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Chemical modification of implant vascular biomaterial. Part II. Antibacterial properties of gentamicin and ciprofloxacin modified vascular prosthesis

Modyfikacja chemiczna wszczepiennych biomateriałów naczyniowych
Część II. Właściwości przeciwbakteryjne protez modyfikowanych gentamycyną i cyprofloksacyną

Infection is the most common cause of biomaterial implant failure in modern medicine. Vascular grafts infections are still infrequent (4% incidence), but there are significant complications in vascular surgery. They are associated with a high rate of limb loss (up to 78%) and mortality (up to 75%) and may be caused by preoperative contamination, postoperative wound infection or systemic bacteriemia [2, 3]. Mainly *Staphylococcus aureus* (~70% cases), but also *Staphylococcus epidermidis* and *Escherichia coli* are responsible for the majority of implants infections [1, 3]. Reduction of the graft infection rate may by achieved by impregnation of the commercial protein-sealed prostheses with antibiotics solutions [1, 7, 8]. Our previous paper (*Chemical modification of implant vascular biomaterial. Part I.*) concerned aminoglicosides and fluoroquinolons immobilization on gelatin-sealed vascular prostheses (made of polyethyleneterephtalate-PET-fibers). A new types of prostheses (carrier-ciprofloxacin and carrier-gentamicin-ciprofloxacin) were created.

The aim of our research was a method of chemical modification antibiotic immobilization on gelatin-sealed vascular prostheses and – as an effect – obtaining an effective and prolonged antibacterial protection of vascular grafts.

MATERIALS AND METODS

BIOMATERIALS

Gentamicin and ciprofloksacin vascular grafts modified due to the procedure described in *Chemical modification of implant vascular biomaterial. Part I* were: Method I (carrier-Gentamicin), Method II (carrier-Ciprofloxacin), Method III (carrier-Gentamicin-Ciprofloxacin).These studies were conducted on Gentamicin sulphate (Polfa-Tarchomin S.A. Poland 40 mg/ml) and Ciprofloxacin hydrochloride (Polpharma, Starogard Gdański, Poland). These antibiotics were immobilized on gelatin-sealed prostheses Uni-Graft® (Braun, Germany) made of polyethyleneterephtalate (PET).

BACTERIAL STRAINS AND MEDIA FOR BACTERIAL GROWTH

We tested the following bacterial strains: *Staphylococcus aureus* ATCC 25923 (methicillin-sensitive), *Staphylococcus epidermidis* ATCC 12228 and *Escherichia coli* ATCC 25992. The media used in the microbiological experiments were: for liquid tests Mueller-Hinton broth (M-H broth), (Oxoid, England) and for plate tests Mueller-Hinton agar (M-H agar), (Oxoid, England).

ANTIBACTERIAL TESTS OF ANTIBIOTIC-MODIFIED PROSTHESIS

Plate antibacterial tests. We assessed *in vitro* the antibacterial activity of a mixed type immobilized antibiotic–modified Uni-Graft® and a control (non modified) Uni-Graft®. These pieces of prostheses were placed in a Petri dish with M-H agar medium (previously inoculated with 0.5°MacFarland of tested bacteria strain). After 18 h of incubation at 37°C zones of inhibited bacteria growth were observed.

Liquid antibacterial tests. Pieces (50 mg) of antibiotic-modified prosthesis and a control (not modified) prosthesis were placed in tubes with 5 ml of M-H broth inoculated with 16 µl of 0.5°MacFarland bacterial suspension. The experiment was performed until the day when bacterial growth appeared in the medium. The presence of living bacteria in the medium was controlled as a CFU/ml (Colony Forming Units/ml) changes and confirmed by application on M-H agar medium and incubated at 37°C for 18 h.

Biofilm test. The presence of bacterial biofilm on biomaterial surface was tested by incubation with 0.001% of 2.3.5- triphenyl tetrazolium chloride (TTC, Sigma USA), in M-H broth for 24 h at 37°C (6). The appearence of a pink (+), red (++) or purple (++++) formazane on prosthesis surface proved a living bacteria presence.

RESULTS

The optimal gentamicin and ciprofloxacin immobilization conditions were fixed (*Chemical modification of implant vascular biomaterial. Part I*). In a further research, the biological effect of bounded antibiotics was tested against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25992 bacterial strains.

It was shown (Table 1) that gentamicin and ciprofloxacin immobilization process did not change their biological properties because the immobilized antibiotics could inhibit bacterial growth. Therefore, pieces of antibiotic–modified prostheses which were placed with bacterial suspension were incubated and the presence of living bacteria in the medium was controlled. Table 1 and figure 1 show that each kind of modified biomaterial (Method I (carrier-Gentamicin), Method II (carrier-Ciprofloxacin), Method III (carrier-Gentamicin-Ciprofloxacin)) inhibited the bacterial growth for at least 5 days (for Method II: ciprofloxacin impregnated prosthesis). The hybride biomaterial bounded covalently with gentamicin and ionically with ciprofloxacin displayed the longest (34 days) antibacterial activity (against *E. coli*), which is clearly shown (Table 1).

In a further research, the modified prostheses after medium infection were analyzed as a bacterial biofilm formation. Data in table 2 indicate that covalent immobilization of gentamicin enables the antibacterial protection of biomaterial from bacterial adhesion. Namely, TTC-reduction test confirmed the absence of living bacteria cells on a covalently-modified biomaterial surface (-). However, ciprofloxacin ionic prosthesis impregnation does not allow full antibacterial control, single living bacteria cells on biomaterial surface are present (+, ++). The biggest bacterial adhesion was obtained for the control (not modified) vascular graft.

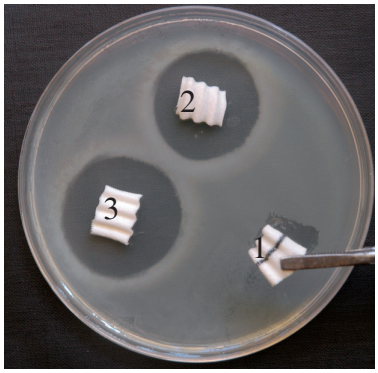

Fig. 1. Zones of bacterial growth inhibition as a confirmation of antibacterial effect of the modified biomaterial: 1) carrier-Gentamicin, 2) carrier-Ciprofloxacin, 3) carrier-Gentamicin-Ciprofloxacin

Table 1. Microbiological effects of gentamicin and ciprofloxacin modified prostheses (the average data obtained from 21 repetitions)

Carrier / Bacterial strain	Carrier-ciprofloxacin		Carrier-gentamicin		Carrier-gentamicin-ciprofloxacin	
	zone of bacterial growth inhibition (mm)	medium infection day ($1{,}5x10^8$ CFU/ml)	zone of bacterial growth inhibition (mm)	medium infection day ($1{,}5x10^8$ CFU/ml)	zone of bacterial growth inhibition (mm)	medium infection day ($1{,}5x10^8$ CFU/ml)
S. epidermidis	24	5	0.2	14	25	29
S. aureus	26	6	0.2	15	27	26
E. coli	34	11	0.2	23	35	34

Table 2. Biofilm formation (TTC-method) as a test of the antibacterial properties of gentamicin and ciprofloxacin modified prostheses (the average data obtained from 9 repetitions)

carrier	*E. coli* adhesion	*S. epidermidis* adhesion	*S. aureus* adhesion
1* Carrier-gentamicin	-	-	-
2* Carrier-ciprofloxacin	+	++	++
3* Carrier-gentamicin-ciprofloxacin	-	-	-
4* Control (not modified)	++++	++++	++++

1* Carrier-gentamicin after 23 days in bacterial medium incubation, 2* Carrier-ciprofloxacin after 11 days in bacterial medium incubation, 3* Carrier-gentamicin-ciprofloxacin after 34 days in bacterial medium incubation, 4* Control (not modified) prosthesis after 24 days in bacterial medium incubation

DISCUSSION

The use of a patient's own tissue material is an optimal solution for the minimalization of a graft rejection risk. The patient's age, general health and vascular system condition must be taken into consideration. These limitations have affected the expansion of biomaterials used in vascular surgery [1, 7].

Vascular prostheses made of polytetrafluoroethylene (PTFE), polymetylomethacrylate (PMMA) and polyethylene terephthalate (PET) sealed with gelatin or collagen are currently in common use in angioplasty. However, after the implantation these grafts may undergo bacterial infections. Mainly

Staphylococcus aureus, but also *Staphylococcus epidermidis* and *Escherichia coli* are responsible for the majority of implant infections [1, 4]. To lower the risk of these postoperative complications, a modification of vascular grafts with antibiotics or other aseptic compounds was proposed. The simple method includes soaking the prosthesis before its implantation in antibiotic solution. In this case, however, the antibacterial agent binds to a graft via weak interactions (adsorption or ionic bonds). Therefore, simple soaking of prostheses in antibiotic solutions gives only a few days' remedial effect [7, 8]. An analogical situation was achieved in our research by prosthesis impregnation in ciprofloxacin (Metod I). After on average 1 week such modified biomaterial was infected. The longest (34 days) antibacterial activity (against *E. coli*) was shown the hybride biomaterial bounded covalently with gentamicin as well as ionically with ciprofloxacin. One month is a sufficient protection time. The patients immunological system and their fibroblasts after 30 days after implantation are active and are able to protect the organism.

CONCLUSIONS

It is possible to create a vascular prosthesis covalently bounded with gentamicin as well as ionically with ciprofloxacin. Such modified prostheses show prolongated antibacterial properties and they do not limit biofilm formation in comparision with control, not modified prostheses.

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SUMMARY

A significant complication in vascular surgery is biomaterial infection. The aim of our study was to obtain prolonged antibacterial protection of vascular grafts, employing covalent immobilization of gentamicin and physical adhesion of ciprofloxacin to gelatin-sealed prostheses. The longest (34 days)

antibacterial activity (against *E. coli*) was shown by the hybride biomaterial bounded covalently with gentamicin as well as ionically with ciprofloxacin. In addition, the covalent immobilization of gentamicin enables an antibacterial protection of biomaterial from bacterial adhesion.

STRESZCZENIE

Istotnym problemem dla chirurgii naczyniowej są infekcje biomateriałów. Celem pracy było uzyskanie przedłużonej w czasie ochrony przeciwbakteryjnej przez kowalencyjne unieruchomienie gentamycyny i jonowe związanie cyprofloksacyny na żelatynowanych protezach naczyniowych. Najdłuższą, bo aż 34-dniową, ochronę przeciwbakteryjną w stosunku do *E. coli* wykazywał hybrydowy biomateriał związany kowalencyjnie z gentamycyną i jednocześnie jonowo z cyprofloksacyną. Dodatkowo kowalencyjna immobilizacja gentamycyny pozwala na przeciwbakteryjną ochronę biomateriału przed adhezją bakterii.