

The influence of steroid sex hormones on collagen composition in post-operative wounds after long-term treatment with anticoagulants

Wpływ płciowych hormonów sterydowych na tworzenie kolagenu w ranach pooperacyjnych po długotrwałej terapii antykoagulantami

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Abstract

Background: Wound healing is a complicated process, mainly dependent on neovascularisation of tissue. New synthesis of collagen filaments is also very important in proper healing of wounds. Widespread use of anticoagulant medication in operative specialties influences the way post operative wounds heal and calls for continuous efforts to find new ways of modifying the findings about this process.

Aim: The main aim of the study was to estimate the influence of early treatment of steroid sex hormones on the healing process of wounds in rats after a long-term anticoagulant therapy. **Material and methods:** The study was conducted on a model of an animal at the Department of High Risk Pregnancy, Medical University of Lodz between 2004-2006. The Wistar rats were used for the study. The influence of heparin and steroid hormones on collagen synthesis in post operative wounds in female rats after bilateral ovariectomy and the intensity of the composition of collagen in prolene mesh in vivo were estimated.

Results: The healing process of post operative wounds in the control group was connected with an increased concentration of collagen in the scar region. Progesterone has no influence on heparin action with respect to total collagen concentration, estrogen administration significantly increased the total concentration of collagen in wounds of rats treated with low-molecular-weight heparin.

Conclusions:

1. Steroid sex hormones in rats after bilateral ovariectomy treated with low-molecular-weight heparin have major influence on post operative wound healing process.
2. Estradiol supplementation in post operative period improves the effect of heparin on the quality of the wound.

Key words: **wound / wound healing / collagen / heparin / estrogens /**

Streszczenie

Leczenie ran jest złożonym procesem zależnym w dużym stopniu od neowaskularyzacji tkanki. Nowa synteza filamentów kolagenu jest również bardzo istotna dla prawidłowego gojenia się ran. Rozpowszechnione użycie antykoagulantów specjalnościach zabiegowych ma wpływ na sposób postępowania z ranami pooperacyjnymi i może spowodować jego zmianę dzięki badaniom w tym kierunku.

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Cel pracy: Celem badań była ocena wpływu zastosowania leczenia za pomocą steroidowych hormonów płciowych na gojenie się ran pooperacyjnych u szczurów poddanych długotrwałej terapii antykoagulantami.

Materiał i metoda: Badania zostały przeprowadzone w Klinice Patologii Ciąży Uniwersytetu Medycznego w Łodzi w latach 2004-2006. Szczury gatunku Wistar zostały użyte do przeprowadzenia eksperymentu. Wpływ heparyny oraz steroidowych hormonów płciowych na syntezę kolagenu na gojenie się ran pooperacyjnych u szczurów płci żeńskiej po obustronnym usunięciu jajników oraz nasilenie syntezy kolagenu na siatkach prolenowych został oceniony.

Wyniki: Gojenie się ran pooperacyjnych w grupie kontrolnej była związana ze wzrostem stężenia kolagenu w okolicy blizny. Progesteron nie miał wpływu na działanie heparyny w odniesieniu do całkowitego stężenia kolagenu, podawanie estrogenów istotnie zwiększało stężenie kolagenu w ranach zwierząt doświadczalnych, u których podawano heparynę niskocząsteczkową.

Wnioski:

1. Steroidowe hormony płciowe u szczurów po obustronnym usunięciu jajników, u których zastosowano niskocząsteczkową heparynę miały znaczący wpływ na proces gojenia się rany.

2. Suplementacja estrogenowa w okresie pooperacyjnym poprawia efekt działania heparyny w odniesieniu do jakości blizny.

Słowa kluczowe: rana / gojenie się / kolagen / heparyna / estrogeny /

Introduction

The process of wound healing may be divided into three phases: inflammatory, proliferative and remodeling. Normal path of this process depends mainly on revascularization of the destroyed tissues [1, 2]. There exist complicated interactions between blood cells and blood vessels elements (platelets, macrophages, neutrophils, endothelial cells, smooth muscle cells), epithelial elements (keratinocytes, melanocytes, Langerhans cells), skin surface elements (external hair osłonka cells, papillomatis cells of hair), proper skin (fibroblast, myofibroblast, neurons) and adipose tissue (adipocytes) [3, 4, 5, 6, 7].

Also, formation of new synthesized collagen filaments plays a very important role in proper healing of wounds as the main part of extra cellular matrix [1, 3, 8, 9, 10]. Anticoagulative treatment, frequently used in operative specialties in anticoagulant prophylaxis, may influence those processes, especially in case of people who were treated with anticoagulants for a prolonged period of time [1, 3, 8, 11]. The estimation of the role of anticoagulative drugs in the process of postoperative wound healing and the possibility of modification of this process by different medications seems to be essential [4, 8, 12, 13, 14, 15].

Aim

The main aim of the study was to estimate the influence of early treatment of steroid sex hormones on the healing process of wounds in rats after a long-term anticoagulant therapy.

Material and methods

The study was conducted on an animal model in the Department of High Risk Pregnancy, Medical University of Lodz, between 2004 and 2006. The Wistar rats were used. Formal written consent of the Bioethics Committee for Experiments on Animals of Medical University of Lodz was obtained.

The influence of low-molecular-weight heparin and steroid hormones on collagen synthesis in post operative wounds in female rats after bilateral ovariectomy and the intensity of the composition of collagen in prolene mesh in *in vivo* conditions of long-term treatment with low-molecular-weight heparin and steroid sex hormones were estimated.

54 female Wistar rats after bilateral ovariectomy were used in the experiment. The ovariectomy was done under general anesthesia. Before the operation the hair had been shaved off. The postoperative wound was closed and the prolene mesh was inserted subcutaneously. The suture was done using Ethibond 2,0 sutures by 4 to 5 single stitches.

The blind trial probe was done after the operation and the rats were divided into four groups.

- 1st group- as controls (n=12) was medicated by heparin – Clexane in the concentration of 1mg/kg of weight and 0.1ml lipophyll solvent for progesterone and estradiol – one dose a day during two weeks.
- 2nd treated group (n=14) was medicated by heparine – Clexane in the concentration of 1mg/kg of weight and 2.5mg of progesterone in 0.1ml of lipophyll solvent– one dose a day during two weeks.
- 3rd group (n=13) was medicated by heparine – Clexane in the concentration of 1mg/kg of weight and 0.25mg of estradiol in 0.1ml of lipophyll solvent– one dose a day during two weeks.
- 4th treated group (n=14) was medicated by heparine – Clexane in the concentration of 1mg/kg of weight and 2.5mg of progesterone and 0.25mg of estradiol in 0,1ml of lipophyll solvent– one dose a day during two weeks.

In 14, 21, 28 and 42 days after the operation the rats were terminated by intraperitoneal Thiopental injection in a fatal dose. The skin from the place near the postoperative scar together with the prolene mesh was removed from hypodermic tissue and taken for biochemical analysis.

In the tissue, the content of water, total concentration of collagen in the tissue and prolene net and the percentage of soluble fraction of collagen were measured.

The concentration of water in the tissue was estimated as the difference of the wet and dry tissue mass. All samples were weighed, dried to the constant substance in the temperature of +90°C and weighed again.

Table I. Mean value of total collagen concentration in wounds.

Observed groups	Time after operation in weeks	Mean concentration	SD	p vs progesterone	p vs estradiol	p vs progesterone + estradiol	p vs controls
progesterone	2	388.0	33.1		<0.05	<0.05	NS
	3	434.3	20.1				
	4	602.9	23.3				
	6	503.8	27.9				
estradiol	2	625.8	87.1	<0.05		NS	<0.05
	3	651.4	51.2				
	4	519.4	37.1				
	6	519.0	32.6				
progesterone + estradiol	2	558.4	41.8	<0.05	NS		<0.05
	3	668.3	21.1				
	4	540.5	48.6				
	6	472.6	22.6				
controls	2	360.4	35.9	NS	<0.05	<0.05	
	3	456.2	23.8				
	4	551.1	23.8				
	6	506.5	68.3				

To estimate the concentration of collagen taken from hypodermic tissue, prolene net was hydrolyzed in 6N solution of HCL acid (3mg/10mg of dry tissue mass). The solution was desiccated again. Newly created dry substance was diluted by distilled water and alkalinized by 5N solution of NaOH forming the solution of 10ml volume and pH= 7.0.

The estimation of hydroxyproline concentration in dry tissue mass was done using the Woessner method with Dąbrowski modification. Hydroxyproline was oxidized to pyrole using chloramine T and then reacted with p-dimetyloaminobenzaldehyde. The optical density was measured using the spectrophotometer Unicam SP 800B at 560 nm. The total collagen concentration was calculated using the 7.46 ratio [16, 17].

The concentration of soluble collagen in the wound was estimated from fresh tissue using the reaction of 0,45 M solution NaCl and incubation at +4°C for 48 hours

To prevent bacterial colonization, Crystalline penicilin (10000iu/sample) was added to all samples. After incubation the solution was centrifuged and supernatant was dried. The rest of the procedure was done using the Dąbrowski method [16].

The obtained results were statistically analyzed using Statistica 6.0 PL program. P<0.05 was deemed statistically significant.

Results

The healing process of post operative wounds in the control group was connected with an increased concentration of collagen in the scar region.

Progesterone has no influence on the action of heparin with respect to total collagen concentration in healing wounds but estrogen administration significantly increased the total concentration of collagen in wound of rats treated with low-molecular-weight heparin.

After 14 days since the initiation of the steroid sex hormones therapy and low-molecular-weight heparin treatment, the differences in total collagen concentration in healing wounds compared to the control group disappeared. (Table I).

The estrogen therapy increased the soluble fraction of collagen contained in healing wounds treated by low-molecular-weight heparin. The increase of collagen soluble fraction was estimated on the 14-th day after the start of the therapy. Similar result was found in concentration soluble collagen fraction in the group treated with estrogen only. Medication by estrogen and progesterone together shows different result – the decrease of soluble fraction in the wound. Those results show a tendency to normalize in two weeks after the sex hormones treatment. (Table II).

Administration of either estrogens or progesterone or both of them, significantly increases the concentration of total collagen in prolene mesh if compared to the control group. The obtained results changed over two weeks after the treatment. (Table III). To summarize the study, we found a significant increase in the concentration of soluble collagen in all treated groups in both – wound tissue and prolene mesh.

Discussion

The process of wound healing is a complicated and still relatively little-known process. It leads to the healing and rebuilding of destroyed tissue. The most important elements, which are necessary for proper healing are: revascularization and new collagen filaments synthesis [1, 3, 4, 5, 6].

Things such proper velocity of the process, long lasting and quality of new tissue might ensure normal wound healing indirectly. From the practical point of view, the possibility of continuing this process, to ensure the new tissue obtains necessary properties, is of vital importance [3, 8, 12, 13, 14, 15].

Table II. Mean value of soluble collagen fraction concentration in wounds.

Observed groups	Time after operation in weeks	Mean concentration	SD	p vs progesterone	p vs estradiol	p vs progesterone + estradiol	p vs controls
progesterone	2	25.3	5.0	NS	NS	<0.05	<0.05
	3	71.1	14.3				
	4	37.4	7.4				
	6	25.3	1.9				
estradiol	2	23.0	4.2	NS	NS	<0.05	<0.05
	3	43.3	2.8				
	4	17.9	3.0				
	6	28.1	4.3				
progesterone + estradiol	2	30.1	3.0	<0.05	<0.05	<0.05	<0.05
	3	38.3	2.7				
	4	41.9	7.3				
	6	24.8	6.1				
controls	2	39.1	2.9	<0.05	<0.05	<0.05	<0.05
	3	51.2	2.9				
	4	70.3	5.1				
	6	22.8	5.9				

Table III. Mean value of total collagen concentration in prolene mesh.

Observed groups	Time after operation in weeks	Mean concentration	SD	p vs progesterone	p vs estradiol	p vs progesterone + estradiol	p vs controls
progesterone	2	115.7	4.2	NS	NS	NS	<0.05
	3	219.9	9.2				
	4	196.2	4.3				
	6	53.5	10.8				
estradiol	2	83.6	15.9	NS	NS	NS	<0.05
	3	159.4	17.1				
	4	145.5	9.9				
	6	157.3	23.0				
progesterone + estradiol	2	99.4	6.6	NS	NS	NS	<0.05
	3	227.3	7.4				
	4	161.2	12.9				
	6	96.7	7.7				
controls	2	45.0	10.5	<0.05	<0.05	<0.05	<0.05
	3	62.4	9.7				
	4	88.0	5.0				
	6	134.4	7.7				

This could be done by helping the process of revascularization in new-built tissue and increasing the content of collagen.

Latest experiments show positive influence of low-molecular-weight heparin treatment on the process of wound healing [1, 3, 18].

Heparin enriches blood distribution, increases fibroblasts penetration to the place of healing and also decreases the possibility of adhesions and scars forming and covers endothelial cells.

The results of our study are similar to those of other authors. Medication with low-molecular-weight heparin treatment as prophylaxis and as treatment of patients from surgical profile departments offers yet another reason to use it. The second important reason that influences the quality of newly constructed tissue is the content of collagen. The collagen concentration could be one of possible ways of assessing wound healing and the quality of tissue [1, 2, 3, 5, 6].

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The main reason of the study was to estimate the influence of steroid sex hormones on the process of post operative wound healing. The positive effect of estrogen treatment on wound healing was shown in some studies [9, 14, 19]. In our study the results were similar to those obtained by other authors [5, 6, 10, 11]. The estrogen treatment increased the concentration of collagen, mucopolisaccharides and hyaluronic acid. By increasing cells adhesion, estrogens helped the increase of angiogenesis and epithelium forming. They decrease local inflammation process by regulating the concentration of pro and anti inflammatory cytokines. Estrogens together with progesterone stimulate the proliferation of keratinocytes and fibroblasts migration. They inhibit apoptosis of epithelial cells and metalloproteinases activity, which are responsible for collagenolysis [4, 7, 9, 10, 11, 12, 13, 15, 18].

The use of estrogens in the process of wound healing is now more common, especially in elderly patients and postmenopausal women.

Conclusions

1. The steroid sex hormones in rats after bilateral ovariectomy treated with low molecular weight heparin has a major influence on the post operative wound healing process.
2. Estradiol supplementation in post operative period improves the effect of heparin with respect to the quality of wound.

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