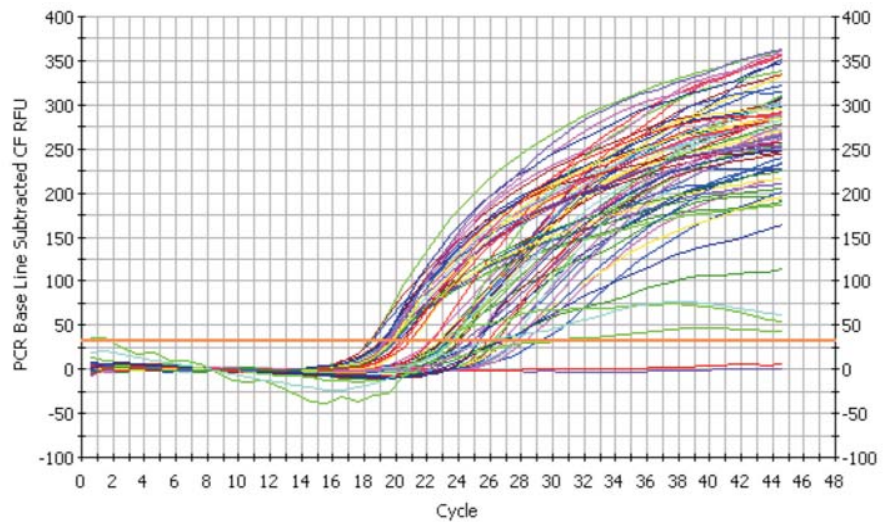


Figure 6. Amplification curve in the case of quantification of the expression of PAI-1 gene



the target gene PAI-1 and the reference gene (actin); in the case of each sample we obtained the following equations:

$$N_{CtPAI-1} = N_{0PAI-1} (1+E)^{CtPAI-1},$$

$$N_{Ctactin} = N_{0actin} (1+E)^{Ctactin},$$

where

- N_{Ct} = number of molecules after C_t amplification cycles,
- N_0 = initial number of molecules,
- E = efficiency of PCR reaction,
- C_t = threshold cycle

For normalization purposes, the ratio between N_{0PAI-1} and N_0 of the reference gene (actin) was calculated with the formula:

$$N_{0PAI-1}/N_{0ref} = k 2^{C_{tref}-C_{tPAI-1}}$$

where k is a correction factor.

The above equation was used for all of the patients, including the normal patient (deemed to be the control patient). In accordance with the equation:

$$\text{Control/patient} = k 2^{C_{trefcontrol}-C_{tPAI-1control}}/k 2^{C_{trefpatient}-C_{tPAI-1patient}}$$

$$= 2^{\Delta\Delta C_t},$$

all of the samples were referred to the control patient, and the diagram in Fig. 7 was obtained.

In compliance with the statistical t-test, two tails, non-paired, all of the values obtained for p were significant ($p < 0.05$). It is apparent from the diagram in Fig. 7 that the relative expression of PAI-1 gene is significantly increased (3-4.5 times) in comparison with control samples.

Relative quantification of the expression of tPA gene. In order to study the relative expression of tPA gene, the Real-Time PCR technique was used as well. The actin gene was used as reference gene again, and the stages run through were the same as in the case of the quantification of expression of PAI-1 gene: optimization of the hybridization temperature of primers, calculation of the efficiency of PCR reaction, and comparison of the expression of tPA of the patients was performed as for the case of the PAI-1 gene.

The PCR conditions were optimized for the primer by varying the annealing temperature between 50-60°C on a gradient thermocycler IQCycler (BioRad). The PCR products were directly analysed by electrophoresis in 2% agarose gels in 1X TAE buffer, stained with ethidium bromide, and visualized under UV light as with the PAI-1 gene.

The optimum temperature of the hybridization of primers

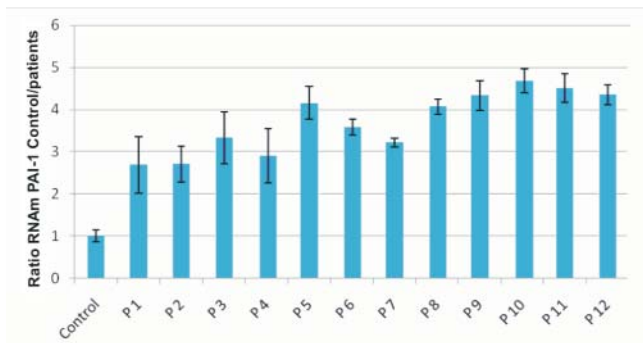


Figure 7. Relative expression PAI-1 gene

was 54°C. In Fig. 8 we have shown the obtained standard curve for tPA gene. The efficiency of the amplification reaction of a fragment of tPA gene was 99%, with a slope of -3.346 and $R^2 = 0.998$.

After the calculation of efficiency, samples from various patients were run in order to quantify the relative expression of tPA gene as a function of the actin reference gene. The samples from each patient were run in triplicate (technical replications). After the samples were run, the program of the PCR instrument established the threshold level and calculated the values of C_t for each sample. In Fig. 9 the amplification curve of all of the samples is presented.

The data obtained was processed in compliance with the equations given above, and the average values obtained – along with the standard deviation – were represented graphically in Fig. 10.

In compliance with the statistic student t-test, two tails, unpaired, all of the values obtained for p were significant ($p < 0.05$). In the case of tPA gene, a level of the expression was obtained, which was 3 to 9 times lower than in the case of the control patients (Fig. 10).

Analysis of the samples of peritoneal liquid

Analysis of the fibrinogen/FDP balance in the samples of peritoneal liquid

The samples were collected over a period of seven days after the operation from the twelve patients in the study group, in which – during the operation – the presence of one of the aggression factors described in Table 1 was ascertained. The

Figure 8. Efficiency of the amplification reaction of a fragment of tPA gene

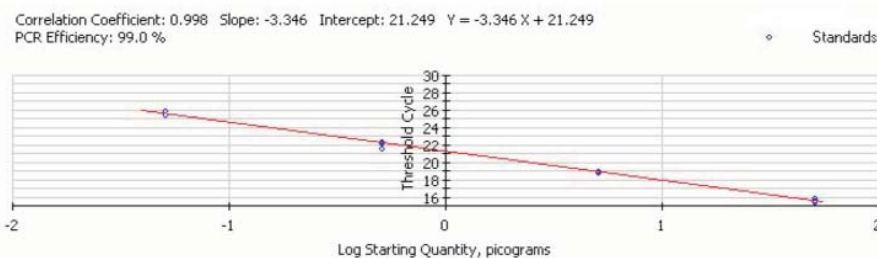
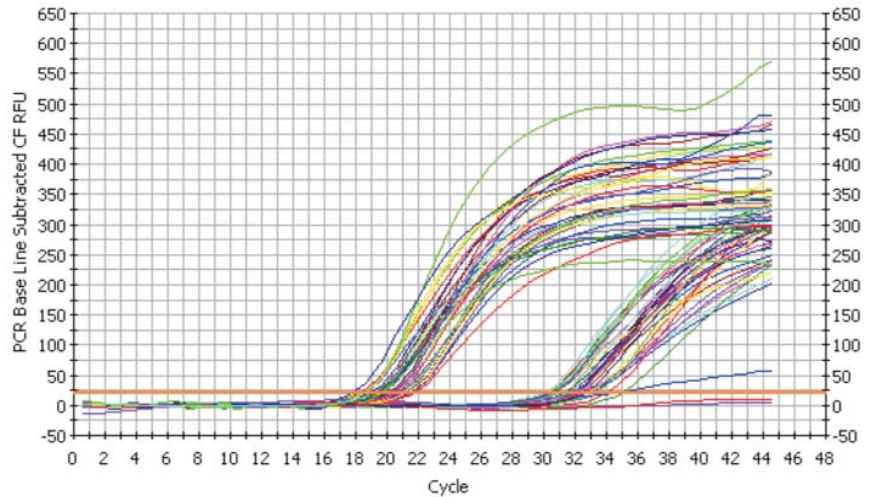


Figure 9. Amplification curve in the case of quantification of the expression of tPA gene



samples from the patients in the control group were collected only in the first three days after the operation, as long as a drainage tube was located in the Pouch of Douglas.

The sample collection and the quantitative determinations were conducted in accordance with the protocol provided in the Materials and methods section. The average age of the patients with factors of peritoneal aggression was 43 years (19 to 71 years), and the average age of the patients in the control group was 29 years (24 to 32 years).

Considering adhesion formation to be a dynamic process, serial sample collections of peritoneal fluid from the same subjects, at different time intervals from the surgical intervention were performed. Levels of fibrinogen and fibrin degradation products (FDP) were analysed in each sample providing more information regarding the evolution of physiological mechanisms and confirming a difference between the study and control groups.

The same variations of the studied parameters were found for all of the patients in the study group, irrespective of the aggression factor involved. It is to be noted that the fibrinogen values exhibit a maximum on the fifth day after the operation, and afterwards they begin to decrease until the seventh day, however without reaching the minimum values noticed in the first day, but – in accordance with the conclusions of a previous study – the absolute values are directly proportional to the risk of adhesiogenesis.

The presence of fibrin d-dimers in the peritoneal liquid was expected, since the fibrinolysis process follows fibrinogenesis sequentially. However, this study was not intended to determine the extent to which FDP levels can reflect the true fibrinolytic activity and can correlate with the risk of adhesiogenesis. In this respect, we notice that the average value of FDP correlates with the mean of the fibrinogen values for each individual case, but the quantitative variation of d-dimers is inversely proportional to the quantitative variation of fibrinogen until the fifth day after the operation and afterwards exhibits the same decreasing trend. These aspects can be found in the diagrams of synchronous variations (Fig. 12 A,B).

One of the drawbacks of this study is the impossibility of

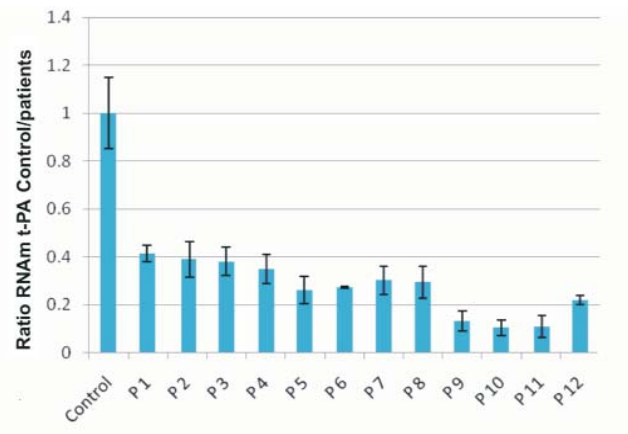


Figure 10. Relative expression of tPA gene

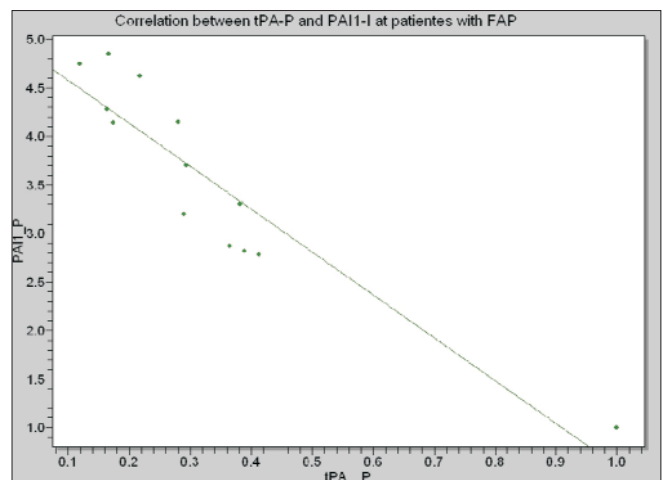


Figure 11. Correlation between relative expression of PAI-1 gene and tPA gene in individual peritoneal samples relative to controls

comparing the Fibrinogen and FDP levels of patients subjected to factors of peritoneal aggression (FPA) with the results of patients in the control group, for the whole period of

Figure 12. (A) Levels of Fibrinogen (FIB) during post-operative period;

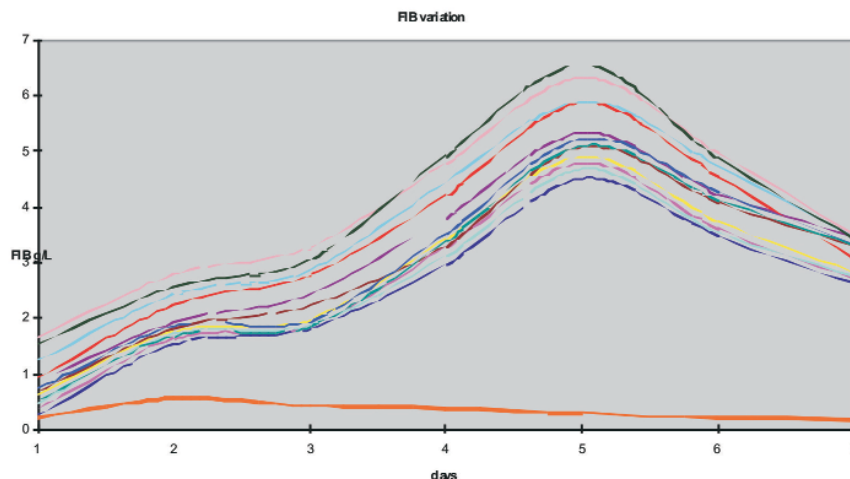
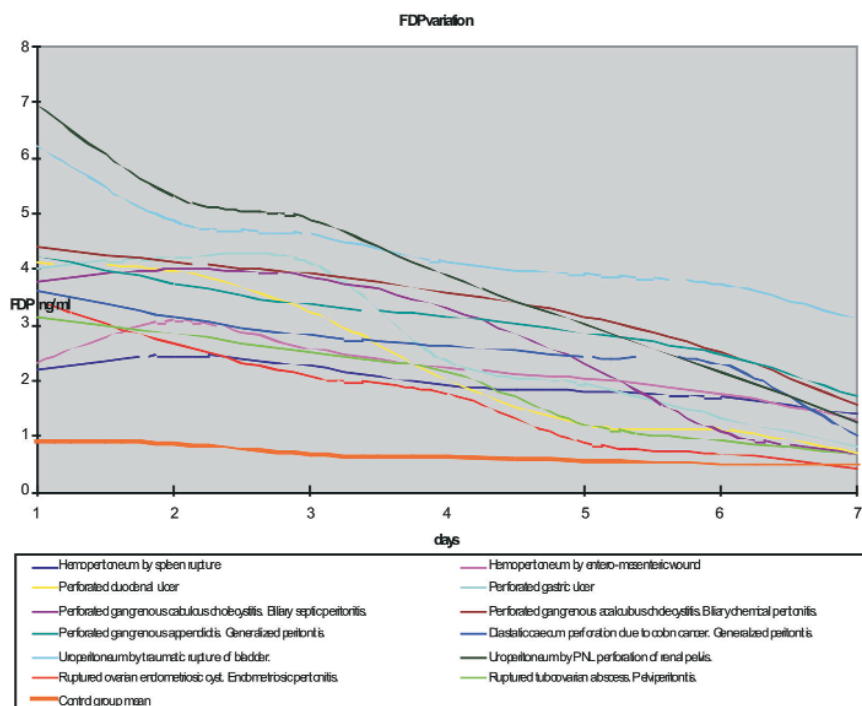


Figure 12. (B) Levels of Fibrin degradation products (FDP) during post-operative period



seven days after the operation. For ethical and medical reasons, this statistical comparison was only possible for the first 2 to 3 days after surgery, as the control patients did not clinically require peritoneal drains for longer than this. For the patients in the FPA group the collections of peritoneal liquid were possible for seven consecutive days or until the last drainage tube was removed from the peritoneal cavity. However, the collection of intraperitoneal exudates from control patients was only possible for as long as the peritoneal drainage was necessary for the patients with appendicitis and serous peritoneal reaction. The peritoneal liquid was collected from one of the patients in the control group for three consecutive days and a descending trend of the values of FIB and FDP was noticed, which could suggest that the prolonged inflammation of the peritoneal serosa, under the influence of aggression factors, represents the main trigger of adhesiogenesis, although

fibrinolytic peritoneal activity was seen in all of the patients in the FPA study group.

Unlike in the case of fibrinogen, FDP values have a higher predictive value in what regards the formation of peritoneal adhesions, depending on the determining aggression factors. It was noticed that in the cases of adnexial pathology (ruptured endometriosis cyst and pelviperitonitis by ruptured tubo-ovarian abscess), FDP values are 1.7 | 1.10 ng/ml and 1.924 | 0.984 ng/ml ($p < 0,05$) and they decrease very slowly, in situations where the quantity of fibrinogen in the peritoneal exudates and, implicitly, the resulting quantity of fibrin are the biggest (3.859 | 1.705 g/L and 3.384 | 1.642 g/L, $p < 0,05$) as compared with the other aggression factors, between their variations existing a negative correlation of -0.72239 and -0.69722 ($|r| < 1$), respectively.

An argument confirming this hypothesis is represented by

Table 2. Fibrinogen levels in patients with factors of peritoneal aggression (FPA) and controls during the post-operative period

Statistical Analysis	Fibrinogen (FIB) – g/L						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
(A) Patients with FPA							
• Average	0.8232	1.9999	2.3411	3.7773	5.3780	4.1944	3.1527
• Std. Dev.	0.4473	0.4056	0.5297	0.6692	0.6584	0.5237	0.3334
• Variation	0.2000	0.1645	0.2805	0.4478	0.4336	0.2742	0.1111
(B) Patients without FPA							
• Average	0.2080	0.5497					
• Std. Dev.	0.0316	0.0862					
• Variation	0.0010	0.0074					
(A)vs (B)							
Bartlett's Test	0.0073	0.0604					
P value							
ANOVA							
P-value		0.0000					
Kruskal-Wallis Test							
P value	0.0094						

the similar FDP values (2.186 | 0.572ng/ml, $p < 0,05$), but in the conditions of a much lower concentration of fibrinogen (2.615 | 1.464g/L, $p < 0,05$) in the patients with post-traumatic hemoperitoneum.

Discussions and Conclusions

The physiological disappearance of the provisional matrix of a clot is as important as its formation. The inadequate removal of fibrin can hinder the normal healing process of wounds and may lead to the formation of fibrous adhesions.

In this study we have demonstrated that factors of peritoneal aggression (chemical, bacterial or traumatic) lead to increased expression of PAI-1 gene in peritoneal tissue of surgical patients compared to control patients who had no factors of peritoneal aggression present at the time of surgery. We have also demonstrated a reduction in the level of tPA gene expression in the peritoneal tissues of patients with factors of peritoneal aggression compared to levels in control patients. An increase in PAI-1 gene expression and a reduction in tPA gene expression at peritoneal tissue level confirm the mechanism of suppression in plasmin dependent fibrinolysis. Increased levels of PAI-1 gene expression correlate with suppression of tPA gene expression in the same peritoneal tissue samples (Fig. 11).

PAI-1 is a glycoprotein of 50kD produced at the level of the endothelial cells, which belongs to the superfamily of superprotease inhibitors (serpin-1) and represents the major inhibitor of the tPA activator (tissue plasminogen activator) and of the activator uPA (urokinase plasminogen activator), at the level of the plasma and of the peritoneum (8,9), determining the formation of the inactive enzymatic complexes PA-PAI. The high levels of expression of PAI-1 and tPA are associated with coronary risk at the level of arteries. The increased activity of tPA is an indicator of increased fibrinolytic potential. The action of PAI-1 is controlled by various cytokines and growth factors, such as TGF- β . Thus,

TGF- β stimulates the secretion of PAI-1 in various cellular lines (10) and in vivo (11,12).

The fibrinolytic system plays an important role in the formation/reformation of peritoneal adhesions. In the first stages of the healing process between two damaged peritoneal surfaces, fibrin bands develop within a fibrinous matrix, which can be removed naturally by fibrinolysis, the result being the formation of fibrin degradation products (FDP). In the pathological processes of adhesion formation, the fibrinolytic mechanisms are inhibited, allowing the formation of persistent adhesions. Our study has demonstrated that this fibrinolysis inhibition is at a gene expression level within peritoneal tissue. Patients with factors of peritoneal aggression demonstrated increased expression of the PAI-1 gene and a reduced expression of the tPA gene compared to control patients (Fig. 10 & Fig. 7).

The quantitative determination of fibrinogen levels in peritoneal fluid acts as an indicator of the inflammation induced by aggression factors within the peritoneal cavity, while the quantitative determination of fibrin degradation products (D-dimers) is an indicator of the fibrinolytic activity. The same patients who had increased PAI-1 gene expression and reduced tPA gene expression in their peritoneal tissue, were also found at a protein level to have increased levels of fibrinogen and fibrin degradation products in sampled peritoneal fluid, confirming an increased level of fibrinogenesis and fibrinolysis activity and a significant difference between the study group and the control group. Patients in the study group with increased propensity for adhesion formation were found to have significantly increased levels of fibrinogen, which peaked on the 5th postoperative day and still had not normalized by the 7th postoperative day (Fig. 12). This supports the argument that adhesion formation occurs within the immediate post-operative period and any potential treatments to prevent adhesion formation would need to be administered during the perioperative and immediate post-operative period. The administration of recombination tPA to promote

Table 3. Levels of fibrin degradation products in patients with factors of peritoneal aggression (FPA) and controls during post-operative period

Statistical Analysis	Fibrinogen (FDP) – ng/ml						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
(A) Patients with FPA							
• Average	4.0333	3.7067	3.3542	2.7567	2.2308	1.8117	1.2175
• Std. Dev.	1.3880	0.8861	0.9378	0.8168	0.9003	0.8676	0.7182
• Variation	1.9267	0.7852	0.8795	0.6672	0.8105	0.7528	0.5158
(B) Patients without FPA							
• Average	0.9000	0.8700					
• Std. Dev.	0.3517	0.2506					
• Variation	0.1237	0.0628					
(A)vs (B)							
Bartlett's Test	0.0852	0.1056					
P value							
ANOVA							
P-value	0.0023	0.0001					
Kruskal-Wallis Test							
P value							

increased fibrinolysis and therefore a reduction in adhesion formation, while found to be of benefit in an in vivo mouse model (6), may unfortunately be impractical due to the increased risk of perioperative bleeding in surgical patients.

The continuation of research is necessary for further establishing the mechanisms involved in adhesiogenesis from a molecular and cellular point of view and for determining with more precision the role of fibrin, as well as the regulation of fibrinolysis and coagulation. By understanding the biochemical and morphological phenomena of normal peritoneal healing, it may be possible to obtain a modality for separating peritoneal surfaces and increasing fibrinolysis. The difference between adhesions that appear at different intra-abdominal or intrapelvic levels must be studied further. A better understanding of adhesions that predispose to complications and the knowledge of the places where these appear more frequently are also necessary. Further research is required for a better knowledge of the newly discovered agents, with special emphasis on the degree of adhesion prevention.

In conclusion, intra-abdominal adhesions continue to cause significant morbidity and despite our increased understanding of their pathology no effective preventative treatment has been found. This study has confirmed a reduction in fibrinolytic activity in patients with increased factors of peritoneal aggression known to cause adhesions, which is mediated through reduced expression of the tPA gene and increased expression of the PAI-1 gene at peritoneal tissue level.

Author contribution

For this published study the contribution of Sebastian Gradinaru, Costel Savlovski and Dragos Serban is equal.

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