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Influence of antibiotic therapy on the level of selected angiogenic factors in patients with benign gynecologic tumors - preliminary report

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An increased fibrin level enhances the activity of proangiogenic factors and may contribute to tumor formation. Formation of new blood vessels during angiogenesis leads to neoplasm development through interaction with factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and interleukins. The aim of this study was to investigate the influence of perioperative antibiotic therapy in women with benign gynecological tumors with regard to basic fibroblast growth factor level, fibrinogen concentration and fibrin viscosity. The influence of clindamycin plus metronidazole therapy (group I) and cephazolin therapy (group II) on fibrinogen concentration, level of bFGF and fibrin viscosity was studied in women diagnosed with nonmalignant myomas and cysts. In patients with benign gynecologic tumors, higher bFGF levels (51.40 ± 13.72 pg/ml), fibrinogen concentration (348.26 ± 64.74 mg/dl) and fibrin viscosity (2.63 ± 0.36 mPa) were observed, as compared with healthy women. There were strong indications that antiangiogenic activity occurred with both clindamycin plus metronidazole and cephazolin, although the response to these particular antibiotic therapies was different. The use of various drug therapies in groups I and II resulted in faster and delayed antiangiogenic effects, respectively. Further research is essential to provide more detailed information about the mechanisms of the induction of antiangiogenic activity by perioperative adjuvant antibiotic treatment.

1. Introduction

The interaction between basic fibroblast growth factor, fibrinogen and fibrin is one of the factors which may influence angiogenesis - a process related, among others, to tumor growth and metastasis (Collen et al. 1998; Mosseson 2005; Palatyńska-Ulatowska et al. 2008; Erdem 2003). In pathological conditions connected with tumor development, increased excretion of proangiogenic factors and uncontrolled endothelial cell proliferation is observed (Wojtukiewicz et al. 2001). In malignant tissue, density of the blood vessel network, elevated blood plasma viscosity and raised fibrinogen concentration accelerate disease progression and may be considered to be independent prognostic factors (von Tempelhoff et al. 2000, 2002).

A new approach to cancer treatment is antiangiogenic therapy (Collen et al. 1998; Markowska and Szala 2004) based on medication, which impedes angiogenesis in tumor tissue by metalloproteinases (MMP) inhibition (Krutovskihk et al. 2002). MMP inhibitors include some nonsteroidal antiinflammatory drugs such as I and II cyclooxygenase (COX) blockers (non-NSAID COX inhibitors - aspirin, piroxicam, meloxicam), furin inhibitors, hydroxamates such as batimastat (BB-94) and marimastat (BB2516) or macrocyclic lactones (bryostatin-1). However, antibiotics are considered to be medications which have a significant antimalignant effect. It has been reported that antibiotics such as tetracyclines, and particularly their semisynthetic derivatives (doxycycline, minocycline) inhibit MMP-1 and MMP-2 activity (Richardson et al. 2005). In addition, various antibiotics, are also administered as perioperative prophylaxis in gynecological oncology (Paszkowski 2007). The selection of proper adjuvant pharmacotherapy with combined antiangiogenic activity might play a significant role not only in prevention of infection but also as an antiangiogenic anticancer treatment.

It has been reported that bFGF induces vascular growth and angiogenesis (Palatyńska-Ulatowska et al. 2008). On the basis of reports indicating that copper levels in tissue may somehow modulate the intensity of the vascular response, Shing observed, that basic fibroblast growth factor appears to have an affinity for copper (Shing 1988). However, he also found that copper was not required for the binding of FGF to heparin and that FGF has separate copper-binding sites. On the other hand, a β-lactam metalloantibiotic - Cefzol (cephazolin) possesses various donor sites for interaction with transition metal ions, such as cobalt, copper, nickel or zinc, and can form various metal complexes (Chohan et al. 2004). Therefore, according to these facts and on the basis of the above investigations we have put forward the hypothesis that the probable mechanisms of competitive metal (copper) bindings with antibiotics depriving metalloproteases of angiogenic properties or decreasing an activity of uncomplexed

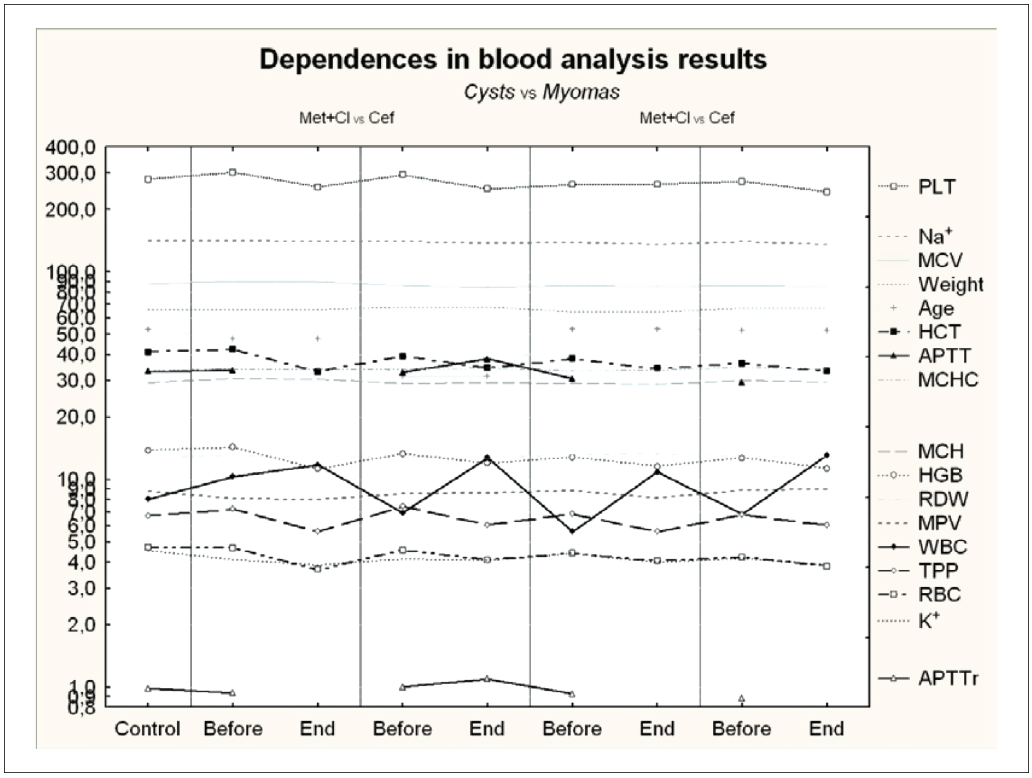

Fig. 1: Basic biochemical blood analysis before and at the end of the evaluation period (6th day of therapy)

to copper heparin is possible. Metal-complexed antibiotics were found to have better antibacterial activity; however their potential antiangiogenic properties, mechanisms of action and clinical implications are not yet known.

With the above hypothesis in mind, the aim of this study was to investigate the effect of selected perioperative antibiotic therapy, administered according to regulatory directives in the 1st Faculty of Obstetrics and Gynecology, on fibrinogen concentration, bFGF level and fibrin viscosity in women with endometrial cysts and myomas before and after the first, third and sixth day of treatment.

2. Investigations and results

Twenty women, aged between 24 and 74 years, with benign gynecological tumors admitted to the II Department of Gynecology, Medical University of Lodz, qualified for the study. All the patients gave their written consent for tissue samples to be taken for evaluation. This work was approved by an Institutional Ethics Board, No RNN/34/08/KE 29.01.2008. The women underwent gynecological surgery to excise benign tumors and received adjuvant antibiotic therapy. In all cases, a histopathological diagnosis confirmed myomas or endometrial cysts. The patients examined were divided into two groups of 10 women each: the first (I), given clindamycin (3 x 600 mg per day) with metronidazole (2 × 0.5 mg per day) intravenous therapy and the second (II), subjected to cephazolin (1 × 1.0 g per day) intravenous therapy. The above medications were administered as perioperative prophylaxis according to regulatory directives in the department. Each group (I and II) was divided equally and included patients diagnosed with myomas and cysts, respectively. Therefore, the four groups selected for the study were as follows: I C (cysts), I M (myomas), II C (cysts), II M (myomas). The third, control, group (III) consisted of 10 healthy women, who were under surveillance in the department. In all the groups investigated, basic biochemical blood tests such as: hematocrit (HCT), hemoglobin (HGB), white blood cell count (WBC), platelets (PLT), mean platelet volume (MPV), red blood cell (RBC) aggregation, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), sodium, potassium and total plasma proteins were performed before and at the end of the treatment. Fibrinogen concentration, basic fibroblast growth factor levels and fibrin viscosity were investigated. Blood samples were collected from each woman before surgery and drug administration, and after the first, third and sixth days of treatment, and subsequently refrigerated at $-70\,^{\circ}C$ for further examination. Pharmacotherapy was administered on the day of surgery, which was also the first day of treatment.

The results of the basic biochemical blood tests before and during the treatment of women with myomas and endometrial cysts, as well as healthy subjects, are presented in Fig. 1. So that the figure can be easily read and because of the amount of data included, standard deviation (±SD) values are not included. So that the results obtained are easily seen, values of each parameter in Y axis are given on a logarithmic scale. Fluctuations of white blood cell values can most probably be explained by healing processes that occurred in patients after surgery and tumor excision.

Factors affecting fibrinogen in all the groups investigated are shown in Fig. 2. Mean ± SD fibrinogen level in healthy women was 269.23 ± 29.01 mg/dl, whereas in patients, the values increased significantly to 348.26 ± 64.74 mg/dl. It can be seen that clindamycin plus metronidazole therapy was more effective on the first day of treatment in both the I C and I M groups. Nevertheless cephazolin therapy produced better long-term effect, did not cause as big an increase in fibrinogen level as did clindamycin plus metronidazole therapy, and in the case of treatment of cysts, it seemed to cause a fall of fibrinogen values in patients' blood after 72 h of treatment.

There was a statistically significant difference in basic fibroblast growth factor level between women with and without

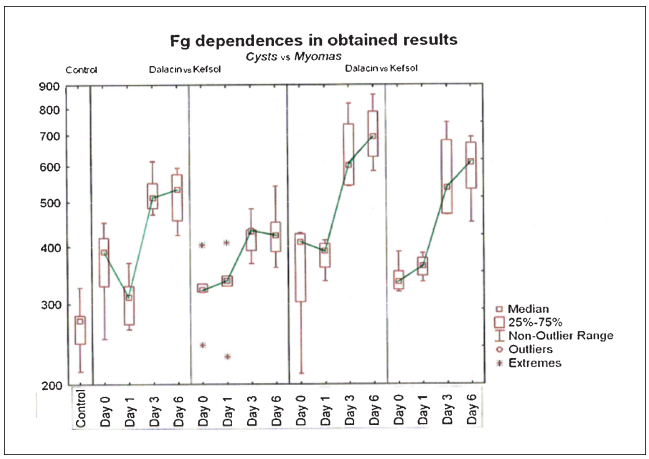

Fig. 2: Fibrinogen dependences in all investigated groups before, in the first, third and sixth day of therapy

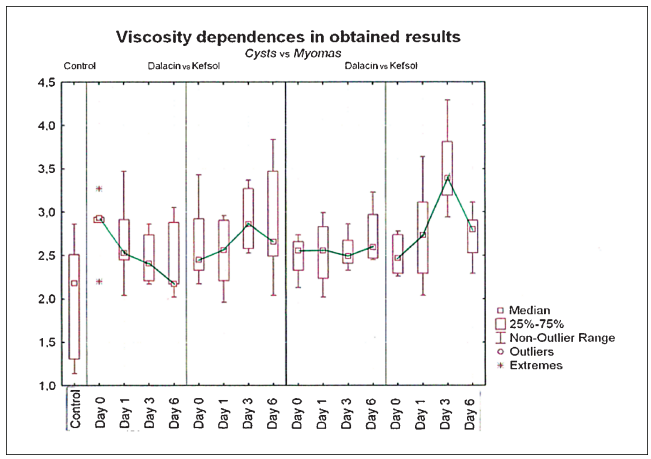

Fig. 4: Viscosity dependences in all investigated groups before, in the first, third and sixth day of therapy

gynecological tumors, being much higher in the affected women (51.40 ± 13.72 pg/ml) than in the control group (9.78 ± 0.88 pg/ml) (Fig. 3). Clindamycin plus metronidazole therapy was more effective in the first 72 h of therapy with a subsequent sudden increase of bFGF level starting from the third day of treatment. This indicates short-term effectiveness of administration of these particular medications, which was not dependent on the kind of tumor investigated. In cases of treatment of cysts, cephazolin therapy statistically significantly decreased the level of this protein beginning from the third day of treatment. The curve of bFGF values in cephazolin therapy of myomas has a similar shape to the fibrinogen value curve in clindamycin plus metronidazole therapy of the same kind of tumors.

Fibrin viscosity was 1.96 ± 0.63 mPa in healthy women, but there was a statistically significant increase to 2.63 ± 0.36 mPa in gynecological patients (Fig. 4). The high peak in the viscosity curve on the third day of cephazolin therapy was characteristic for all patients with either kind of tumor diagnosed. A decrease of viscosity over the whole period of treatment was observed only in group I C. Also values of fibrin viscosity in patients given metronidazole plus clindamycin therapy were lower compared with group II.

3. Discussion

Two angiogenic factors which influence the process of angiogenesis are fibrin and basic fibroblast growth factor. Fibrin not only plays an important role in hemostasis, but also takes an active part in the development of malignancy. The three-dimensional network of the fibrin permits tissue factor expression and binding with endogenous growth factors (bFGF, VEGF), which protect the fibrin from proteolytic degradation and inactivation (Sahni et al. 1998). Fibrin stimulates proliferation and migration of endothelial cells and increases angiogenesis. In the primary tumors, as well as in neighbouring lymph nodes, expression of tissue factor (TF), activation of blood coagulation and increased generation of fibrin occur. An increased level of bFGF and changes in the fibrin structure have been observed in many malignancies (Carr 1996; Laurens et al. 2006; Sahni et al. 1999; Zimering and Thakker-Varia 2002).

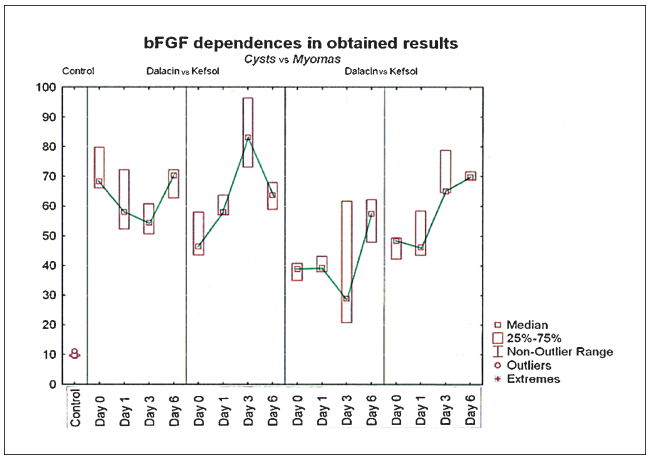

Fig. 3: Fibroblast growth factor level in all investigated groups before, in the first, third and sixth day of therapy

In the group of subjects with benign gynecological tumors, increased levels of basic FGF and fibrinogen and also an increase of fibrin viscosity compared with healthy women were observed. According to this study, increased bFGF and fibrinogen levels and higher fibrin viscosity in the treated patients could indicate intensified tumor neoangiogenesis. Tempelhoff et al. (2000, 2002) also stated that in gynecological neoplasms there are significant changes in fibrin viscosity, and these correlate with increased blood platelet aggregation and elevated fibrinogen level. In sick patients, those changes are called "Trousseau's syndrome" (coagulopathy induced malignancies) and they are strictly related to hypercoagulation and metastasis (von Tempelhoff et al. 2002). In pathological changes, increased fibrin viscosity correlates with an increase in blood viscosity accompanying the malignancy process and metastases. Long-term evaluation has produced evidence that hyperviscosity in gynecologic cancer patients is significantly associated with poor prognosis of the malignancy (von Tempelhoff et al. 2000). The results obtained also confirm the conjecture that high concentrations of bFGF as well as increased levels of fibrinogen and fibrin hyperviscosity can be diagnostic markers in the development of benign gynecologic neoplasia.

Some antibiotics, particularly tetracyclines, inhibit metalloproteinases activity. It has been shown that chelating bonds between antibiotics and the active center of the metalloproteinases act as MMP inhibition mechanisms (Kołomecki 2000). The studies conducted were intended to investigate the influence of combination metronidazole plus clindamycin and cephazolin therapy on the level of bFGF, fibrin viscosity and the fibrinogen concentration before treatment and during six days of drug administration in women with gynecological benign neoplasia. The antibiotics mentioned were chosen according to the general guidelines for perioperative prophylaxis used in the department. The results may confirm that metronidazole plus clindamycin medication (group I) administered in

perioperative gynecologic tumor therapy was more effective in reducing bFGF level and fibrin viscosity during the first three days of therapy compared with cephazolin, which induced an effect delayed for 72 h (group II). Although the probable mechanism of competitive metal binding and depleting metalloproteases of the angiogenic properties of cephazolin have not been proved, there were strong indications that clindamycin plus metronidazole therapy resulted in antiangiogenic activity as well as cephazolin treatment, but in a different way. According to our study, drug administration in groups I and II resulted in a faster or delayed response in bFGF and fibrinogen levels, respectively. Further research is needed to provide more detailed information about mechanisms inducing antiangiogenic activity and clinical aspects of the antibiotic treatment indicated. More advanced studies based on blood samples taken from patients with diagnosed malignant gynecologic tumors are planned.

4. Experimental

4.1. Fibrinogen

Citrate plasma (1:9) was used to determine the fibrinogen concentration. The study used an ACL Advance system with the PT-Fibrinogen Recombinant kit 0020005000 (Instrumentation Laboratory Company, Lexington, USA) according to the Clauss method (Clauss 1957).

4.2. Basic fibroblast growth factor

Basic fibroblast growth factor concentration was evaluated in serum with the ELISA method (Enzyme-Linked Immunosorbent Assay; Quantikine bFGF Elisa, R&G Systems, Abingdon, UK). A standard curve was calculated for the concentration range 10–640 pg/mL. Basic FGF concentration level was interpreted from the regression equation $y = 0{,}0021x + 0{,}0154$ ($R^2 = 0.9913$). Measurement of bFGF concentration was performed by an Elx800 platelet reader at a wavelength of 450 nm (Bio-Tek Instruments, Inc.)

4.3. Fibrin viscosity

Fibrin viscosity was evaluated according to the Marchi method (Marchi 2006). Three speciments were investigated: 250 µl of human citrate plasma (1:9), 250 µl of 50 mmol/L calcium chloride (PPH Polish Chemical Reagents, Poland) and 125 µl of 12.8 U/mL thrombin (Biomed, Poland). Viscosity measurements were performed with a DV-III cone–plate type digital rheometer, version 3,0 (Brookfield), combined with a PGW E-1 bath thermostat (Medingen), in which the sample material was inserted in the slot between the cone and the plate surface. The flow curve for the configuration examined is based on a non-Newtonian fluid mechanism.

4.4. Statistical analysis

All the results obtained during evaluation were verified with different statistical methods such as the t-Test ($p < 0{,}01$ except $p = 0{,}097$ for bFGF in myomas with clindamycin plus metronidazole treatment, Day 3) and the Shapiro-Wilks Test ($p > 0{,}05$). All tests were performed with StatSoft Statistica 8,0 PL software.

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