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## THE STUDY OF MICROCIRCULATION IN THE SUBCUTANEOUS TISSUE BY THE METHOD OF TISSUE CLEARANCE $Na^{131}I$ IN DIABETIC PATIENTS WITH MEDICATION ACTING ON THE CONDITION OF BLOOD HAEMOREOLOGICAL PROPERTIES

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The study is focused on the possibility of early diagnosis of diabetic microangiopathy. Authors used examination of microcirculation by the method of tissue clearance of  $Na^{131}I$ . Some drugs can influence the status of vessels and haemoreological attributes of the blood. These drugs include glycosaminoglycane sulodexide, indobuphen, serotonin receptors antagonists, naphtidrophuryl, pentoxyphyllin, alprostadiil. The aim of the study was to prove their influence on blood perfusion in capillary course.

**Key words:** *Diabetes mellitus, Microangiopathy, Microcirculation, Tissue clearance of  $Na^{131}I$ .*

### Introduction

The patients with diabetes mellitus gradually suffer from functional and morphological changes of their vascular system in the form of microvascular and macrovascular affection [1,2]. The observation shows that as a consequence of these morphological changes, there are changes in microcirculation [3,4]. There are also changes in haemoreological characteristic features of the blood. Some drugs have the capacity to influence this state in the sphere of microcirculation.

The fibrinolytical system is influenced by sulodexide, that is natural glucosaminoglycane. It influences the endothelial dysfunction acting on blood elements and haemoreology. It is a combined medication containing in 80% a material of heparin features of low molecular weight and in 20% dermatan sulphat that acts on the level of vascular endothelium. In the sphere of trombogenesis, as an antiplatelet agent acetylsalicyl acid with irreversible inhibition of COX-1 in platelets is mostly applied.

Some of its features are of no advantage, therefore, an effective substance was searched for that would have low side effects. One of such substances is indobufenum. Indobufenum selectively inhibits tromboxan ( $TXA_2$ ) in the platelets by a competitive inhibition of cyclooxygenase. Its acting is reversible, the platelet functions are modified within 12–24 hours after withdrawal of the medication. Naphtidrophuryl hydrogenooxalate acts as vasodilatant, haemoreologic and antagonist of serotonin receptors (it blocks the serotonin receptors –  $5HT_2$ , that are found in the cells of the smooth muscles of the arteries, in the membranes of thrombocytes and erythrocytes). The effect of the substance results from the characteristic features of the active substance. In narrowed arteries, it inhibits the local vasospasm, but does not act vasodilatory on normal arteries, does not create the steal phenomenon. As a consequence of this, it does not influence the blood pressure, it increases the supply of oxygen to the tissues, and this allows a better

usage of glucose. It lowers the creation of lactate, the creation of ATP. It decreases the vascular permeability, decreasing this way the outer pressure on the arteries. The haemoreological effect consists in the inhibition of aggregation of thrombocytes. It decreases their rigidity, impedes the creation of thrombs. The endothelial dysfunction is influenced also by an inhibition of the proliferation of smooth muscle cells of the arteries, decreases the progression of the creation of atherosclerotic plates. Within the acting on vasa nervorum, it regenerates the nerves, makes better the muscle trophy. The result is a better blood supply to the tissues, better objective symptoms and lesser subjective difficulties.

Pentoxifyllin belongs to the group of haemoreologics. An improvement of the reological characteristic features of the blood is given by a better deformability of the erythrocytes and leucocytes, the result of this is a decrease in the blood viscosity. At the same time, and antiaggregatory effect is present, as well as an influence on the fibrinolysis and a vasodilatory effect. The result of this is a better microcirculation and on the whole, a better blood supply in the tissues, and better oxygen and nourishment supply of the affected areas.

Alprostadil is a synthetic version of prostaglandin E<sub>1</sub>. It has a vasodilatory effect on the arterial walls (relaxation of the smooth muscles of the arteries), haemoreological (higher flexibility of erythrocytes), anti-thrombotic (inhibition of the aggregation of thrombocytes, more fibrinolytical activity of the plasma, inhibition of the activation of the leukocytes, less proteasis, cytosine, toxic radicals, less cholesterol accumulation in the arterial walls), which positively influences the microcirculation [1,5].

The study is focused on the possibility of early diagnosis of diabetic microangiopathy using examination of microcirculation by the method of tissue clearance of Na<sup>131</sup>I. Some drugs can influence the status of vessels and

haemoreological attributes of the blood. These drugs include glycosaminoglycane sulodexide, indobuphen, serotonin receptors antagonists, naphthidrophuryl, pentoxifyllin, alprostadil. The aim of the study was to prove their influence on blood perfusion in capillary course.

### Material

In the group of 15 patients of 2<sup>nd</sup> type diabetes (average age 62,2), sulodexid was administered (Vessel DUE F) according to a recommended diagram (the first 14 days parenteral, then per-oral 2 x 1 tbl daily) during 5 months respecting the indications and counter-indications. In the group of 19 patients with 2<sup>nd</sup> type diabetes (average age 53,9), Indobufen (Ibustrin) was administered during 5 months, 400 mg per day. In the group of 40 patients (average age 65,1), naphthidrophuryl (Enelbin retard) was administered in the amount of 400 mg per day during 5 months. In the group of 35 patients with 2<sup>nd</sup> type diabetes (average age 63), pentoxifyllin (Trental) was offered in 800 mg per day. In the group of 18 patients with 2<sup>nd</sup> type diabetes (average age 66,3), alprostadil (Alprostan) was given parenteral according to the recommended diagram during 14 days, taking into account the counter-indications. The medication was given to the patients with serious changes in the peripheral vascular system. Due to counter-indications or other reasons, it was not recommended to solve the state of health of these patients by surgical ways.

### Methods of examination

The examined patients with diabetes had no signs of cardiac decompensation, no hypertension, no illnesses of thyroid, they did not have varices in the lower extremities, nor signs of venous insufficiency. Diabetes mellitus was in a compensated state. In the laboratory tests, the values of fibrinogene, Quick test, microalbuminuria and lipids were tested. The control group were 36 clinically healthy people between 20 and 60 years (average age 36,9). The examination was performed lying, in a stable room temperature and

smoking was prohibited.

All examined patients with diabetes, before the beginning of the treatment and after 5 months of treatment with the particular medications (in case of Alprostan after 14 days of treatment), angiological examination of the arteries of the lower extremities was performed by palpation, reopletysmographically, sonographically measuring the systolic peripheral pressure above the arteries arteria dorsalis pedis (ADP), arteria tibialis posterios (ATP), arteria poplitea (AP), measuring the grade of pressure. The reographical examination allows to differ the functional changes from the organic ones. The exact location of the most affected part of the arteries is done by means of the ultrasound method [5,6,7]. We can find out about the changes in microcirculation by capilaroscopy, by flood fuxmetry, transcutaneous measuring of the tension  $O_2$  thermometrically. We examined the microcirculation of the subcutaneous tissues of the shin and calf muscles by the method of tissue clearance  $Na^{131}I$  [8,9]. The low molecule nuclear medication  $Na^{131}I$  is absorbed from the interstitial of the examined tissue (subcutaneous, from muscle) into the blood by the microcirculatory flood [8,9]. The quickness of absorption may be estimated from the rest of radioactivity measured by a scintillation detector above the particular location as a, so called, half absorption ( $T_{1/2}$ ). The higher the value, the slower the microcirculation.

A low dose  $Na^{131}I$  is used (4

$\mu Ci Na^{131}I$ ) in 0,1 ml isotonic solution NaCl. This is administered subcutaneously in the examination of the subcutaneous tissues, intramuscular in the examination of the muscle tissues. The radioactivity above the location of the application is measured by a scintillation detector which is connected to a spectral analysis device and a writing device. The number of impulses is recorded from the first minute after application during 14 minutes. The slope of the line indicates the quantitative ability of local circulation to absorb and carry away the nuclear medication. The rate of decrease in radioactivity is a measure of capillary blood flow.

### Results

In the control group of healthy people, the half absorption of  $Na^{131}I$  was between 9 to 18 minutes, with the average 14,7 minutes in the examination of subcutaneous tissues and the average of 11,9 minutes in the examination of muscle tissues. The most common finding in patients with diabetes mellitus was a slow

Table 1

Number of examined individuals treated with particular medications

	Vessel Due F	Ibustrin	Enelbin	Trental	Alprostan
Number of examined	15	19	40	35	18
Improved microcirculation	13	17	33	28	14
Not improved microcirculation	2	2	7	7	4

Table 2

Average values  $T_{1/2}$  in minutes subcutaneously and in the calf muscle, before treatment and after treatment, during the administration of particular medications

	Vessel Due F	Ibustrin	Enelbin	Trental	Alprostan
Subcutaneous $T_{1/2}$ before treatment	43,4	40,5	34,1	35,5	49,6
Subcutaneous $T_{1/2}$ after treatment	35,1	29,1	20,6	25,2	36,1
Muscle $T_{1/2}$ before treatment	25,3	28,3	21,6	21,9	27,9
Muscle $T_{1/2}$ after treatment	19,0	21,4	18,8	18,7	22,3

Na<sup>131</sup>I absorption (longer half of Na<sup>131</sup>I absorption). After the administration of particular medications, we found out there was an improvement in the radioactive substance resorption in cases of patients with diabetes with a changed Na<sup>131</sup>I absorption. This would show an improvement of the blood flow, that is to say a positive influence of the microcirculation (Table 1, Table 2).

After 5 months treatment with Vessel DUE F, we found improvement in microcirculation in the subcutaneous tissues of the tibia region and in the calf muscle in case of 13 patients, in patients with diabetes, the microcirculation did not change. The average value T S in the subcutaneous region before the treatment was 43,4 minutes, after 5 months treatment 35,1 minutes. The average value T S in the calf muscle before treatment was 25,3 minutes, after 5 months treatment 19,0 minutes (Figure 1).

In reopletismographical examination, we did not find any significant changes in the morphology of the curve and mathematical indexes of the reographic curve. The improvement in the repletion was proved by an increase of the values of pressure quotients (TKQ) in the arteries of lower extremities (ADP, ATP, AP), in the comparison of values before and after treatment even though these changes are statistically not significant (Figure 2). The subjective improvement (less paresthesia, fatigue, claudication distance extension) was stated by 13 patients.

The laboratory tests compared the average values of Quick test, fibrinogen and lipids before treatment and after 5 months treatment. The changes of the studied

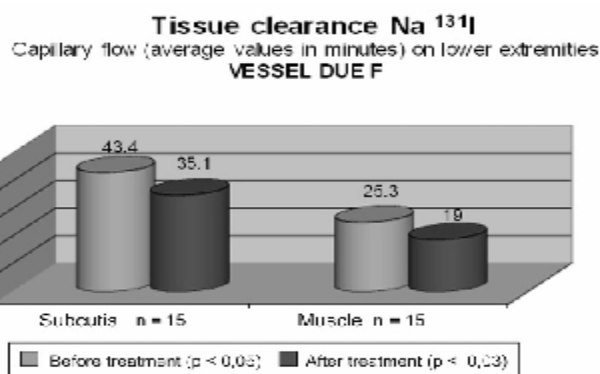


Fig. 1. Average value T ½ (min) in the subcutaneous tissues and in the calf muscle before treatment and after 5 months treatment with vessel due f (the changes are statistically significant), capillary flow (average values in minutes) in low extremities, before therapy, after therapy.

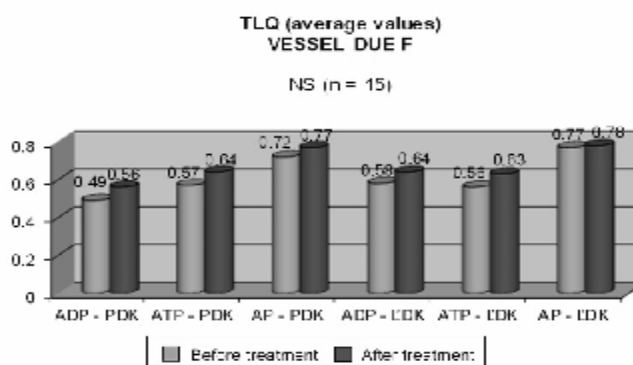


Fig. 2. Values of pressure quotients (for ADP, ATP, AP) right lower extremity and left lower extremity before treatment, after 5 months treatment with VESSEL DUE F (The changes are statistically not significant p > 0,05).

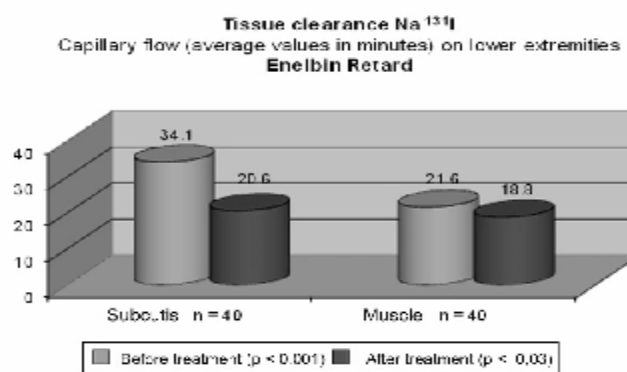


Fig. 3. Average value T ½ (min) in the subcutaneous tissues and in the calf muscle before treatment and after 5 months treatment with Enelbin retard (the changes are statistically significant).

parameters were statistically not significant. In 15 patients also microalbuminuria was examined. After the treatment, microalbuminuria decreased in 6 patients. The average value before the treatment was 22,52 mg/min, after 5 months of treatment 20,08 mg/min. The tolerance of the

medication was good, there were no serious side effects.

Before the initiation of the treatment with Indobufen, the average value T S in the subcutaneous region of the tibia was of 40,5 minutes, after 5 months treatment it was 29,1 minutes. The T S value in the calf muscle was before treatment of 28,3 minutes, after 5 months of treatment 21,4 minutes. The changes are statistically significant.

Neither a reopletismographic nor a sonographic examination, when comparing the findings before treatment and after treatment, proved significant changes in the arteries of the lower extremities.

In the microcirculation examination in the subcutaneous tissues of the tibia region and in the calf muscle after the medication Enelbin retard in 33 patients with diabetes, we found elevated microcirculation, in 7 patients, the microcirculation did not change significantly. The average value T S in the subcutaneous tissues of the tibia was before treatment of 34,1 minutes, after 5 months of treatment 20,6 minutes. The average value T S in the calf muscle was before treatment 21,6 minutes, after 5 months treatment 18,8 minutes (Figure 3).

The change in the perfusion in the lower extremities (ADP, ATP, AP) showed in the change of pressure quotients before treatment and after 5 months treatment (Figure 4). Several patients stated subjective improvement.

After 5 months treatment with Trental, we found an improvement of slow microcirculation in case of 28 patients. In 7

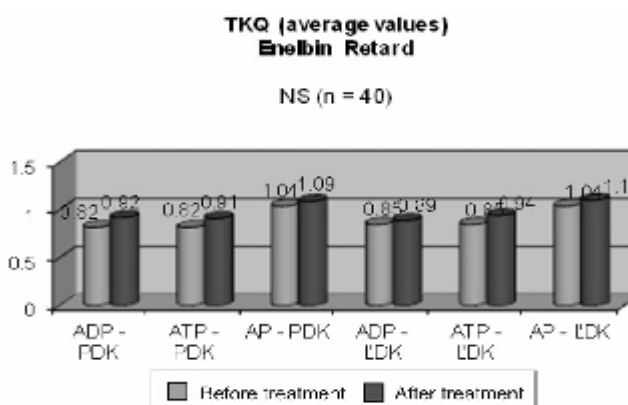


Fig. 4. Values of pressure quotients (for ADP, ATP, AP) right lower extremity and left lower extremity before treatment, after 5 months treatment with ENELBIN RETARD (The changes are statistically not significant  $p > 0,05$ ).

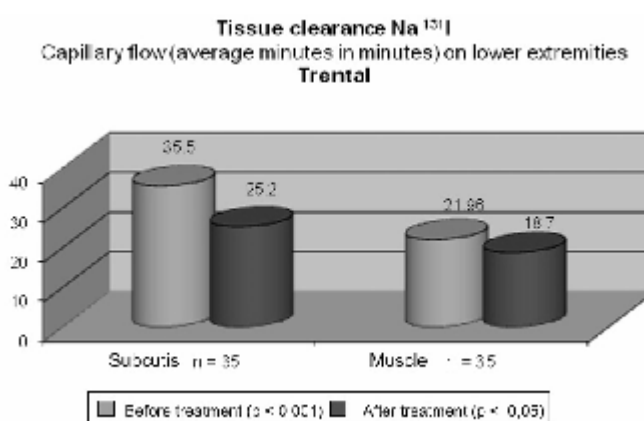


Fig. 5. Average value T  $\frac{1}{2}$  (min.) in the subcutaneous tissues and in the calf muscle before treatment and after 5 months treatment with trental (the changes are statistically significant).

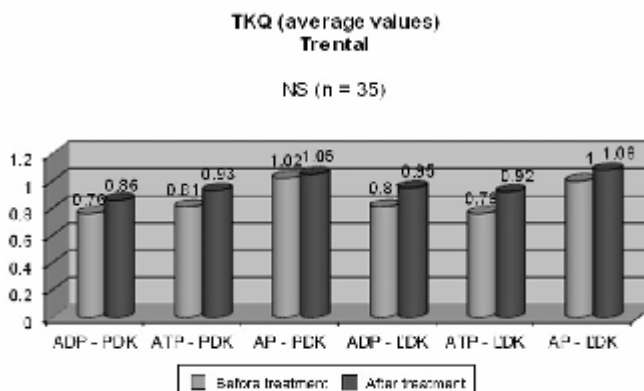


Fig. 6. Values of pressure quotients (for ADP, ATP, AP) right lower extremity and left lower extremity before treatment, after 5 months treatment with TRENTAL (The changes are statistically not significant  $p > 0,05$ ).

patients, the microcirculation did not change significantly. The average value T S in the subcutaneous tissues of the tibia region before the treatment was of 35,5 minutes, after 5 months of treatment of 25,2 minutes. The average value T S in the

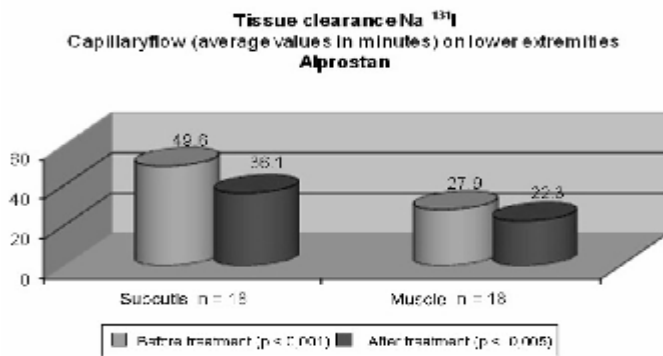


Fig. 7. Average value  $T_{1/2}$  (min) in the subcutaneous tissues and in the calf muscle before treatment and after 5 months treatment with alprostan (the changes are statistically significant).

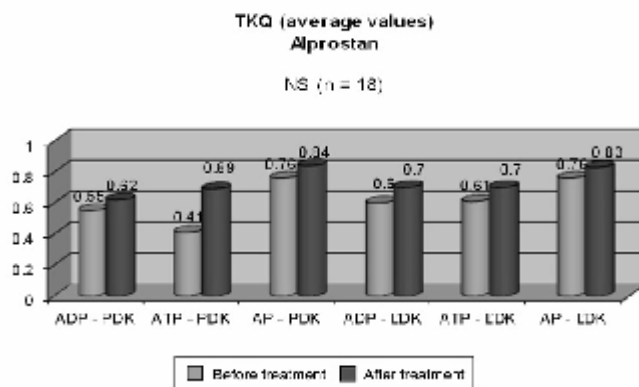


Fig. 8. Values of pressure quotients (for ADP, ATP, AP) right lower extremity and left lower extremity before treatment, after 5 months treatment with alprostan (The changes are statistically not significant  $p > 0,05$ ).

calf muscle before treatment was 21,9 minutes, after 5 months treatment 18,7 minutes (Figure 5).

TKQ values improved (Figure 6). The majority of the examined stated a subjective improvement (retreat of paresthesia, fatigue, claudication distance extension).

After one treatment with Alprostan we found an improvement in the microcirculation both in the subcutaneous tissues as well as in muscle tissues in case of 14 patients. In 4 individuals the microcirculation did not change 5 months after the treatment. The average value of TS in the subcutaneous tissues of the tibia region was before treatment of 49,6 minutes, after treatment 36,1 minutes. The average value TS in the calf muscle was before treatment 27,9 minutes, after treatment 22,3 minutes (Figure 7). In a control examination after one month, the TS values were similar like those after the treatment in case of 12

patients.

The change in perfusion showed in the change of the pressure quotient after treatment when compared with the values before treatment (Figure 8). 10 patients stated a subjective improvement. In 2 patients within one month it was no longer necessary to perform an amputation of the lower extremity.

### Discussion

The notion microangiopathy is used for specific changes in the capillary and pre-capillary walls, that is in the microcirculation. Microangiopathic changes are changes which include, apart from the thickening of the base membrane also the proliferation of the endothelium, dequamation of the endothelium into the arterial lumen, creation of microaneurisma, perivascular inflammatory reactions. A consequence of these is a change in the microcirculation, even in an early state of the illness. The changes in the microcirculation can be found by a capillaroscopy, fluxmetry, transcutaneous measuring of  $O_2$  pressure, thermometrically, or by the method of tissue clearance  $Na^{131}I$  (10,11).

The method of tissue clearance  $Na^{131}I$  was used and modified by Pechór (1971). He examined the microcirculation in hypertension and the possibility of influencing the microcirculation [9]. We have been using this method in our department in the examination of patients with diabetes since 1997. The thickening of the base membrane can cause an increase of the intracapillary pressure and with that a higher permeability of the capillary walls. The initial stages of changes in microcirculation are characterized by a rapid  $Na^{131}I$  absorption, higher permeability of the capillary walls. The referred changes are shown in a rapid  $Na^{131}I$  absorption. A

consequence of other microangiopathic changes is a lower microcirculation, shown by a slow  $\text{Na}^{131}\text{I}$  absorption. A clear evidence of microangiopathy is given through histological methods from excision of subcutaneous or muscle tissues [3, 10]. We believe that a change in the microcirculation shows an early state of diabetic microangiopathy in the lower extremities. After the application of particular medication, we found a statistically significant improvement of the blood percussion, that is to say a positive influence in the microcirculation. This positive influence may be introduced eliminating the functional disorders, for example, with less spasm and extension of micro-vasculature. It can also be an improvement of the haemoreology [12, 13, 14].

Macroangiopathy in the lower extremities is shown by atherosclerosis and medial calcinosis [15]. Histological changes in macroangiopathy do not differ much from atherosclerosis in case of non-diabetes patients. The reason for defining this are especially differences in the affection according to sex and place of changes. In case of patients with diabetes, the distal parts of the extremities are most likely to be affected. Macroangiopathy in the lower extremities was diagnosed reopletismographically and sonographically. The reopletismographical examination allows us to evaluate the quality of the vascular walls, differ the functional changes from the organic ones. The ultrasound method allows us to locate exactly the most affected part of the arterioles.

### Conclusion

We found a statistically significant improvement in the blood percussion, a positive influence of the microcirculation in all mentioned medication. This positive influence could have been introduced by eliminating the functional disorders, for example with less spasm (enhancing the micro-vasculature). It could also have been an improvement of haemoreology.

We consider the improvement in blood percussion in the lower extremities as found by measuring TKQ in the lower extremities arteries as a positive finding, even though the changes are statistically not significant. They correlate with the improvement of the subjective state of the patients. It is possible that the changes occurred with the retreat of spastic influences, or with the improvement of the collateral circulation. Further improvement in the percussion can be expected after a longer application of the medication.

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#### Резюме

ИССЛЕДОВАНИЕ МИКРОЦИРКУЛЯЦИИ  $Na^{131}I$  В ПОДКОЖНОЙ ТКАНИ У ДИАБЕТИЧЕСКИХ ПАЦИЕНТОВ ПРИ ПРИМЕНЕНИИ ЛЕКАРСТВЕННЫХ СРЕДСТВ, ДЕЙСТВУЮЩИХ НА ГЕМОРЕОЛОГИЧЕСКИЕ СВОЙСТВА КРОВИ

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Целью исследования является возможность ранней диагностики диабетической микроангиопатии. Авторы использовали исследование микроциркуляции методом клиренса ткани  $Na^{131}I$ . Некоторые лекарства могут влиять на состояние сосудов и гемореологические свойства крови. Эти лекарственные средства включают гликозаминогликановый сулодексид, индобуфен, антагонисты серотониновых рецепторов, нафтидрофурил, пентоксифиллин, алпростадил. Целью исследования было доказать их влияние на перфузию крови в капилляр-

ном русле.

**Ключевые слова:** сахарный диабет, микроангиопатия, микроциркуляция, клиренс  $Na^{131}I$ .

#### Резюме

ДОСЛІДЖЕННЯ МІКРОЦИРКУЛЯЦІЇ  $Na^{131}I$  У ПІДШКІРНІЙ ТКАНИНІ У ДІАБЕТИЧНИХ ПАЦІЄНТІВ ПРИ ЗАСТОСУВАННІ ЛІКАРСЬКИХ ЗАСОБІВ, ЩО ДІЮТЬ НА ГЕМОРЕОЛОГІЧНІ ВЛАСТИВОСТІ КРОВІ

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Метою дослідження є можливість ранньої діагностики діабетичної мікроангіопатії. Автори використовували дослідження мікроциркуляції методом кліренсу  $Na^{131}I$ . Деякі ліки можуть впливати на стан судин і гемореологічні властивості крові. Ці лікарські засоби включають глікозаміноглікановий сулодексид, індобуфен, антагоністи серотонінових рецепторів, нафтідрофурил, пентоксифілін, алпростаділ. Метою дослідження було довести їх вплив на перфузію крові в капілярному руслі.

**Ключові слова:** цукровий діабет, мікроангіопатія, мікроциркуляція, кліренс  $Na^{131}I$ .

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