CILOSTAZOL FOR TREATMENT OF PATIENTS WITH DIABETIC LEG ULCERS

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Abstract.
Aim. The research was conducted for the purpose of improving treatment outcomes, reducing time on treatment, declining in disability, improving life quality of patient with diabetic ulcers.
Methods. In this research two groups of patients with diabetic leg ulcers were used: I group (51 patients) – treated with a standard procedure (control group) and second group (52 patients) – whether treatment was supplemented cytostazol (100 mg twice a day). For the evaluation the life quality of patients a questionnaire «SF – 36 Health Status Survey» was used during the treatment, the rate of the wound healing was calculated by the formula and 120 histological preparations were analyzed (20 patients of each group).
Results. After analysing the questionnaire results, significant differences were observed between groups of patients, manifested by de-emphasized role of physical problems, improved viability and psycho-emotional state with less manifestation of the differences in the control group. The reliable increase in the rate of wound healing was found in patients who took cilostazol. It was observed appearance of carbohydrate determinants in the endothelium of newly formed blood vessels after two weeks of treatment while in the most of the patients' drugs of the control group there were no such obvious manifestations of regeneration.
Discussion. Regarding the results of the research, cilostazol is worth to be examined as perspective means for treatment of patients with diabetic ulcers. In turn, the results of lectinohistochemicals researches can be one of the objective tests of violation of balance of restorative-reparatory and destructively-necrotizing processes for patients with leg ulcers.
Keywords: trophic, chronic, leg, ulcer, wound, healing, regeneration, diabetic, cilostazol, lectines.

1. Introduction

Despite the progress of medicine and the utilisation of the modern medicines products, treatment of diabetic ulcers is not often accompanied by satisfactory results. The number of patients with diabetes is rapidly increasing around the world. A large percentage of this population can be expected to develop diabetic ulcers. The economic, social, and public health burden of these ulcers is immense. This problem is tackled every day by many healthcare workers all over the world [1]. Patients with diabetic leg ulcer have a greater than twofold increase in mortality compared with nonulcerated diabetic patients [2].

The outcome of management of diabetic leg ulcers remains a challenge, and there remains continuing uncertainty concerning optimal approaches to management [3]. Cilostazol is one of the preparations that finds out vasodilate and antithrombotic properties. The efficiency of application of cilostazol at the chronic ischemia of legs, intermittent claudication is confirmed by evidential medicine [4]. Own experience of using this preparation testifies about perspective of its application for patients with the diabetic leg ulcers [5, 6]. Expediency of its application for patients with the diabetic leg ulcers needs profound confirmation, although there are reports that specify on credible potential of the use of preparation at treatment of this category of patients [7, 8].

The research was conducted for the purpose of improving treatment outcomes, reducing time on treatment, declining in disability, improving life quality of patients with diabetic ulcers.

3. Materials and Methods

The research was executed at the Department of surgery No. 2 of Danylo Halytsky Lviv National Medical University, based on the Department of Surgery No. 1 and the Department of Vascular Surgery of the Lviv Regional Hospital during 2016–2017. The analysis of the treatment effectiveness in patients with diabetic lower extremity ulcers was conducted. Wounds were concidered as a chronic (nonhealing) if the positive dynamics was not observed or its changes were minimally expressed after four weeks of treatment [9]. The inclusion criteria for the study were chronic diabetic leg ulcers not less than 25 cm^2, preliminary treatment and lack of its positive dynamics. In this research two groups of patients were used: I group (51 patients) – treated with a standard procedure (control group) and second group (52 patients) – whether treatment was supplemented cytostazol (100 mg twice a day). The rate of the wound healing was calculated by the formula [10]. The first measurement was conducted after necrectomy, measurement was repeated after 14 days of the first one. The average size of ulcers was 114 cm^2 (26–688 cm^2). For the evaluation the life quality of patients and its changes a questionnaire «SF – 36 Health Status Survey» was used during the treatment [11].

Exclusionary criteria were cancer, severe concomitant diseases, and lesions of osteoarticular apparatus.

Statistical analysis of the presented data was performed using Student`s T-test. Differences between mean values were concidered significant if the probability was at least 95 % ($p < 0.05$).

For this research 120 histological preparations were analyzed (20 patients of each group). All patients gave their consent to participate in this research. Tissue samples for histological preparations were taken from the most visually healthy areas of the wound before treatment, after two and four weeks of treatment. Tissues dissected with blade and entire piece was taken on the border of the skin and the wound surface (fragment size: 1×0,5×0,5 cm). Fixation of tissue samples was performed in 4 % neutral formalin. Histological preparations were stained with hematoxylin and eosin. In order to clarify the fundamental mechanisms of regenerative processes in the patient of control group and ones who used cilostazol additionally the lectins histochemical investigation was performed using lectins of different carbohydrate specificity labeled peroxidase. Lectins were produced in the laboratory «Lectintest» (Danylo Halytsky Lviv National Medical University). Lectins panel includes: Con A – specific to DMan, HPA – to NAcDGal, PNA – to βDGal, LABA – to αLFuc, SBA – to NAcDGal, WGA – to NAcDGlc. Visualization of lectin receptors was carried out in the system H2O2-3'3'-diaminobenzidine tetrahydro chloride[12,13]. Microscopic examination of histological preparations was performed using a microscope Olympus BX-41, and Carl ZEISS Jena Ng with digital camera Canon IXUS 700.

4. Results

The quality of life questionnaire «SF - 36 Health Status Survey» was used in order to evaluate the effectiveness of cilostazol before and after four weeks of the treatment. After analysing the ques-

tionnaire results, significant differences were observed between groups of patients, manifested by de-emphasized role of physical problems, improved viability and psycho-emotional state with less manifestation of the differences in the control group (**Table 1**).

Table 1

Evaluation of the quality of life of patients with diabetic ulcers using questionnaire «SF – 36 Health Status Survey» (in points)

Quality of life: SF-36	Control group		+ cilostazol	
	Before treatment	After 4 weeks of treatment	Before treatment	After 4 weeks of treatment
Physical activity	25.2±3.4	54.0±5.2*	25.4±.9	60.8±5.4*
Role of physical problems	16.6±3.0	33.9±3.6*	16.4±2.5	45.5±4.2* $^{\Delta}$
Pain	42.4±3.9	61.4±5.8*	43.1±4.0	72.0-±5.9*
General health	31.0±3.1	42.4±3.8*	31.2±3.0	53.3±4.4*
Viability	18.1±2.0	31.8±3.2*	18.0±2.2	42.9±4.1* $^{\Delta}$
Social activity	12.0±1.8	37.1±3.6*	12.1±1.7	43.9±4.2*
Role of emotional problems	11.1±2.2	40.2±4.1*	11.2±2.1	52.8±4.9* $^{\Delta}$
Mental health	21.4±2.0	43.0±3.9*	21.6±2.9	55.0±4.8* $^{\Delta}$

*Note: * – reliability in relation to the initial level (p<0.05); $^{\Delta}$ – reliability in relation to the control group (p<0.05)*

Based on the calculation relative rate of wound healing in groups was established: a control group – (0.253±0.020) %/day; +cilostazol – (0.331±0.024) % /day (p<0.05).

Histologically it was found that thickening of epidermis, epithelial vacuolization of the germ layer of epidermis were peculiar to diabetic ulcers before treatment. Fibrin region in the papillary dermis, stasis in blood vessels, swelling around blood vessels and hemorrhage were observed. Disturbances of microcirculatory was noted and as a result – degenerative changes of fibrous structures around the vessels. Desquamation of the surface layer of epidermis was reported.

Lectin-histochemical research have shown that before the treatment of patients with diabetic ulcers, a positive reaction with Con A in thick bundles of collagen fibers of reticular dermis against the background of weak binding of thin bundles of collagen fibers can be stated. There is also high expression in mannose glycans in local groups of white blood cells and the basal layer of the epidermis, epitheliocytes of germinal layer of the epidermis and the minor one in the nuclei of the surface layers.

Mosaicity of the receptors' binding of mannose specific lectin Con A with bundles of collagen fibers or their loss by the last may indicate their degradation in the areas of inflammation. High expression of mannose glycans in the cells of the Leukocyte infiltrates indicates that they are involved in the synthesis of pro-inflammatory interleukins. After 14 days of treatment, weak binding of Con A with collagen fibers of the dermis was observed. Meanwhile, emergence of mannose glycans in endotheliocytes of newly formed vessels of microcirculation circle and their slight expression in elastic fibers is noted. After 28 days of treatment a moderate expression of mannose glycans was observed in the bundles of collagen fibers, and high expression in endothelium of vessels of microcirculation circle and cellular elements of connective tissue - fibroblasts was also observed. Along with the above mentioned process, high expression of mannose glycans in germinal layer of epidermis was stated which may indicate that mannose glycans are involved in proliferative and adhesion processes. During the promotion of epithelial cells to the surface of the epidermis, in the cytoplasm of the last the number of receptors Con A is decreased. In the papillary layer of dermis a high expression of mannose glycans in the cells of fibroblastic row is observed which indicates the increase of synthetic processes in them involving mannose glycans.

NAcDGal-receptors of the specific lectin HPA before treatment were observed in the areas of necrosis of reticular dermis and cellular detritus. Weak expression of lectin receptors HPA is observed in degraded fibrous structures. After 14 days the weak binding of lectin with the fragments of collagen fibers of the reticular dermis layer was diagnosed. After 28 days of treatment, a local strengthening of HPA lectin receptors with collagen fibers was observed which indicates a partial recovery of bundles of collagen fibers since it is known that carbohydrate determinants are involved in merger of collagen fibrils into collagen fibers. Before the treatment, mosaicity of αL-Fuc specific lectin LABA expression in degraded fibrous structures and cell detritus was also mentioned. On the 14th day of treatment a weak binding of lectin with elastic fibers in the area of the partial restoration of the dermis was observed. Probably on the background of partial regeneration of the dermis there are areas of degraded collagen fiber bundles with the advent of low-positive LABA cells of fibroblastic row. On the 28th day the emergence of carbohydrate determinants in organized bundles of collagen fibers and germinal layer of the epidermis was observed. A similar binding specificity on the studied stages was noted with the lectins SBA, WGA and PNA.

6. Discussion

It was observed substantial positive changes in the state of patients who took cilostazol that was represented by the increase of the level of the quality of life. The reliable increase in the rate of wound healing was found in patients with diabetic ulcers who took cilostazol.

The lectin-histochemical research indicates that carbohydrate determinants as αD-man, βDGal and to a lesser extent, L-Fuc, NAcDGal, NAcDGlc are involved in the regeneration process of both the epidermis and dermis. A significant difference was detected on the 14th day of treatment. Evidence of this is the appearance of carbohydrate determinants in the endothelium of newly formed blood vessels after two weeks of treatment while in the most of the patients' drugs of the control group there were no such obvious manifestations of regeneration.

Regarding the results of the research, cilostazol is worth to be examined as perspective means for treatment of patients with diabetic ulcers. In turn, the results of lectinohistochemicals researches can be one of the objective tests of violation of balance of restorative-reparatory and destructively-necrotizing processes for patients with the ulcers of feet that respond to treatment in difficult way and can be applied for prognostication of efficiency of the treatment.

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