Use of textile biomaterials for the topic
treatment of chronic venous disease

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DACIANA ELENA BRĂNIŞTEANU¹, MARIETA NICHIFOR²,
CARMEN MIHAELA DOROBÂŢ³, DANIEL CONSTANTIN BRĂNIŞTEANU⁴,
FLORIN DUMITRU PETRARIU⁵, ANDREEA DANA MOLODOI⁶,
DORU CEZAR RADU⁷, DANIEL BODA⁸

¹ Discipline of Dermatology, „Gr. T. Popa” University of Medicine and Pharmacy, Iaşi; ² Department of Bioactive and Biocompatible Polymers, „P. Poni” Institute of Macromolecular Chemistry, Iaşi, Romania; ³ Discipline of Infectious Diseases, „Gr. T. Popa” University of Medicine and Pharmacy, Iaşi, Romania; ⁴ Discipline of Ophthalmology, „Gr. T. Popa” University of Medicine and Pharmacy, Iaşi, Romania; ⁵ Discipline of Primary Health Care and Epidemiology, „Gr. T. Popa” University of Medicine and Pharmacy, Iaşi, Romania; ⁶ Department of Dermatology, Municipal Hospital, Roman, Romania; ⁷ Faculty of Textile and Leather Engineering „Gh. Asachi” Technical University, Iaşi, Romania; ⁸ Dermatology Research Laboratory, “Carol Davila” University of Medicine and Pharmacy Bucharest, Romania

Correspondence adress: dradu@tex.tuiasi.ro; radu_cezardoru@yahoo.com

Abstract

This scientific work summarizes and concludes the efforts of three regional research centers having regard to discovering an innovating topic treatment for the chronic venous disease. New textile biomaterials have been prepared, by chemical or physical application of biopolymers on the textile material, and the load of the biopolymers and biomaterials with rutin, troxerutin and heparine have been tested using physical or chemical methods. The pharmacologically active substance was then gradually released, in a controlled way, at the level of venous endothelium, as a consequence of the prolonged contact of the biomaterial with the skin. There have been performed in vitro and in vivo animal studies, in order to choose the most efficient biomaterial. The textile material chosen must keep intact both the chemical structure and the shape of the grafted polymer, as well as the structure of the drug included in it.

Finally the efficiency, tolerance and safety of the applied treatments have been analyzed in a rigorous, scientific manner on the patients in different stages of the chronic venous disease.

Key words: biomaterial, biopolymer, elastic compression, chronic venous disease, troxerutin

Introduction

The chronic venous disease (CVD) is being defined as a long term decompensation of the venous circulation, with the occurrence of venectasia, reticular veins and hydrostatic varicose (secondary to venous stasis), stasis in the lymphatic system and various skin changes: vesperal or permanent edema, eczematous dermoeipidermitis (stasis chronic eczema), pigmented purpuric dermatitis, dermohypodermitis with scleerous development (lipodermatosclerosis), Milian’s white atrophy and leg chronic ulcer [1]. The aim of this research is to establish the chemical, biochemical, pharmacological, medical and technological aspects of the design of the most adequate polymers systems with controlled release of frequently used drugs in the CVD treatment. This was possible in the common research of the three research groups („Petru Poni”
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Institute for Macromolecular Chemistry in Iasi, „Gr. T. Popa” University of Medicine and Pharmacy Iasi and „Gh. Asachi Technical University” Iasi), whose complementary activity allowed obtaining new biomaterials comfortable for the patient. These biomaterials were made by grafting the polymers on textile supports and including the drugs used in the CVD topic treatment in the obtained grafted structures. Many aspects that may impact on the biomaterials efficiency have been investigated: type of textile, fiber composition, finishing technology, the way of grafting the polymer on the textile support and the way of including the drugs. In particular, we were interested to test different venoactive drugs in the same biomaterial to establish the most potent one. The obtained biomaterials have been tested in vitro and in vivo (on laboratory animals) from the point of view of the release speed, biological activity, tolerance and toxicity. Finally, the most performant biomaterial was selected for being tested on patients. CVD is a „frontier” disease, in its diagnosis and treatment being involved, besides dermatologists, a range of other specialists: internal medicine physicians, radiologists, vascular surgeons, ophthalmologists etc. The data in the speciality literature show an increasing high incidence of CVD. At the European level the prevalence of CVD is estimated at 25-50%. [2]. In Romania, the data regarding the incidence of this disease was absent until 2004, when the research entitled SEPIA (Epidemiological Study on the Prevalence of chronic venous insufficiency in Romania in Ambulatory) demonstrated, in a rigorous scientific manner, that CVD was a public health problem in Romania as well (32% of the patients enrolled in the study presented symptoms of CVD) [3]. The early stage therapeutically action, even from the first CVD stages, may considerably diminish the social and medical impact of the disease and increase the patient’s quality of life. Actual therapeutic options target the control of symptoms and skin lesions and also to prevent related complications of CVD. The therapeutic means suggested for CVD are: external elastic compression, the supine, sclerotherapy, flebotonics, surgical, balneary treatment and hygiene. Nevertheless, the leg ulcer still raises problems from the therapy point of view.

The external elastic compression (elastic stockings, elastic bandages or edema reducer) is at the moment the most modern method of fighting against venous stasis, being used in approximately 71% of the CVD patients. It is leading to treating phlebohypertony, normal return circulation, reduces edema and determines the healing of skin trophic lesions [1]. The increase of the people’s quality of life is nowadays a predominant and continuous concern for the international scientific research. The specialists try to replace the classical treatments with the modern ones, more efficient and which increase the patients’ compliance towards them. The controlled release systems of a bioactive principle (PBA) include protecting PBA against degradation or metabolization and prolonging its optimal duration of action (sustained release) [4].

The procurence of textiles with biomedical activity has had so far 2 obvious directions: transdermal systems used for the topic administration of drugs with systemic activity. In this case, the skin is just a barrier which has to be penetrated by the active principle, in the presence of permeation enhancers, the drug’s activity happening after this one arrives in the blood circulation (transdermal patches such as Nicoderm® and Transderm Nitro®); textiles (clothes) processed by means of adding on their surface multifunctional layers / grafts with the role of barrier against some factors that may affect skin and, implicitly, the human body (fire, temperature differences, UV rays, insects, microbes etc.). The use of textiles as a support for controlled and sustained release of drugs in CVD was not mentioned in the speciality literature. This innovating type of textiles is not being commercialized neither at national or international levels [2, 4]. Oligosaccharides and polysaccharides are often used in making the textiles for the aim mentioned above. Thus, cyclodextrins (CyD), which have a hydrophobic cavity capable of including many hydrophobic substances, were physically or...
chemically placed on knitted materials or fibers, for including insecticides, flavors and antimicrobial composites [5]. The chitosan (Ch)(the most abundant polysaccharide after cellulose), deriving from chitin, was very often used in making textiles, for its intrinsic antibacterial properties and for improving the quality of textiles coloring. At the same time, microcapsules of chitosan containing flavors and hydrating products were commercialized under the name of Skintex [6, 7]. These textiles last for only 2-3 washing cycles, after that the active oligo and polysaccharides being removed. Their use for the controlled release of drugs in the dermatological area was not mentioned in the speciality literature. The topic of our research is the interface for several fields of activity, very important to the scientific research: fine organic synthesis, polymers science, pharmacology, biochemistry, textiles and dermatology [5, 6, 7].

Materials and Methods

Obtaining of textile biomaterials

The initial research aimed at obtaining biomaterials with a sustained action in CVD. In this context the textile materials had to be functionalized, in order to create on the surface reservoirs, from which enough biomolecule can be released to its action site. Two biomaterials that were called biomaterials I and II, were prepared by chemical or physical treatment of knitted material based on a mixture of polyamide/elastic.

Among the topically active drugs in CVD, the rutin, troxerutin and heparine were tested. Biomaterials I were obtained by grafting functionalized compounds with acrylic double bonds on the textile material. For this purpose, new acrylic derivatives were synthesized or commercial monomers were used. All monomers were characterized by Nuclear Magnetic Resonance (NMR) and Fourier Transformed Infrared (FTIR) spectroscopy and thin layer chromatography.

Acryloyl troxerutin (AT) and Acryloyl β-cyclodextrin (A-CyD) were synthesized by direct esterification of troxerutin or cyclodextrin with acryloyl chloride in dimethylformamide - DMF (figure 1, where R-OH is troxerutin or CyD). As a function of molar ratio troxerutin/acryloyl chloride, two derivatives were obtained: AT 1 and A-CyD 1, with 2 acryloyl groups/molecule, or AT 2 and A-CyD 2 with a higher content of the acryloyl groups/molecule.

Glycidyl methacrylate (GMA) is a commercial monomer, with a functional glycidyl group able to chemically bind nucleophilic molecules such as troxerutin.

Type I biomaterials were obtained by grafting the acrylic monomers on the polyamidic material, according to the reaction scheme presented in figure 2, followed, when necessary, by complexation (A-CyD) or chemical binding (GMA) of troxerutin. After the impregnation with an initiator solution, the textile fabric was either sprayed on one face or immersed into the monomer solution. Thus, a material with a bioactive layer on one face or on both faces was obtained. [8] Then the material was left for 6 hours in thermostated oven at 60-70°C. The...
amount of grafted polymer was determined by the difference of weight between the treated and not treated material, and it was of 4-6 g% for the spraying procedure and 10-25 g% for the immersion procedure, both for AT and A-CyD derivatives, but lower than 2 g% for GMA.

Materials based on CyD monomers were finally immersed in 1% (g/v) drug solution in methanol/water (6/1), for 48 hours. The resulting PA-T biomaterials contained up to 25 g% troxerutin, PA-CyD 0.16-0.46 g troxerutin and 13 g% rutin. The higher rutin retention was due to its higher hydrophobicity [8, 9, 10].

Synthesized biomaterials were characterized by FTIR reflexion spectroscopy, elemental analysis, optical and scanning electron microscopy (SEM), as previously reported [1, 11, 12].

Biomaterials II were obtained by covering the surface of the textile material with microparticles that can retain the drug through electrostatic interactions or physical adsorption. The microparticles were synthesized from different polysaccharides: i) Chitosan microparticles containing troxerutin or heparin. Drug loading was realized either after microparticle synthesis (CH-a and CH-b), or by synthesis of microparticles from the pre-formed mixture of chitosan-drug. CH-a and CH-b microparticles contained 16-20g% heparin and 33g% troxerutin; CH-b1 retained 50g% heparin and 15g% troxerutin; ii) Carboxymethyl dextran (CMD) microparticles, which bound about 22g% troxerutin by chemical attachment; iii) Carboxymethylated pullulan/sulfo-propionate in association with cyclodextrin microparticles retained an unsignificant amount of troxerutin. The microparticles were characterized by elemental analysis, water retention capacity and optical microscopy. Their fixation on the textile material surface was realized with a binder (polyethyleneglycol – PEG MW 4000, epoxysiloxane), followed by drying at room temperature. The fixation yield was almost 100% and optical microscopy indicated PEG as the best binder.
Textile material

"Gh. Asachi" Technical University Iasi (TU) was involved in producing the thread with elastic and grafting capacity, the knitted structure with compression capacities as well as the final product grafting (contention stockings) with the products provided by "Petru Poni" Institute for Macromolecular Chemistry Iasi (IMC). The in vitro and in vivo preliminary researches were conducted at the „Gr.T.Popă” University of Medicine and Pharmacy Iasi (UMPh).

The textile product was assessed functionally, aesthetically and chemically, from the point of view of the polymers and drugs integrity.

Results and discussion

In vitro release of drugs from biopolymers and biomaterials

In vitro release tests of troxerutin from the biopolymers or biomaterials were performed by IMC in solution simulating legs sweat content (0.73 meq NaHCO₃/l, 2.41 meq KCl/l, 30.60 meq NaCl/l, pH 5.35).

Biomaterials I released troxerutin faster than their precursor biopolymers, with a burst effect in the first 1-2 hours, followed by a slow release, with almost zero release rate.

CH-a and CH-b released troxerutin very fast (90% in 20 minutes), while CH-b₁ released about 40 – 50% troxerutin within the first 60 minutes, but after that a slower and more constant release was observed (about 0.5%/hour).

The release of troxerutin chemically linked on the carboxymethyl dextran microparticles takes place much slower than the release of the drug physically included into the microparticles. Thus, approximately 10 days later, only 10-12% of the troxerutin quantity is released. This release is conditioned by breaking the esteric connections between the polymer network and troxerutin. The relatively slow release of troxerutin may be an advantage in obtaining a system with a prolonged drug release [8].

In vivo tests of biomaterials on laboratory animals

In order to get information on biomaterials capacity of acting as controlled release systems on the skin, several biomaterials were prepared, containing either troxerutin or fluorescently marked heparin, in order to be tested on lab animals and establish which biomaterial is the most efficient, in terms of the release speed and the drug quantity arriving at the level of the venous endothelium for a longer period of time (sustained continuous release) [8, 9, 10].

For preparation parts a compression stocking were used, with a surface of approximately 28 cm². The features of the patterns sent for testing are presented in table 2. All materials were treated on one face. The drug release from the biomaterial and its presence in the skin and the legs venous network was tested on laboratory animals: rats/rabbits/guinea pigs. The textile material was placed on their back, after being shaved. The experiments were focused on the release of the active pharmacological principle from the fabric impregnated with biopolymers. Biopsies were taken from the treated skin, fixed with paraformaldehyde, cut at the cryostat and then examined by means of epifluorescence microscopy and confocal laser microscopy. The presence of the fluorescent drug molecules were examined in different skin layers, in dynamics, at 3 and 6 hours. The animal tests were approved by the Ethics Committee of “Gr.T.Popă” University of Medicine and Pharmacy Iasi, and the laboratory animals were used because there were no other research alternatives. There were chosen methods involving a small number of animals and a treatment minimally brutal, the protection of those involved in the experiment being also provided. These activities were performed by UMPh (animal tests) and IMC which provided the biomaterials. Two sets of biomaterials with
characteristics included in Table 1 and 2 were tested, in order to choose the biomaterial with the most adequate features to the CVD treatment [1, 8].

Table 1. The characteristics of biomaterials (set I) synthesized by IMC and tested at UMPh

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Biomaterial I with physically embedded rutin (PA-CyD)</td>
</tr>
<tr>
<td>B1</td>
<td>Biomaterial I, with chemically bound trexorutin (PA-T)</td>
</tr>
<tr>
<td>C1</td>
<td>Biomaterial II (1) with fluorescently marked heparin (CH-a)</td>
</tr>
<tr>
<td>C2</td>
<td>Biomaterial II (2) with fluorescently marked heparin (CH-b)</td>
</tr>
<tr>
<td>C3</td>
<td>Biomaterial II (3) with fluorescently marked heparin (CH-b₁)</td>
</tr>
</tbody>
</table>

Table 2. The characteristics of biomaterials (set II) synthesized by IMC and tested at UMPh

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Type biomaterial</th>
<th>Biopolymer</th>
<th>Binding mode</th>
<th>Contents trexorutin, g%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>I</td>
<td>PA-CyD</td>
<td>physical</td>
<td>0.46</td>
</tr>
<tr>
<td>T2</td>
<td>I</td>
<td>PA-T</td>
<td>chemical</td>
<td>25.0</td>
</tr>
<tr>
<td>T3</td>
<td>II</td>
<td>CH-a</td>
<td>physical</td>
<td>33.0</td>
</tr>
<tr>
<td>T4</td>
<td>II</td>
<td>CH-b</td>
<td>physical</td>
<td>15.4</td>
</tr>
<tr>
<td>T5</td>
<td>II</td>
<td>CH-b₁</td>
<td>physical</td>
<td>9.8</td>
</tr>
<tr>
<td>T6</td>
<td>II</td>
<td>CMD</td>
<td>chemical</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Clinical studies with functionalized compression stockings

For the synthesis of high amounts of biopolymer used for the treatment of compression stockings, acryloyl trexorutin was synthesized, in 5 turns of 20g of trexorutin initially in the reaction medium, according to the method described for AT-2 (soluble acryloyl trexorutin in a mixture water/ethanol 1/2 and containing several groups of acryloyl). The average efficiency per turn was of 80%. The product was used by the Technical University for the impregnation of 5 contention stockings, according to the immersion procedure elaborated by IMC [1, 8].

The obtained stockings were used for the clinical studies on patients (UMPh). These studies were approved by the Ethics Committee of “Gr.T.Popa” University of Medicine and Pharmacy Iasi, being followed all individual rights and being provided the protection against side effects. The tested patients had been correctly informed on the nature of the experiment and gave their consent fully conscientious about their participation in the research activity [1].

For this research, 42 patients in different stages of CVD had been selected, except for the VI CEAP class (active chronic leg ulcers), because of the difficulty in performing the venous doppler ultrasound in this stage and of elastic contention [1].

Before treatment, the patients were clinically examined from the dermatological point of view (establishing the CVD stage) and the general status (finding associated diseases or diseases which may turn it into more serious the CVD – cardiologic and ophthalmologic examination).

In order to assess the patients from the cardio-vascular point of view, the complete clinical examination was performed (heart and peripheral arteries), as well as the ECG examination, in order to exclude the eventual association of the heart ethiology edema, drug origine edema (e.g. calcium channel blockers) or peripheral artery diseases. The aim of the ophthalmological examination was double: on one hand it was assessed the patient’s peripheral vascular status, which facilitated the general clinical evaluation, and on the other hand it was obvious its evolution during the clinical evaluation, as well as the occurance of the eventual retine side effects to the studied medication. Besides the clinical examination of the back segment,
secondary parameters were also followed: previous segment aspect (lens, cornea), visual acuity (VA), intraocular pressure (IOP) and chromatic feeling. The data obtained represent arguments for the lack of ocular problems during the treatment. Moreover, no obvious disturbances were noticed at the level of the retina’s structure or the back segment components and no problems were found from the point of view of VA, IOP or chromatic feeling [1].

There were added some paraclinical data regarding the functionality and localization of the profound and superficial leg venous system disturbances (biological investigations and leg venous doppler ultrasound). The CVD evolution under the well known treatments have been monitored and recorded for six months. Then, for other six months, the patients undertook a treatment with the new elastic compression stocking releasing in a controlled way the pharmacologically active active substance, used in CVD treatment. At scheduled intervals of time there have been appreciated the tolerance and toxicity of the new treatment. The most efficient drug was recorded or association of drugs embedded in polymers and released in a controlled manner from the fabric. Manometry was used to appreciate the fabric quality and its capacity to perform optimal elastic contention. The clinical and paraclinical results were evaluated and the statistical analysis was performed [1].

Conclusions

The synthesis of type I and II biomaterials and their in vitro and in vivo behavior demonstrated the following aspects: i) type I and II biomaterials preparation included setting the synthesis conditions of the biopolymer and their precursors (3 monomers), as well as establishing the most adequate procedure for polyamide fabric impregnation. In vitro release experiments showed that both biomaterials types (I and II) with chemically bound troxerutin might ensure a sustained drug release; ii) in vivo experiments on animals with biomaterials I and II with physically or chemically linked troxerutin demonstrated that the biomaterial I with chemically linked troxerutin released the drug for a longer period of time (about 10 days) and with a higher concentration than the one obtained for the physically linked troxerutin or for troxerutin administered as ointment; iii) biomaterial I with chemically linked troxerutin was the most adequate for obtaining medical stockings, which cumulate the elastic contention effects with the ones of controlled and prolonged transfer of troxerutin. The results of the clinical testing confirmed the viability of this material to be used on local treatment for CVD.

The purpose of this research was gathering in one treatment two of the most modern therapies for CVD: elastic contention and topic treatment (with phlebotonic drugs, capilarotrophic drugs, heparinoids, anti-inflammatory drugs etc.).

Using the controlled and sustained release of the pharmacologically active principles, their efficiency will be optimized and a substantial drug economy will be made, which will be available where and when it is needed.

The increase of patient’s compliance with the new therapy will be based on the increased efficiency of the treatment which is easy to be administered. The performance of this treatment, without any risks, in prophylactic scope, to the patients with high risks in developing CVD, will be an efficient method of preventing the disease.
References