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The effect of antiplatelet and antiproliferative agents on endothelial dysfunction under conditions of the formation of arteriovenous anastomoses in an experiment

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The structural and functional features of the endothelium during arteriovenous anastomosis formation in rabbits, and the effect of antiplatelet and antiproliferative agents on endothelial dysfunction over time (on the 10th, 30th, and 90th days after surgery), were studied. The experiment confirmed the pleiotropic properties of platelet P2Y12 receptor inhibitors, including clopidogrel and phosphodiesterase III, as well as the antiplatelet activity of cilostazol, particularly their effect on the synthesis of markers of endothelial dysfunction. It was found that monotherapy with antiplatelet agents is accompanied by a decrease in the content of endothelin-1 (ET-1) and inducible NO synthase (iNOS) in the blood serum by 44–71% and 46–79%, respectively, during observation (most significantly on the 10th and 90th days after anastomosis formation) compared to the control (formation of a connection without pharmacological correction). The effectiveness of topical application of a biodegradable film saturated with the calcineurin inhibitor tacrolimus is most strongly manifested on the 30th day of observation. Local application of tacrolimus in combination with antiplatelet therapy provides optimal indices of the endothelium functional state throughout the observation period. On the 30th day of the examination, the content of ET-1 under conditions of combined therapy decreased by 21 and 28%, and iNOS decreased by 32 and 23%, respectively, compared to the values of similar indexes against the background of using only clopidogrel or cilostazol, respectively. Under the studied conditions, on the 10th day of observation, the NO_2^- content in the blood serum of the animals in the control group exceeded baseline values (i.e., values before surgery) by 37%. In contrast, against the background of pharmacological correction, the values of the index were 14-33% lower. Morphological examination of the surgical site under the conditions of combined use of antiaggregant and immunosuppressive drugs revealed a decrease in the intensity of smooth muscle cell proliferation and extracellular matrix in the subendothelial layer, confirming the effectiveness of the proposed method for preventing stenotic processes in the vascular anastomosis site. Important aspects include preservation of the structure of the vascular walls with good adventitial vascularization in groups undergoing complex correction. The obtained results are confirmed by the data of ultramicroscopic examination of the vascular wall components' structure. Thus, experiments have proven the effectiveness of combining antiaggregant and topical immunosuppressive therapies in reducing the risk of endothelial dysfunction developing at arteriovenous anastomosis sites.

Key words: structural and functional features of the endothelium; endothelial dysfunction; arteriovenous anastomosis; pharmacological correction.

INTRODUCTION

The development of neointimal hyperplasia in the site of arteriovenous anastomoses is one of the key factors limiting the duration of use of permanent vascular access in haemodialysis patients. The onset of the pathological process is closely associated with the development of endothelial dysfunction in the site of surgical intervention. Therefore, it remains relevant to study the mechanisms of its development in order to find effective methods for the prevention of occlusive-stenotic processes in the site of arteriovenous anastomosis.

The tone of vascular wall is determined by the complex interaction between vasodilators and vasoconstrictors, which affect the smooth muscle cells of the media and regulate the lumen of the vessel, determining the velocity and volume of blood flow. The main endogenous vasodilators are nitric oxide (NO), prostacyclin (PGl2) and endothelium-dependent hyperpolarising factor (EDHF). The key regulator of vascular wall tone is NO, which is synthesized in the human body with the participation of NO synthases (NOS). There are three main isoforms of the enzyme: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) [1]. Activation of eNOS provides a short-term increase of NO level, which causes vasodilation of the vascular wall under physiological conditions, prevents excessive proliferation of smooth muscle cells in the media, inhibits platelet adhesion and has an anti-inflammatory effect. In contrast, iNOS activation occurs in response to vascular damage after 6-12 h and is induced by pro-inflammatory cytokines: tumour necrosis factor (TNF-α), interleukins (IL-1β), interferon-γ (IFN-γ), lipopolysaccharides (LPS). The amount of NO synthesized in this case is significantly higher, which leads to the development of oxidative stress, cumulation of toxic peroxynitrite ($ONOO^-$), cell apoptosis, and stimulation of inflammatory processes in the area of damage. Excessive iNOS formation under such conditions causes a decrease of eNOS activity, leading to endothelial dysfunction and the development of neointimal hyperplasia of the vascular wall due to excessive synthesis of smooth muscle cells [2, 3]. Therefore, regulation of eNOS and iNOS synthesis after surgery is an important factor for the prevention of development of occlusive-stenotic lesions in the site of arteriovenous anastomoses.

Among vasoconstrictors, endothelin-1 (ET-1), angiotensin II, noradrenaline, and thromboxane A_2 (TXA_2) are the most important. ET-1 plays a decisive role in both the regulation of vascular tone and the pathological remodelling of the vascular wall during inflammatory processes due to the stimulation of proliferation of smooth muscle cells of the media and their migration into the intimal layer, which leads to its thickening [4]. There are two main types of ET-1 receptors – ET_A (located in smooth muscle cells of vessels) and ET_B (located in both smooth muscle cells and the vascular endothelium). The activation of these receptors in smooth muscle cells is accompanied by vasoconstriction. In contrast, the binding of ET-1 to receptors on the endothelium leads to an increase the synthesis of NO and prostacyclin. Damage of the vascular wall triggers the development of oxidative stress, eNOS inhibition, accompanied by a decrease of NO synthesis and its bioavailability, and an increase of ET-1 production. It is precisely this imbalance between vasodilators and vasoconstrictors that leads to pathological proliferation of smooth muscle cells, which is the basis for neointimal hyperplasia and the development of haemodynamic disorders in sites of vascular anastomoses [5, 6].

The aim – to study the effect of antiplatelet and antiproliferative agents on the development of endothelial dysfunction and changes of the structural organization of arteriovenous anastomoses in an experiment.

METHODS

The study was conducted on male Chinchilla rabbits (18 animals in each group), that an end-to-side arteriovenous fistula was formed between the common carotid artery and the external jugular vein. The surgical intervention was performed under intramuscular anaesthesia with thiopental sodium (“Arterium”, Ukraine, 20 mg/kg) and xylazine (Sedazin, “Biowet Pulawy Sp. z o.o”, Poland, 10 mg/kg), as well as additional infiltration anaesthesia in the incision area on the anterolateral surface of the neck (Lidocaine, 2%, “Darnytsia”, Ukraine). For prevention the development of infectious complications, animals received parenteral perioperative antibiotic prophylaxis (Cefazolin, “Arterium”, Ukraine, 50 mg/kg).

Intraoperatively, animals received systemic heparinisation (1000 U intravenously). The functioning of the reconstruction site was assessed by the pulsation of arterial blood flow in the fistula area. In the postoperative period rabbits of the 1st group received the platelet P2Y12 receptors inhibitor clopidogrel (Plavix, SANOFI, France, 20 mg daily, per os), 2nd – the platelet aggregation inhibitor cilostazol (Pletol, "Adamed Pharma s.a.", Poland, 20 mg/kg daily, per os), animals of the 3rd during the surgery in the anastomosis site were locally applied with a film saturated with the calcineurin inhibitor tacrolimus (Prograf, "Astellas Pharma", Japan, 1 mg), 4th - combined tacrolimus (locally) and clopidogrel, 5th - tacrolimus (locally) and cilostazol. Rabbits of the control group did not receive pharmacological correction in the postoperative period. The animals were withdrawn from the experiment on the 10th, 30th, and 90th days after the surgery by overdosing with thiopental sodium. Biochemical markers of endothelial dysfunction (ET-1, iNOS and NO metabolite - NO_2^-) were exemined in the blood serum. For determination ET-1 and iNOS standart sets of reagents Rabbit ET-1 (Endothelin 1) ELISA Kit and Rabbit NOS2/iNOS (Nitric Oxide Synthase 2, Inducible) ELISA Kit (Wuhan Fine Biotech Co., Ltd., China) were used. Blood was collected from rabbits before anastomosis formation and during withdrawing from the experiment. Morphological changes of the artery and vein walls in the reconstruction site were determined using light microscopy, and ultrastructural features – electron microscopy of previously removed corresponding sections of the vessels. Histological sections from paraffin blocks were stained with haematoxylin and eosin [7, 8]. Photographic documentation of micropreparations was performed using a MICROS MC300 (XT) microscope (Austria), a ToupCam 5.1M UHCCD C-Mount Sony digital camera, an AMA075 adapter in ToupTek ToupView software (V3.7.1398). Ultra-thin sections obtained on an ultramicrotome ("Tesla", Czech Republic) were mounted on copper blends with a diameter of 1 mm, contrasted with a solution of uranyl acetate with Reynolds' mixture, and studied using a PEM-100 electron microscope ("SELMI OJSC", Ukraine) with subsequent photography. The study was conducted in accordance with the current Law of Ukraine "On the protection of animals from cruel treatment", the provisions of "The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" after the experiment design was approved by the Ethics Committee of Ivano-Frankivsk National Medical University (Protocol No. 130/22 dated 22 November 2022).

Statistical data processing was performed using the Excel computer program of the Microsoft Office 365 ProPlus package. For each of the samples, it was determined whether the distribution of the studied index is normal using the Shapiro-Wilk criterion. All data corresponded to the normal distribution type, therefore, to assess the reliability of the difference of results in the two compared samples, the corresponding Student's t-criterion was used. The difference was considered statistically reliable at $P < 0.05$.

RESULTS AND DISCUSSION

As a result of the analysis of biochemical markers on the 10th day of the study, the lowest ET-1 values were found in animals of the 1st and 2nd research groups (were on monotherapy with antiplatelet drugs using clopidogrel and cilostazol, respectively). At the same time, a decrease of ET-1 content by 21.34 and 14.73% ($P_{3\text{-}4,5} < 0.05$) was observed in the blood serum of rabbits that were on combined local and systemic therapy (4th and 5th research groups, respectively) compared to animals that received tacrolimus only topically (3rd research group) (Fig. 1A). On the 30th day of the examination, an increase of ET-1 content was found in the blood serum of animals of all research groups that received disaggregate therapy. Under these conditions, a decrease of ET-1 content in the blood serum of rabbits in the 4th and

5th research groups by 20.68 and 28.42% ($P_{1\text{-}4,5} < 0.05$) was established compared to the values of the similar index in animals of the 1st and 2nd. These results may indicate a positive effect of combination therapy compared to monotherapy with antiplatelet agents. On the 90th day of the examination, the tendency towards a decrease of ET-1 content in the blood serum of animals in the control and research groups persisted, with the exception of the 3rd group, where the index reliably increased by 82.79% which may be associated with complete resorption of the film and cessation of the local effect of tacrolimus.

During the analysing the features of iNOS formation on the 10th day of the examination, attention is drawn to a decrease of the index in the 1st research group (clopidogrel monotherapy) by 90.76% compared to the control and by 71.86% in comparison with the 2nd group (cilostazol monotherapy) (see Fig. 1B). On the 30th day, a reliable decrease of iNOS in the blood serum was observed in animals of the 4th and 5th research groups by 46.63 and 61.02%, respectively, compared to the values on the 10th day. Persist a tendency towards a higher efficacy of combined systemic administration of antiaggregants and cytostatic topically compared to the monotherapy with clopidogrel or cilostazol, reflecting a reliable decrease of iNOS by 45.87% (1st) opposed to 78.23% (4th) and by 55.05% (2nd) opposed to 78.75% (5th) groups relative to the control. On the 90th day of the experiment, iNOS content in animals of the 4th and 5th research groups was lower by 45.93 and 54.87% ($Pc_{-4,5} < 0.05$) compared to the values in the control group.

Under the studied conditions, on the 10th day of observation, the NO_2^- content in the blood serum of animals in the control group exceeded the initial values by 37.54% ($P < 0.05$). Against the background of pharmacological correction, the values of the index were by 14.48-32.72 % lower in relation to the control. The same pattern persisted on the 30th day of the experiment (except the 3rd group), but the NO_2^- content in the blood serum was by 12.80-21.15% lower than before the formation of anastomosis. On the 90th day, a reliable dynamic of increasing the formation of signalling molecules was

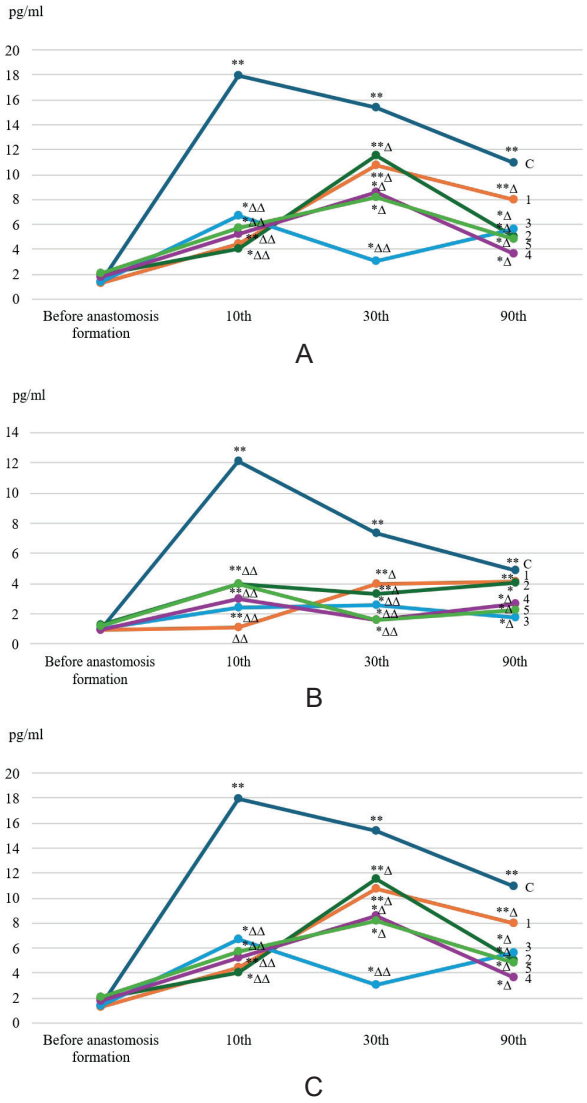


Fig. 1. Changes of endothelin-1 (ET-1, A), inducible NOS (iNOS, B), nitrite ion (NO_2^-, C) content in the blood serum of rabbits that underwent arteriovenous anastomosis formation: C – without pharmacological correction in the postoperative period (control group), 1 – under conditions of clopidogrel administration (1st group), 2 – cilostazol (2nd), 3 – topical use of tacrolimus (3rd), 4 – clopidogrel and tacrolimus locally (4th), 5 – cilostazol and tacrolimus locally (5th) before anastomosis formation, on the 10th, 30th, and 90th days of the study (M ± m); *P < 0.05, **P < 0.01 – relative to the values before anastomosis formation, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ relative to values in animals of the control group

established, in contrast to the control group, where a decrease of the index was observed (see Fig. 1C).

Against the background of change in biochemical markers of endothelial function in the vascular wall of animals of the 1st research group on the 30th day, the perimeter of the lumen is clearly delineated by a chain of endothelial cells on the basement membrane. Separate loci of neointimal transformation are represented by diffusely distributed smooth myocytes. In the middle membrane, there are oedematous changes (Fig. 2A). In the vascular wall of animals of the 2nd group at the same period, the endothelium is represented by a basophilic strip, unevenly expressed around the perimeter of the lumen. There are areas of protrusions alternating with thinning. In the middle membrane, smooth myocytes are ordered, in some fields of view they are stratified by oedematous connective tissue structures. In oedematous altered loose connective tissue of the adventitia the multiple blood vessels are located (Fig. 2B). Electron microscopic examination of the vascular walls of animals of the 2nd group on the 90th day reveals endothelial cells with cytoplasm of medium electron density on the uniform undulations of the internal elastic membrane. Connective tissue fibres surround smooth myocytes as a framework (Fig. 2C).

In animals of the 3rd research group on the 10th day of the study, endothelial cells are located on the basement membrane. Smooth myocytes of the middle membrane are surrounded by the ground amorphous substance. There are loci of lymphocytic infiltration. Vasa vasorum are clearly visible in the loose connective tissue of the adventitial layer (Fig. 3A). Submicroscopically, on the 30th day after anastomosis formation, the internal elastic membrane is exposed, with small areas of diffuse deposits. The sarcolemma of the smooth myocytes of the middle membrane forms variously shaped protrusions. The nucleus of the smooth myocyte is oval, with invaginations of the nuclear membrane. The connective tissue fibres of the vascular wall are oedematous (Fig.

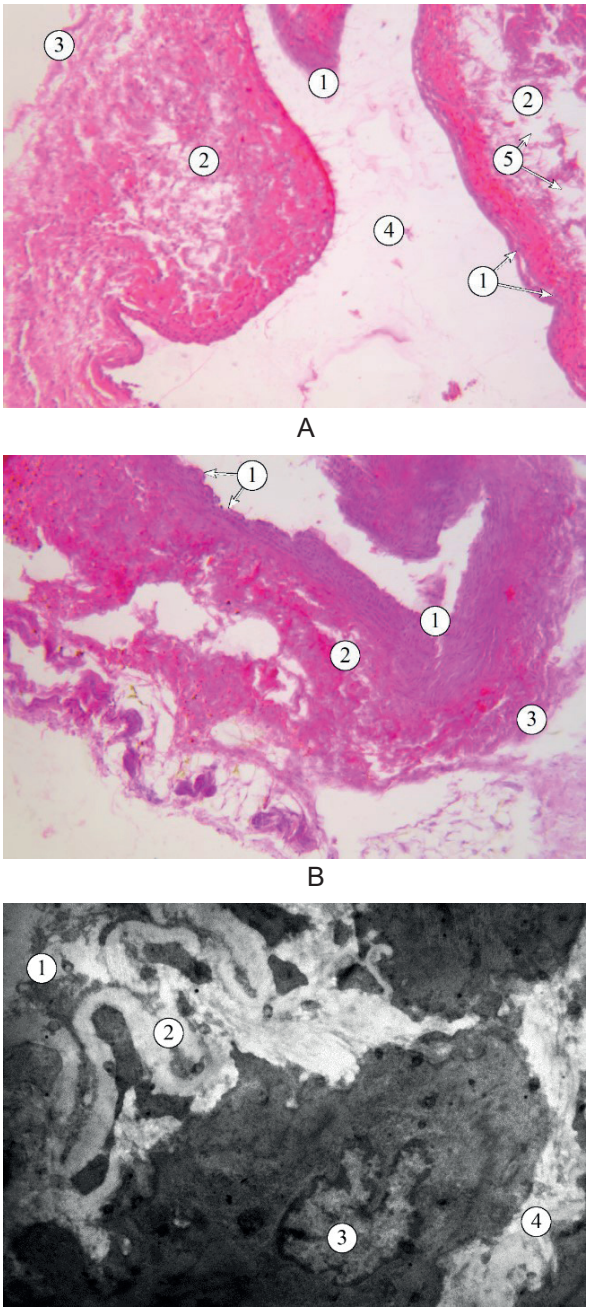


Fig. 2. Structural features of the vascular wall in animals that received clopidogrel (1st group) and cilostazol (2nd group) on the 30th day of the study - A, B, on the 90th (2nd group) - C. Staining: haematoxylin and eosin (A, B). A, B ×100, C ×4800 (electron micrograph). On A: 1 - endothelial cells, 2 - middle membrane, 3 - adventitial membrane, 4 - vessel lumen, 5 - oedematous changes; on B: 1 - endothelial cells, 2 - media, 3 - adventitia; on C: 1 - endothelial cells, 2 - internal elastic membrane, 3 - smooth myocyte nucleus, 4 - connective tissue layers

3B). In animals of this research group, on the 90th day, the undulations of the internal elastic membrane are covered with flattened endothelial cells on the basement membrane. In the media, areas of pericellular oedema of smooth myocytes are visible (Fig. 3C). Submicroscopically, erythrocyte sludge and platelet associations are visible in the lumen. The inner elastic membrane has loci of desquamation and island-like enlightenment of the subendothelial layer (Fig. 3D).

In the vascular wall of animals of the 4th research group on the 10th day of observation, ultrastructurally the electron-transparent deposits are found on the uniform undulations of the internal elastic membrane. The nucleus of smooth myocytes with numerous invaginations of the nuclear membrane and margination of heterochromatin. Sarcoplasm is granular. Connective tissue fibres surround smooth myocytes (Fig. 4A). On the 30th day, endothelial cells are located on the undulations of the luminal protrusions of the connective tissue elements of the wall. Smooth myocytes of the middle membrane are ordered. Blood vessels with signs of stasis are visible in the enlightened adventitia (Fig. 4B). On the 90th day the vascular wall is structured into layers. The intima is in the form of a weakly basophilic strip, with signs of neointimal transformation in some areas. The

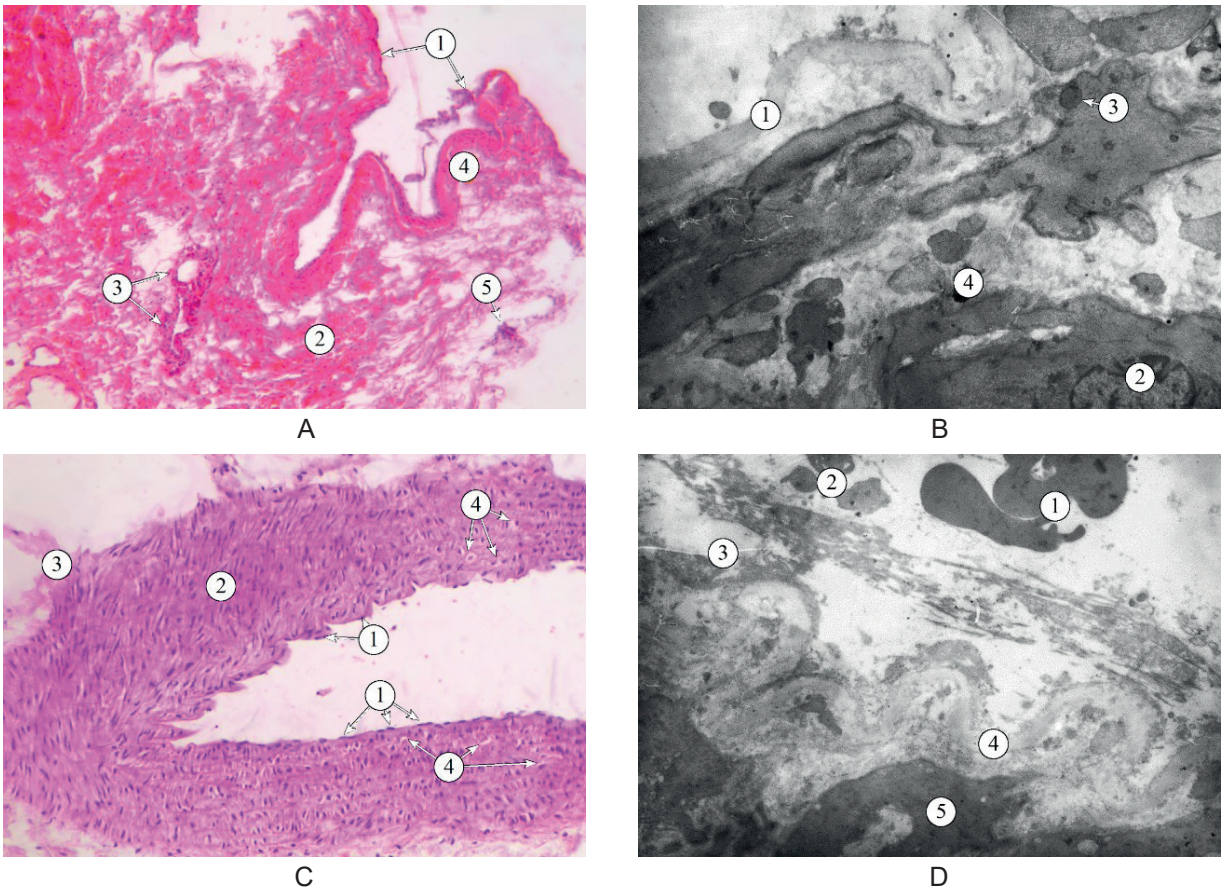
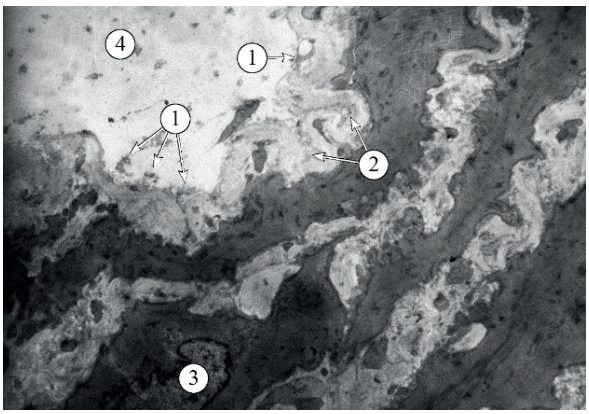
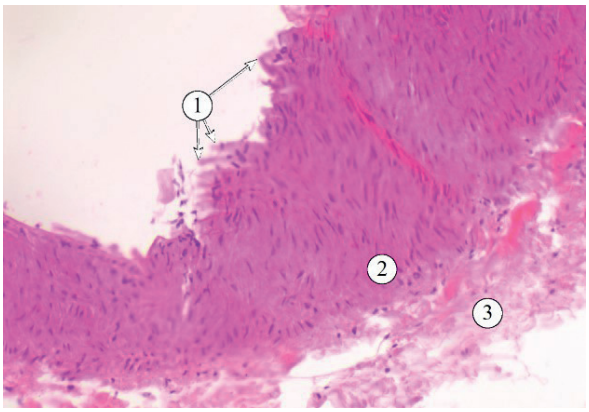


Fig. 3. Structural features of the vascular wall in animals treated with tacrolimus-saturated film (3rd group) on the 10th day of the study - A, on the 30th day - B, on the 90th day - C, D. Staining: haematoxylin and eosin (A, C). A ×100, B ×4000, C ×200, D ×3200 (B, D - electron micrographs). On A: 1 - endothelium, 2 - middle membrane, 3 - vasa vasorum, 4 - oedematous changes, 5 - lymphocytic infiltration; on B: 1 - internal elastic membrane, 2 - smooth myocyte nucleus, 3 - mitochondria in the sarcoplasm of smooth myocytes, 4 - oedematous connective tissue elements; on C: 1 - endothelial cells, 2 - media, 3 - adventitia, 4 - pericellular enlightenments; on D: 1 - erythrocyte sludge, 2 - platelets, 3 - endothelial cells, 4 -internal elastic membrane, 5 - smooth myocyte



A



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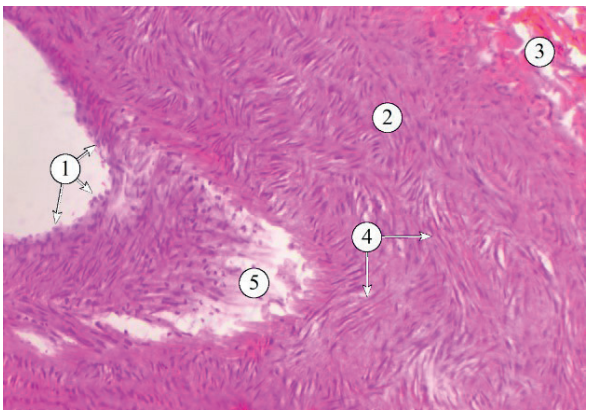

C

Fig. 4. Structural features of the vascular wall in animals that received clopidogrel and tacrolimus topically (4th group) on the 10th day of the study - A, on the 30th day - B, on the 90th day - C. Staining: haematoxylin and eosin (B, C). A ×2400 (electron micrograph), B, C ×200. On A: 1 - electron-transparent deposits, 2 - internal elastic membrane, 3 - smooth myocyte nucleus, 4 - lumen; on B: 1 - endothelial cells, 2 - middle membrane, 3 - adventitial membrane; on C: 1 - endothelial cells, 2 - media, 3 - adventitia, 4 - multidirectional connective tissue fibres, 5 - oedema

middle layer is represented by smooth myocytes surrounded by multidirectional connective tissue fibres. Islands of optical enlightenment are visible in the wall (Fig. 4C).

In animals of the 5th research group, on the 10th day after the formation of anastomosis, elongated by perimeter of the lumen basophilic nuclei of endothelial cells, located on the basement membrane with a thin subendothelial layer are observed. Smooth myocytes of the middle membrane are surrounded by oedematous stratifications (Fig. 5A). On the 90th day, the light optically thinned endothelium is located on the basement membrane as a continuous flattened layer. Smooth myocytes of the middle membrane with clearly defined nuclei. The adventitia is well vascularised (Fig. 5B). Submicroscopically, the luminal surface of the endothelial cells forms multiple spiral-like protrusions into the lumen. The cytoplasm contains scattered mitochondria of various shapes, most of which have a diffuse matrix, while some have cristae, which are sometimes incomplete. The cisternae and tubules of the granular endoplasmic reticulum are shortened. Lysosomes of varying electron density (Fig. 5C).

One of the basic approaches aimed at preventing the development of occlusive-stenotic lesions of vascular reconstruction sites is the use of antiaggregant therapy. The purpose of using the drugs of this group is to prevent the development of thrombotic complications and improve the functional state of the endothelium. Acetylsalicylic acid remains a quite common antiplatelet drug, which inhibits the synthesis of the vasoconstrictor TXA_2 by suppressing the activity of cyclooxygenase-1, thereby disrupting platelet aggregation [9]. In the current recommendations of the European Society of Vascular Surgeons, use of clopidogrel is the first-line drug for preventing the haemodynamic disturbances of vascular access for haemodialysis. The pharmacodynamics of the drug is based on the inhibition of the P2Y12 ADP receptor, which manifests as an antiplatelet effect and indirectly preserves the functional state of endothelial

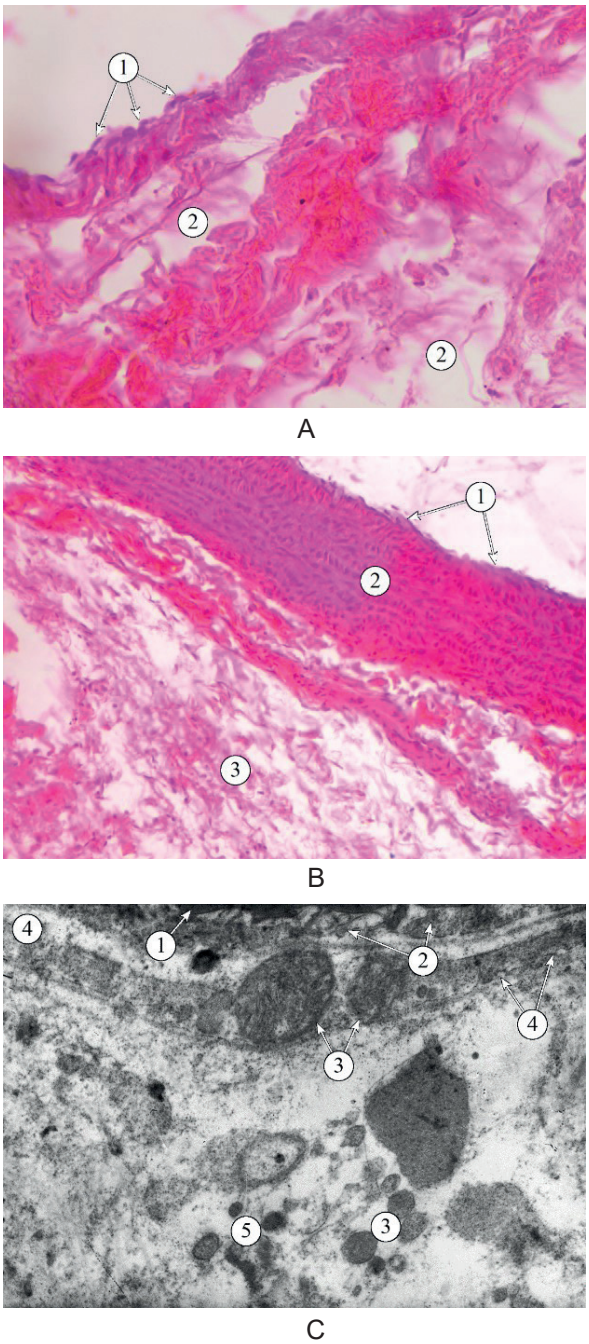

Fig. 5. Structural features of the vascular wall in animals that received cilostazol and tacrolimus topically (5th group) on the 10th day of the study - A, on the 90th day - B, C. Staining: haematoxylin and eosin (A, B). A ×200, B ×400, C ×16000 (electron micrograph). On A: 1 - endothelial cells nuclei, 2 - oedematous changes in the intima and media; on B: 1 - endothelial cells, 2 - media, 3 - adventitia; on C: 1 - lumen, 2 - luminal protrusions, 3 - mitochondria, 4 - granular endoplasmic reticulum, 5 - lysosomes

cells [10]. Cilostazol is a highly potent agent that provides vasodilatory effect and prevents the development of pathological remodelling of the vascular wall. The experimentally established results are likely to reflect its effect on endothelial cells under conditions of the inflammatory trigger TNF-α, causing an increase of eNOS activity and suppression of excessive ET-1 synthesis [11].

Studies reflecting the effect of local application of immunosuppressive drugs for prevention the pathological remodelling of the vascular wall after reconstructive surgery are promising [12]. There is experimental evidence confirming the effectiveness of using sutures that ensure prolonged release of tacrolimus directly in the anastomosis site, which ensures a reduction the thickness of neointima of the vascular wall by 22% compared to the control. The authors justify the better safety profile of topical application of cytostatic compared to systemic use in animals. The mechanism of action of the drug is based on the inhibition the synthesis of pro-inflammatory cytokines (IL-2, IFN-γ, TNF-α), which in turn reduces the activity of T-lymphocytes and macrophages in the site of damage [13]. In addition, tacrolimus indirectly inhibits the proliferation of smooth muscle fibres of vascular wall, induces eNOS activity, reduces oxidative stress indicators and decreases ET-1 expression, thereby preventing the development of endothelial dysfunction and neointimal hyperplasia. We observed some of these effects of the drug under the conditions of its local application as part of a film.

CONCLUSIONS

The formation of arteriovenous anastomosis is accompanied by the development of endothelial dysfunction. The use of antiplatelet therapy is accompanied by a decrease the content of ET-1 and iNOS in the blood serum (most significantly on the 10th and 90th days after anastomosis formation) in relation to the control (formation of a connection without

pharmacological correction). The effectiveness of topical application of a biodegradable film saturated with the calcineurin inhibitor is most evident on the 30th day of observation. Local application of tacrolimus in combination with antiplatelet therapy provides optimal indexes of functional state of endothelium throughout the observation period. Morphological examination of the surgical site under conditions of combined use of disaggregant and antiproliferative drugs revealed a decrease the intensity of proliferation of smooth muscle cells and extracellular matrix in the middle layer of the vascular wall, confirming the effectiveness of the proposed method for preventing stenotic processes in the anastomosis site. Important aspects are the preservation of the structure of the vascular layers with good adventitial vascularisation against the background of complex correction.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ВПЛИВ АНТИТРОМБОЦИТАРНИХ І АНТИПРОЛІФЕРАТИВНИХ ЗАСОБІВ НА ЕНДОТЕЛІАЛЬНУ ДИСФУНКЦІЮ ЗА УМОВ ФОРМУВАННЯ АРТЕРІОВЕНОЗНИХ АНАСТОМОЗІВ В ЕКСПЕРИМЕНТІ

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Вивчали структурно-функціональні особливості ендотелію за умов формування артеріовенозних анастомозів у кролів і вплив антитромбоцитарних та антипроліферативних засобів на ендотеліальну дисфункцію в динаміці (на 10, 30 і 90-ту доби після оперативного втручання). В експерименті підтверджено плейотропні властивості інгібіторів P2Y12-рецепторів тромбоцитів клопідогрелю та фосфодіестерази III з антитромбоцитарною активністю цилостазолу, зокрема їх вплив на синтез маркерів ендотеліальної дисфункції. Встановлено, що застосування монотерапії антитромбоцитарними засобами супроводжується зниженням вмісту ендотеліну-1 (ET-1) та індуцибельної NO-синтази (iNOS) у сироватці крові на 44–71 і 46–79% відповідно впродовж спостереження (найсуттєвіше на 10-ту та 90-ту доби після формування анастомозу) щодо контролю (створення з'єднання без фармакологічної корекції). Ефективність топічного застосування біодеградуючої плівки, насиченої інгібітором кальциневрину такролімусом, найбільшою мірою проявляється на 30-ту добу спостереження. Місцеве застосування такролімусу у поєднанні з антитромбоцитарною терапією забезпечує оптимальні показники функціонального стану ендотелію на всьому періоді спостереження. На 30-ту добу дослідження вміст ET-1 за умов поєднаної терапії знизився на 21 і 28%, iNOS – на 32 і 23% відповідно щодо значень аналогічних показників на тлі застосування тільки клопідогрелю чи цилостазолу відповідно. За досліджуваних умов на 10-ту добу спостереження вміст NO_2^- у сироватці крові тварин контрольної групи перевищив вихідні значення (до оперативного втручання) на 37%, тоді як на тлі фармакологічної корекції значення показника були на 14–33% меншими. При морфологічному дослідженні ділянки хірургічного втручання за умов поєднаного застосування дезагрегантних та імуносупресивних препаратів виявлено зменшення інтенсивності проліферації гладком'язових клітин і екстрацелюлярного матриксу в субендотеліальному шарі, що підтверджує ефективність запропонованого методу попередження стенотичних процесів у ділянці судинного анастомозу. Важливими аспектами є збереження структури оболонок судин при добрій васкуляризації адвентиції у групах комплексної корекції. Отримані результати підтверджені ультрамікроскопічно дослідженням структури компонентів судинної стінки. Таким чином, експериментально обгрунтовано ефективність поєднаного застосування дезагрегантної та топічної імуносупресивної терапії для зниження ризиків розвитку ендотеліальної дисфункції ділянок артеріовенозних анастомозів.

Ключові слова: структурно-функціональні особливості ендотелію; ендотеліальна дисфункція; артеріовенозний анастомоз; фармакологічна корекція.

REFERENCES

1. Pautz A, Li H, Kleinert H. Regulation of NOS expression in vascular diseases. Front Biosci. (Landmark Ed). 2021; 26(5): 85-101. doi:10.52586/4926.
2. Wierońska JM, Cieślik P, Kalinowski L. Nitric oxide-dependent pathways as critical factors in the consequences and recovery after brain ischemic hypoxia. Biomolecules. 2021; 11(8): 1097. doi :10.3390/biom11081097.
3. Campbell A, Kuonqui KG, Ashokan G, Rubin J, Shin J, Pollack BL et al. Role of inducible nitric oxide (iNOS) and nitrosative stress in regulating sex differences in secondary lymphedema. Front Physiol. 2024; 15: 1510389. doi :10.3389/fphys.2024.1510389.
4. Planas-Rigol E, Terrades-Garcia N, Corbera-Bellalta

M, Lozano E, Alba MA, Segarra M et al. Endothelin-1 promotes vascular smooth muscle cell migration across the artery wall: a mechanism contributing to vascular remodelling and intimal hyperplasia in giant-cell arteritis. Ann Rheum Dis. 2017; 76(9): 1624-34. doi:10.1136/annrheumdis-2016-210792.

5. Yang L, Li X, Ni L, Lin, Y. Treatment of endothelial cell dysfunction in atherosclerosis: a new perspective integrating traditional and modern approaches. Front Physiol. 2025; 16: 1555118. doi :10.3389/fphys.2025.1555118.
6. Dunaway LS, Mills WA, Eyo UB, Isakson BE. The cells of the vasculature: advances in the regulation of vascular tone in the brain and periphery. Basic Clin Pharmacol Toxicol. 2025; 136(5): e70023. doi :10.1111/bcpt.70023.
7. Bagriy MM, Dibrova VA. Methods of morphological research: monograph; 2016: 328. [Ukrainian].
8. Voronych VO, Popadiuk OYa, Popadynets OH, Kotyk TL Morphological and functional features of different types of arteriovenous anastomoses in rabbits. Fiziol Zh. 2025; 71(3):18-25. doi: 10.15407/fz71.03.018.
9. Badimon L, Vilahur G, Rocca B, Patrono C. The key contribution of platelet and vascular arachidonic acid metabolism to the pathophysiology of atherothrombosis. Cardiovascul Res. 2021; 117(9), 2001-15. doi: 10.1093/cvr/cvab003.
10. Scanavini-Filho MA, Berwanger O, Matthias W, Aguiar MO, Chiang HP, Azevedo L et al. Effects of Ticagrelor and clopidogrel on coronary microcirculation in patients with acute myocardial infarction. Adv Ther. 2022; 39(4): 1832-43. doi :10.1007/s12325-022-02061-0.
11. Xue Y, Li X, Wang Z, Lv Q. Cilostazol regulates the expressions of endothelin-1 and endothelial nitric oxide synthase via activation of the p38 MAPK signaling pathway in HUVECs. Biomed Rep. 2022; 17(3): 77. doi:10.3892/br.2022.1560.
12. Park DS, Na MH, Jeong MH, Sim DS, Jin YJ, Kee HJ et al. Efficacy and safety evaluation of Tacrolimus-Eluting stent in a porcine coronary artery model. Tissue Eng Regen Med. 2024; 21(5): 723-35. doi:10.1007/s13770-024-00646-0.
13. Parikh KS, Josyula A, Inoue T, Fukunishi T, Zhang H, Omiadze R. Nanofiber-coated, tacrolimus-eluting sutures inhibit post-operative neointimal hyperplasia in rats. J Control Release. 2022; 353, 96-104. doi: 10.1016/j.jconrel.2022.11.020.

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