GEORGIAN MEDICAL NEWS


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INFLUENCE OF VASCULAR STENT SURFACE TREATMENT WITH AN ADAPTIVE COMPOSITION (AdC) FOR IMPROVING ITS BIOCOMPATIBILITY AND RESTENOSIS PREVENTION

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Abstract.

**Aim:** The article describes a method of implant surface treatment that reduces the risk of an inflammatory reaction to vascular implants.

**Materials and methods:** The research was conducted on 34 male rabbits of the "Flemish Giant" breed weighing 2.5-3.0 kg, following the standards of bioethical principles. The blood vessels of the experimental animals were previously provoked by the administration of endogenous pyrogenic solution according to a predetermined protocol. Under general anesthesia, the animals were endovascularly (via femoral access into the abdominal aorta) implanted with standard Z-shaped stents made of 316L stainless steel. To obtain indicative results, 10 rabbits were implanted with non-treated stents, while another 12 rabbits had stents pre-treated with the adapting composition (AdC) implanted. After 8 weeks, the animals were withdrawn from the experiment.

**Results and discussion:** Vessel wall morphometry revealed that the treatment of stents with AdC before their placement into the vessel resulted in a reduction of vessel wall thickness at the site of their implantation.

**Conclusions:** The clinical application of AdC aimed at improving the biocompatibility properties of implants with respect to the recipient's body is characterized by a 100% (95% CI 78.2% - 100%) likelihood of absence of complications.

**Key words.** Endovascular stenting, restenosis, implants, rejection, biocompatible materials, adaptive composition, aorta.

Introduction.

Every year, surgeries for the installation of various implants are performed worldwide. In 25-30% of cases, implants are not accepted by the recipient's body [1-4].

In cardiovascular and interventional radiology surgery, implants in the form of stent grafts or stents are used to treat stenotic and occlusive lesions of the arterial lumen due to atherosclerosis and/or diabetes. One of the reasons for restenosis or reocclusions is the body's reaction to the implantation of stents.

The basis of the foreign material rejection reaction is aseptic inflammation, which results from the immune response of the body to the surface of implants. Specific "binding sites" called epitopes exist on the surface of implants, which are recognized by the recipient's immune system's protective elements, immunoglobulin class G (IgG), as foreign. Therefore, upon initial contact of the body's tissues with the implant, the epitopes on its surface are recognized by IgG, a component of the recipient's humoral immune system. The strength of the binding between IgG and the surface is directly proportional to the quantity of epitopes on it that are "sensitive" to a specific pool of IgG. The formation of an affinity bond between the surface of the prosthesis and IgG leads to the activation of the recipient's cellular immune response. This results in the formation of an isolating capsule, the thickness of which is directly proportional to the degree of inflammation. The adhesion of IgG to the epitopes on the surface of the implant itself is accompanied by the generation of peroxide radicals, which play a leading role in the degradation of the prosthesis surface [5-7].

The presence of such rejection reactions to stent placement necessitates the search for ways to solve the problem of these reactions in order to increase the patency of the angioplasty zone with stenting and reduce the number of re-interventions.

One of the options for reducing reactions to implants is the search for and use of more biocompatible materials with the recipient's organism [8]. In the search for effective methods of preventing restenosis at stent implantation sites, the use of special coatings on the surface of the implant, such as stents coated with "Paclitaxel" (a mitotic inhibitor used in cancer chemotherapy), is also being explored. However, according to the meta-analysis of randomized controlled trials, despite the reduction in the inflammatory response to the stent and, as a result, the increase in primary patency of the stenting area, there is data on an increased risk of mortality from the use of such coating methods [9,10]. These studies have become the second reason for seeking alternative methods aimed at preventing restenosis at stent implantation sites.

Several studies have demonstrated that the introduction of the recipient serum albumin solution modifies the surface of implants, reducing the frequency of side effects.

Albumin solution has also been successfully used to block epitopes that did not react during ELISA analysis [11-13].

**The aim of this study** was to develop a method of coating the surface of vascular implants (stents) to reduce the risk of inflammation reaction (rejection), enhance their biocompatibility, and prevent restenosis.

Materials and Methods.

The experiments were conducted in accordance with the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (No. 3447–IV dated February 21, 2006) and adhered to the requirements of the European Parliament and Council (2010). The research was carried out on 34 male rabbits of the "Flemish Giant" breed with a weight of 2.5–3.0 kg. The animal experiments were performed in the vivarium of the Department of Surgery and Transplantology named after O.O. Shalimov of the National Academy of Medical Sciences of Ukraine, following the standards of bioethical principles [14,15].
Under general anesthesia, rabbits were subjected to endovascular procedures via femoral access to the abdominal aorta. Standard Z-shaped stents made of 316L stainless steel (Figure 1) were implanted using a 4F catheter. To ensure reliable results, a control group of 10 rabbits received non-coated stents, while an experimental group of 12 rabbits received stents pre-coated with AdC.

To create an atherogenic model according to the developed methodology, rabbits were intramuscularly injected with a solution of Pyrogenal at a dose of 1.25 mg every other day for 2 weeks. Then, in the postoperative period, a dose of 1.25 mg was administered once a week for 8 weeks. The inflammation model was chosen for the experiment based on the works of Aleksyeyeva T.A., Lazarenko O.N., et al. [16,17].

The technology for preparing AdC and the method of implant surface treatment to enhance their biocompatibility are described in the European patent and the methodological recommendations titled "Clinical Application of Implant Surface Treatment with an Adaptation Composition to Improve their Biocompatibility in Reconstructive and Restorative Surgery" 58.16/140.16 [13,17]. The main processing stages included the following procedures.

Blood was collected from the experimental animals through a vein using a vacuum container. After clot formation for 10-16 minutes, it was centrifuged using an NF 200 centrifuge (Nuve, Turkey) for 7 minutes at 500g. Then, 2 ml of the obtained serum, which had been pre-filtered through a membrane filter with pore sizes of 0.22 μm (Minisart, Sartoriusstedium, Biotech corp.), was mixed in a sterile container with 18 ml of sterile physiological solution (0.9% NaCl).

In molar terms, the concentration of albumin in the resulting serum is an order of magnitude higher than that of immunoglobulins. The adsorption kinetics of albumin to the surface is also higher than that of immunoglobulins. Therefore, during the preparation of AdC, it is not necessary to get rid of serum immunoglobulins, which simplifies the process of its preparation.

After the AdC is prepared, it is poured into a sterile container (cuvette, tray, denture packaging container, etc.) suitable for immersion of the prosthesis and kept in it for 5 minutes, after which it is implanted.

The vessel or container for immersing the prosthesis should have a volume sufficient for complete immersion of the prosthesis. A packaging container for a voluminous prosthesis is an optimal solution for its immersion in AdC, as it requires minimal quantity for immersion.

The immunohistochemical and histomorphological examination was performed 8 weeks after implantation.

The composition of the proposed AdC is presented in Table 1. For statistical data analysis, the software packages MedCalc v.17.2 (MedCalc Software Inc, Broekstraat, Belgium, 2017) and MedStat (Lyakh Yu.Ye., Guryanov V.G., 2004–2011) were used. The impact of risk factors was evaluated using the indicator of Absolute Risk Reduction (ARR) and by constructing and analyzing logistic regression models. The quality of the models was assessed by constructing Receiver Operating Characteristic (ROC) curves and calculating the Area Under the Curve (AUC), as well as the Positive and Negative Predictive Values. The corresponding 95% Confidence Intervals (95% CI) were also calculated.

Results and Discussion.

The results of vascular wall morphometry revealed that stents pre-treated with AdC led to a reduction in vessel wall thickness at their placement site. The data are presented in Table 2.

The results of the research have demonstrated that the proposed method, which involves the prior treatment of stents with AdC, leads to a reduction in the reaction of surrounding tissues by modifying the surface of the implants. This also decreases the thickness of neointimal growth, indicating the absence of inflammation processes and the formation of fibrous tissue around the implant (Figures 2 and 3).

On the Figure 4., Immunohistochemical staining illustrating the native (healthy) rabbit tissue (A), tissue around the AdC-treated implant (B), and tissue around the implant without AdC treatment (C).

<table>
<thead>
<tr>
<th>Protein fractions of the recipient’s serum:</th>
<th>The absolute amount of proteins in 20 ml of AdC is grams per 20 ml (± grams per 20 ml).</th>
<th>The ratio of protein components, % (± %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>albumins</td>
<td>0.94 ± 0.26</td>
<td>61.1 ± 16.7</td>
</tr>
<tr>
<td>α1-globulins</td>
<td>0.04 ± 0.002</td>
<td>2.4 ± 0.12</td>
</tr>
<tr>
<td>α2-globulins</td>
<td>0.14 ± 0.02</td>
<td>9.6 ± 1.3</td>
</tr>
<tr>
<td>β-globulins</td>
<td>0.18 ± 0.015</td>
<td>11.5 ± 1.0</td>
</tr>
<tr>
<td>γ-globulins</td>
<td>0.24 ± 0.06</td>
<td>15.4 ± 3.9</td>
</tr>
</tbody>
</table>
the tissues around the implant showed that the application of immunohistochemical and histomorphological examinations of changes associated with AdC stent processing. The results of compared to untreated implants. The Absolute Risk Reduction (ARR) is 35.7% (6.8% – 61.2%) of intra- and post-operative complications when using AdC. the level of local inflammation, thus decreasing the frequency to implantation using the proposed technology reduces (p=0.04) therefore, the processing of implants with recipient serum prior to implantation using the proposed technology reduces (p=0.04) the level of local inflammation, thus decreasing the frequency of intra- and post-operative complications when using AdC. The Absolute Risk Reduction (ARR) is 35.7% (6.8% – 61.2%) compared to untreated implants. Thus, no animals exhibited pathological histomorphological changes associated with AdC stent processing. The results of immunohistochemical and histomorphological examinations of the tissues around the implant showed that the application of the proposed AdC stent processing method reduces the level of pro-inflammatory changes. There is a significant decrease in the vessel wall thickness at the site of implantation after AdC stent processing (p<0.05) compared to untreated implants. A number of researches have shown the benefits of in vitro treatment of artificial surfaces and the advantages of in vitro treatment of artificial surfaces in contact with blood using autologous endothelial cells have been demonstrated. This was exemplified by Zilla et al. over 20 years ago when they successfully reduced the occurrence of stenosis and reocclusions after femoropopliteal polytetrafluoroethylene grafting [18-22]. However, the clinical application of this strategy was complicated by the challenges of obtaining endothelial cells through additional invasive procedures, such as harvesting from healthy native vessels. Other approaches to cell isolation included extracting endothelial progenitor cells (EPCs) from bone marrow, which required invasive and painful bone marrow aspiration [23], as well as cultivating microvascular endothelial cells from dermal tissue. The latter also remained problematic due to difficulties in obtaining a pure cell population without fibroblast contamination, low yield of endothelial cells, and the short lifespan of isolated cells [24]. Due to the fact that peripheral blood is readily available, the creation of AdCs is a relatively simple procedure that can provide results in preventing vascular and tissue reactions to foreign material. There have also been studies aimed at improving the neoendothelialization of arterial grafts, such as the application of a proactive VEGF (vascular endothelial growth factor) design on the surface of PTFE (polytetrafluoroethylene) grafts [25]. However, these results have not been widely implemented in clinical practice. On the other hand, the results of our research clearly demonstrate the advantages of using AdC-treated vascular stents and hypothetically stent grafts. In the case of stent graft implantation into the aorta for aneurysms, there is an oversized contact area with the aorto-iliac segment, which may trigger reactions to foreign agents. The probability of the absence of complications associated with vascular implant rejection after AdC treatment in our study reaches 100% over 12 months (95% CI 78.2% – 100%), and the overall clinical effectiveness of the proposed technology achieves a moderate level (AUC = 0.69; p <0.001). These research findings correlate with the results of Chen Z’s study [26], in which the immobilization of serum albumin and peptide aptamer for EPC on a polydopamine-coated titanium surface was investigated for enhanced in situ endothelialization. Therefore, based on the experimental data, it can be concluded that the surface treatment of various types of vascular implants with AdC to enhance their biocompatibility with the recipient's organism is justified.

**Conclusion.**
The research has confirmed that the implantation of any exogenous material is a trigger for the activation of the immune response by the recipient's body. This is due to the presence of epitopes on the surface of implants - antigenic determinants that recognize the recipient's IgG, leading to the activation of his or her humoral and cellular immunity.

Treating the implant with the recipient's blood serum solution right before the surgery effectively blocks active sites on its

**Table 2. Changes in rabbit aortic wall thickness depending on the type of stent treatment.**

<table>
<thead>
<tr>
<th>Groups (N = 34)</th>
<th>The wall thickness of the aorta (μm)</th>
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</thead>
<tbody>
<tr>
<td>Control (N = 12)</td>
<td>124 ± 32</td>
</tr>
<tr>
<td>Untreated (N = 10)</td>
<td>305 ± 82</td>
</tr>
<tr>
<td>AdC Treatment (N = 12)</td>
<td>140 ± 28</td>
</tr>
</tbody>
</table>

N - Number of animals in the group (p<0.005)

**Figure 3.** Rabbit aortic wall. Stent pre-treated with AdC prior to implantation. Hematoxylin-eosin staining. Magnification approximately 20x, field of view 20x.

**Figure 4.** Immunohistochemical staining of tissues near the implant site after 8 weeks of stent placement: A - collagen fibers; B - slight accumulation of lymphocytes; C - intense lymphocytic infiltration.

The tissue of a healthy experimental animal, depicted in Figure 4 (A), is uniformly stained with clear visualization of collagen fibers. In Figure 4 (B), a slight accumulation of lymphocytes is observed in the area of the implant element, while in Figure 4 (C), there is intense lymphocytic infiltration.

We suggest that the albumin layer on the surface of implants can lead to the shielding of binding sites with IgG and block the initial stage of the immune response. As a result, the immune system doesn't "react" to the implant as a foreign object. Therefore, the processing of implants with recipient serum prior to implantation using the proposed technology reduces (p=0.04) the level of local inflammation, thus decreasing the frequency of intra- and post-operative complications when using AdC. The Absolute Risk Reduction (ARR) is 35.7% (6.8% – 61.2%) compared to untreated implants. Therefore, based on the experimental data, it can be concluded that the surface treatment of various types of vascular implants with AdC to enhance their biocompatibility with the recipient's organism is justified.

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Treating the implant with the recipient's blood serum solution right before the surgery effectively blocks active sites on its
surface, preventing adhesion of recipient's immunoglobulins and the activation of the immune response. The outcome of using this proposed technique is a significant reduction in the recipient's body reaction to foreign material.

The clinical application of the adapting composition (AdC) for implant adaptation to enhance their biocompatibility with the recipient's body is characterized by a 100% (95% CI 78.2% - 100%) likelihood of complication-free outcomes.

Acknowledgement.

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Authors’ contributions. According to the order of the Authorship

Conflicts of interest. The Authors declare no conflict of interest.

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ВПЛИВ ОБРОБКИ ПОВЕРХНІ СУДИННИХ СТЕНТИВ АДАПТУЮЧОЮ КОМПОЗИЦІЄЮ (AdC) ДЛЯ ПОЛІПШЕННЯ ЇЇ БІОСУМІСНОСТІ ТА ПРОФІЛАКТИКИ РЕСТЕНОЗУ (експериментальне дослідження)

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Резюме. В роботі висвітлюється метод обробки поверхні імплантатів, що знижує ризик виникнення реакції запалення на судинні імплантати. Матеріали і методи. Дослідження проводили на 34 кролях, самцях, порода «сірий велетень» масою 2,5-3,0 кг у відповідності до стандартів біоетичних принципів. Судини експериментальних тварин були попередньо спровоковані введенням ендогенно розчину пірогеналу по загально визначений схемі. Тваринам під загальною анестезією були ендовасккулярно (через стегновий доступ у абдомінальну аорту) встановлені стандартні Z-подібні стенти із нержавіючої сталі 316L. Для отримання показових результатів 10 кролям встановлені не оброблені стенти, іншим 12 кролям були встановлені стенти попередньо оброблені адаптуючою композицією (AdC). Через 8 тижнів тварин виводили з експерименту.

Результати та їх обговорення. Морфометрія стінки судини показала, що обробка стентів AdC перед встановленням їх до судини призводить до зменшення товщини судини у місці їх встановлення.

Висновки. Клінічне застосування AdC з метою поліпшення біосумісних властивостей імплантатів по відношенню до організму реципієнта характеризується 100% (95% БI 78,2%-100%) вірогідністю відсутності ускладнень.

Ключові слова: ендовасккулярне стентування, рестеноз, імплантати, реакція відторгнення, біосумісні матеріали, адаптуюча композиція, аорта.