Original paper

Impact on porcine heart valve tissue treating with carbodiimide

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Summary

Objectives: There are many treatment protocols used these days, but seems like no one ensures a hundred percent effectiveness for lifelong prosthesis function. The aim of the study was to investigate whether some new method could mitigate calcification better than usually used and to compare impact on different valve tissues.

Methods: Porcine aortic and mitral valve cusps were treated with glutaraldehyde and carbodiimide. Control group without any treatment was also used. Cusp pieces were implanted subcutaneously into 90 rats for 60 days. Tissue calcification was assessed by atomic absorption spectrophotometry.

Results: The most calcification was found when not used any treatment, and the lowest calcium level was in group when treated with carbodiimide. Glutaraldehyde in low concentration lowered significantly residual calcium level, but not so effectively as carbodiimide. All data differ statistically significantly ($p < 0.05$).

Conclusion: Calcification was minimal treating porcine valve tissue with carbodiimide comparing to glutaraldehyde or no treatment.

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Introduction

Usage of bioprosthesis becomes more and more ordinary, but valve tissue treatment did not change much. Due to degradation and calcification there are limited groups of patients to whom these implants are safe by means of longevity, since many studies show 15 to 20 years in best cases free from valve related problems [1]. So usually bioprosthesis implantation is indicated in patients older than 70 years. Standart tissue treatment is with glutaraldehyde and some additives [2]. There were also investigations with quite new substances such as carbodiimide for tissue treatment with good results [3].

It remains unclear how this agent affects different tissues: aortic valve and mitral valve. So in our study we compared standard treatment with glutaraldehyde with carbodiimide on different valve tissues in rat experimental model.

Materials and methods

For our study we used 200 g Wistar rats ($N = 90$). We implanted 60 samples of untreated porcine aortic and mitral valve tissues in 30 rats and 60 more samples treated with glutaraldehyde or carbodiimide, and untreated in next 60 rats. Thus in total we had 120 control group samples and 60 samples from each treatment group. All anaesthetic and surgical procedures conform with the principles outlined in the Declaration of Helsinki and approved by the local ethics committee, and complied with the Principles of Laboratory Care and the Guidelines for the Care and Use of Laboratory Animals.

Data were expressed as mean ± SEM. Statistical analysis was performed using the Tukey–Kramer HSD test with SPSS software version 9. A significance level $p < 0.05$ (two-tailed) or less was accepted as statistically significant.

Tissue fixation

Porcine aortic roots were harvested at local slaughterhouse. Conduits were kept in cold saline

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Table 1.
Residual calcium amount in valve leaflets, 60 days after implantation

	Control group ($N = 120$)	Glutaraldehyde treatment ($N = 60$)	Carbodiimide treatment ($N = 60$)
Aortic valve leaflet	92.2 ± 6.1	27.3 ± 3.1	0.6 ± 0.1
Mitral valve leaflet	26.8 ± 2.1	16.2 ± 1.4	2.5 ± 1.1

All data differs statistically significant, $p < 0.05$.

(4°C). Further treatment was done in two protocols and control group without any treatment. First one had treatment with glutaraldehyde in low concentration (0.1%). The second one included basic treatment with carbodiimide 0.3 M with N-hydroxysuccin-imide 0.1 M for 24 hours adding poly(propylene glycol)bis 2-(aminopropyl) ether (Jeffamine) 0.1 M for 3 hours under shaking conditions, later on treating with L-alanine 100 mM in PBS; pH 7.6; 37°C for 24 hours.

After treatment completion all tissue samples were washed in sterile saline and kept in PBS in 4°C until implantation, which was performed within 24 hours.

Rat implants

Anaesthesia was performed with ketamine hydrochloride. Using sterile technique, incisions were made in the skin bilateral to the spine, and subcutaneous pockets were made. Approximately one square centimeter samples from treated or untreated porcine aortic and mitral valves were cut and implanted. Each rat received three aortic and three mitral valve samples and there were more samples implanted as control group from both tissues as described before in additional rats. The incisions were sutured. 60 days after implantation, animals were sacrificed and the implants retrieved for residual calcium analysis by atomic absorption spectrophotometry.

Calcium analysis

Hitachi 175-50 (Japan) flame atomic absorption spectrometer equipped with hollow cathode lamps was used for the analysis. The instrumental parameters were adjusted according to the manufacturer recommendations. A calcium hallow cathode lamp operating at 240.7 nm was used as the radiation source. The lamp current was set at 10 mA. The flame composition was acetylene (gas pressure 2.94×10^4 Pa) and air (gas pressure 1.28×10^4 Pa).

For the fragmentation of analyzed samples the method of dry mineralization has been selected (temperature of burning was 800–850°C). The residue was dissolved in diluted HCl (1:1). Calcium levels were expressed as mg/g dry mass of tissue.

Results

Experimental data show good impact on valve tissue when treated with carbodiimide. Residual calcium level in aortic valve leaflet was 0.6 mg/g and in mitral valve leaflet 2.5 mg/g.

Treating with low concentration glutaraldehyde we have got significant calcification, 27.3 mg/g in aortic valve leaflet and 16.2 mg/g in mitral. To estimate the total calcification level we used control group without any treatment. In this case residual calcium amount in aortic valve cusps was 92.2 mg/g and 26.8 mg/g in mitral. All data differ statistically significantly when comparing in each group separately or between groups. All results are shown in Table 1.

Discussion

As the results show, the usage of chemical treatment before implantation is very important for tissues. In experimental model tissues without treatment due to ghost reaction become calcified. The level of calcification depends on which treatment agent is used. Glutaraldehyde without any doubt mitigates calcification, it is proved and used in bioprosthesis production, but important factor for tissue quality is concentration. Higher concentrations impair tissue properties, but also more efficiently prevent calcification. Thus trying to prevent tissue damage we used low concentration glutaraldehyde. Comparing to animal study reported by Connolly et al. [4] residual calcium level using single glutaraldehyde in subdermal implants was significantly higher in our series. The main difference between ours and Connolly's protocols is that we used even less concentration of glutaraldehyde – 0.1% vs 0.6%. These results confirm importance of concentration, the higher it is the less calcification is expressed.

Carbodiimide is another agent used in our study. According to theory this substance crosslinks collagen without zero length cross

links, thus collagen is stabilized, but at the same time stays more elastic comparing to glutaraldehyde treatment. Adding any more substances could give better results, but we were interested just in single carbodiimide action. Again, comparing animal studies results with Zilla et al. [5] results are quite similar, calcification level is low comparing to control or glutaraldehyde groups.

Interesting part of this study was mitral valve leaflet treatment with those agents, since there are no reports on this topic. Bioprostheses are produced from aortic valves – trileaflet, implantation is very similar to aortic. There were studies that show calcification of these aortic bioprosthesis cusps calcification after implantation in mitral valve position [6]. But again in this study aortic trileaflet valve was used. In our research we used porcine mitral valve tissue and got promising results. As usually, non-treated samples had the most calcium deposits. Glutaraldehyde had significant impact on calcification prevention as residual calcium level lowered twice, but comparing to treatment with glutaraldehyde of aortic cusps – calcification lowering was more than three times. So this fact also points that due to mitral valve anatomical-histological structure, cusps contain more fibrous, elastic and collagen based material suitable for calcium deposits formation. This is confirmed when using carbodiimide, calcification level lowered almost ten times in mitral valve tissues comparing to untreated samples whereas in aortic cusps more than one hundred times. According to obtained data we could think that mitral valve leaflet tissue is not the best material for bioprosthesis production.

Conclusion

Treatment of aortic valve leaflets with carbodiimide significantly reduces residual calcium level comparing to glutaraldehyde. Mitral valve leaflet treatment with carbodiimide also lowered calcium deposits, but not so substantially as in aortic cusps. Treatment agents, their concentrations and tissue structure play important role in calcification prevention.

Statement of conflict of interest

The authors state no conflict of interest.

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