WAYS OF PREVENTING CALCIFICATION OF BIOLOGICAL HEART VALVE PROSTHESES

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Porcine aortic xenobioprostheses preserved with glutaraldehyde are widely used at the present time in surgery of heart valve defects. However, besides their positive properties, whereby they are superior to mechanical prostheses, surgeons have found various disadvantages, of which the chief is calcification of the biological tissue of the prosthesis. The frequency of development of this complication is particularly high in children and young persons, and this fact is limiting the use of biological prostheses in patients of this category [6, 9, 12].

Factors of the recipient and factors of the graft both participate in calcification [13]. Factors of the graft include structural and biochemical transformations which the value tissue undergoes during conservation; factors of the recipient include the age, sex, and individual features of his calcium metabolism.

The aims of the present investigation were to study the possibility of acting upon both groups of factors by optimizing methods of conservation of the biological valve and the use of the drug xydiphon, which belongs to the diphosphonate group.

The diphosphonates, which are analogs of natural polyphosphates, prevent the development of soft tissue calcification by blocking the transformation of amorphous calcium phosphates into hydroxyapatite and inhibiting aggregation of hydroxyapatite crystals into large clusters [1, 3].

EXPERIMENTAL METHOD

Cusps of porcine aortic valves, treated by three different methods, were used in the experiment: 1) by standard conservation with 0.625% glutaraldehyde solution, 2) by the same method followed by treatment with papain, and 3) by a combination of the 2nd method and subsequent immobilization of heparin on the tissue of the prosthesis.

The experimental part of the work was done on 30 noninbred female rats weighing 110-130 g.

Technique of Implantation

Under ether anesthesia and aseptic conditions, six subcutaneous pockets were formed from three incisions carried along the spine (three pockets on each side of the spine). Into each pocket was placed a disk 0.6 cm in diameter, cut from the cusp of a xenogeneic valve, conserved by one of the methods indicated above, and washed in sterile 0.9% sodium chloride solution. In this way two samples of tissue, conserved by the same method, were implanted into each animal.

The animals were divided into two groups: the rats of group 1 were subjected to no procedures/ the rats of group 2 received xydiphon (the sodium salt of hydroxyethylidenediphosphonic acid) perorally in a dose of 10 mg/kg daily for 21 days , in the form of a 1% aqueous solution. After 21 days the implanted cusps were removed from the subcutaneous pockets of the animals; the material was kept in 0.625% glutaraldehyde solution.

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TABLE 1. Effect of Method of Conservation and of Xydiphon on Calcium and Phosphorus Concentrations (in μg/kg) in Tissues of Xenogeneic Valves Implanted Subcutaneously in Rats

Characteristics of material	Method of conservation								
	1-st			2-nd			3-rd		
	Ca	P	Ca/P	Ca	P	Ca/P	Ca	P	Ca/P
Implanted valves									
1st Group	18,58±2,40	7,43±0,93	1,93	17,74±3,01	7,54±1,34	1,82	4,05±0,51	3,27±0,42	0,95
2nd Group	3,56±0,43	5,81±0,32	0,47	4,24±0,41	6,48±0,34	0,50	1,83±0,41	3,37±0,27	0,42
Unimplanted	1,89±0,19	1,08±0,10	1,35	1,86±0,20	1,07±0,11	1,35	1,86±0,18	1,07±0,09	1,35

Each disk was washed with distilled water and dried for 6-8 h at 100°C. Each specimen was weighed and hydrolyzed in 2 ml of 0.1 N hydrochloric acid. Calcium and phosphorus were determined quantitatively by the method of Leont'ev et al. [4].

Altogether 98 tissue specimens were studied.

EXPERIMENTAL RESULTS

It follows from the data given in Table 1 that cusps treated by the 1st and 2nd methods, in animals of group 1, were virtually identical as regards their calcium and phosphorus content. The Ca/P ratio in these specimens of xenogeneic tissue was 1.82-1.93. Hence it could be concluded that calcium and phosphorus are deposited here in the form of hydroxyapatite — a compound with a perfect crystalline structure.

Enzyme treatment thus did not reduce the ability of the tissue of the cusps to undergo calcification. The reason is evidently that the nucleator of calcium phosphate is collagen itself [5], especially when treated with oxidizing agents and glutaraldehyde [7, 8, 10].

In native cusps regions with a natural tendency toward calcification are known to be protected by glycosaminoglycans [14] or proteoglycans [7], bound with collagen and capable of blocking nucleation. Glutaraldehyde destroys these protected structures. Additional treatment with enzyme probably intensifies this process. However, such treatment greatly improves the biomechanical properties of the valve [2].

To remove the disadvantages of this combined treatment and, at the same time, to enhance the resistance of the valve tissues to thrombosis, heparin was immobilized on the valve cusps, thereby forming heparinized cusps. It was postulated that heparin, as a glycosaminoglycan, "attached" to collagen, thereby becomes an artificial structure which, like the natural structure, prevents nucleation of calcium phosphates.

The results of biochemical investigation of the heparinized cusps confirmed the theoretical assumptions: it was found that the calcium concentration in these cusps was reduced by 4.5 times compared with cusps conserved by glutaraldehyde by the standard method (Table 1). The Ca/P ratio was 0.95 and, consequently, the compound in whose composition the calcium was present was not hydroxyapatite.

However, the calcium concentration in these cusps was nevertheless twice as high as in unimplanted cusps. Consequently, this method of conservation cannot completely prevent calcification processes.

In cusps conserved by the standard method and with additional treatment with xydiphon the calcium concentration was reduced by 4.5 times compared with levels obtained in group 1 (Table 1). The Ca/P ratio was 0.47-0.50, so that the presence of hydroxyapatite in the tissues could be ruled out.

The use of heparinization of the cusps in this group enabled the combined effect of the procedures on implant factors and on recipient factors to be obtained. With respect to the calcium concentration these cusps were virtually indistinguishable from unimplanted cusps, and the Ca/P ratio was 0.42 — the least of all the batches of specimens studied.

It can thus be concluded from the results of these experiments that a combination of immobilization of heparin on the valve tissue with the use of diphosphonates enables the development of calcification in the biological tissue of the prosthesis to be inhibited by the greatest possible degree. The proposed method can be regarded as promising for the prevention of this complication when biological material is used in cardiovascular surgery.

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ULTRASTRUCTURAL CHANGES IN CNS AXONS IN EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS

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Amyotrophic leukospongiosis (AL) is a distinctive progressive spinal amyotrophy that is inevitably fatal, which belongs to the group of spongiform encephalopathies, caused by a non-classical virus [2]. In guinea pigs in which the infection is produced, disappearance of neurons (mainly spinal motoneurons), proliferation of astrocytes, and death of axons without any evidence of their demyelination are observed. Disturbances of the blood-brain barrier and penetration of immunocompetent blood cells into the CNS have not been observed [2, 3].

In the investigation described below, conducted on guinea pigs with experimental AL, the white matter of the spinal cord was studied in order to establish the specificity of degeneration of the central axons; other features studied included the response of the glial component and functioning of oligodendrocytes, astrocytes, and microglia as phagocytes, i.e., their role in the distinctive form of immunologic surveillance in a barrier organ such as the CNS.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs weighing 250-300 g, into which the liquid phase of a 10% suspension from the brain of patient D., dying from AL, was injected. The method of preparing the homogenates and of infecting the animals was described previously [4]. Three groups of animals were used in the experiments. In group 1 the nine guinea pigs were infected by the retro-orbital route in order to preserve the integrity of the blood-brain barrier. With this method of infection, clinical manifestations of the disease such as loss of hair, muscle atrophy, the development of pareses and paralyses of the limbs and trunk, oc-

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