4. K. A. Dzhivanyan, Biol. Zh. Arm., No. 2, 128 (1984).
5. N. L. R. Bucher and M. N. Swaffield, Proc. Natl. Acad. Sci. USA, 72, 1157 (1975).
6. Choi Sook Chong and J. C. Hall, Cancer Res., 34, 2351 (1974).
7. J. B. Price, Takeshige Kotohito, M. H. Max, et al., Surgery, 72, 72 (1972).
8. R. V. Shah, B. Pilo, M. V. Asnani, et al., J. Ahim. Morphol. Physiol., 21, 132 (1974).
9. K. Takeshige, H. Suzuki, Y. Fukaya, et al., World. J. Surg., 5, 441 (1981).
10. L. M. Molina Trigueras, J. A. de Diego Carmona, J. F. del Canzo Lopez, et al., Cir. Esp., 35, 69 (1981).

EFFECT OF GLUTARALDEHYDE PRESERVATION ON IMMUNOGENIC, PHYSICOMECHANICAL, AND FUNCTIONAL PARAMETERS OF ARTIFICIAL AORTIC HEART VALVES

L. S. Barbarash

UDC 615.361.132.03:616.126.3-089.28./014.41

KEY WORDS: heart valves; prostheses; preservation; immunity; strength; hydraulics.

The use of artificial heart valves, treated with glutaraldehyde (GA), in heart surgery has been followed by publication of a number of investigations in which different concentrations of this preservative, in the composition and pH of the buffer, and in the technology and conditions of preservation, have been evaluated [3, 6, 8, 10]. However, the extremely important comparative data, whereby the optimal values of these parameters can be established, are virtually not to be found.

The aim of this investigation was to study the rate of absorption and the quantity of GA absorbed by the tissue, and also to study changes in the immunogenic properties and physical mechanical and functional parameters of artificial heart valves preserved with GA solutions of various concentrations.

EXPERIMENTAL METHOD

The effect of stabilized 0.25, 0.625, and 1% solutions of GA (from Serva, West Germany), made up in phosphate buffer at pH 7.4, on the tissue of valve prostheses made from pig aorta was investigated. The rate of absorption of GA by the artificial valve tissue was estimated by determining its concentration by iodometric titration by the method in [5]; 10 experiments were carried out with GA solutions of the same concentration.

The immunogenic properties of native and preserved tissues were studied in active anaphylaxis experiments conducted on 113 guinea pigs, and by radial immunodiffusion in agar gel with antisera obtained by immunization of four rabbits. The methods of these investigations were described previously [1]. To determine the protein concentration by Lowry's method extracts of six samples of tissue preserved in GA solutions of different concentrations were used.

As criteria of the effect of GA on the physicomechanical parameters of the prosthesis, the tensile strength and the reserve of deformability of the valve cusps, measured on an "Instrom-1122" tensile testing machine, were used. Each investigation was conducted on 43-74 tissue samples. Parameters of function of the preserved valves were assessed during work in vitro on a bench with constant flow of fluid, by the method described by the writer previously [2]. Eight valves were used in each group of tests.

No. 1 Department of Surgical Diseases, Kemerovo Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 747-749, June, 1985. Original article submitted June 12, 1984.

0007-4888/85/9906-0813 $09.50

TABLE 1. Antigenic Activity of Tissue Extracts of Native and Preserved Heterologous Aortic Valve Prostheses

Type of sensitizing antigen	Concentration of GA solution	Severity of shock after reacting injection of antigen				
		AGN	AGP			
			0,25 % GA	0,625 % GA	1 % GA	0,625 % GA + formaldehyde
AGN	—	4,2/30	0,2/6	0,2/5	0,5/6	0/5
AGP	0,25%	0,8*/9	0/6	—	—	—
AGP	0,625%	0,8*/10	—	0/6	—	—
AGP	1%	1,7*/11	—	—	0/6	—
AGP	0,625% + formaldehyde	0,4*/9	—	—	—	0/5

Legend. AGN) Tissue antigens of native valves, AGP) the same, of preserved valves. Numerator – mean anaphylactic index or severity of shock, assessed by a 5-point system; denominator, number of animals. Results of sensitization of animals with AGP that differ statistically significantly from results of sensitization with AGN, are marked by an asterisk.

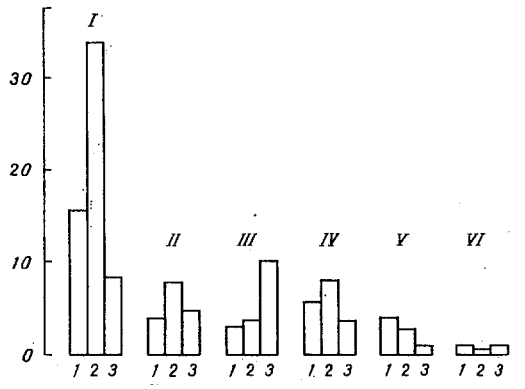

Fig. 1. Time course of absorption of preservative (in mg/g tissue) at different times of preservation of valves in GA solutions of different concentrations. Ordinate, amount of absorption of preservative (in mg/g). 1-3) Data relating to preservation in 0.25, 0.625, and 1% GA solutions respectively. I-VI) Periods of preservation: from 0 to 12 h (I), from 12 to 24 h (II), from 24 to 48 h (III), from 48 to 72 h (IV), from 72 to 168 h (V), and from 7 to 28 days (VI).

TABLE 2. Effect of Preservation of Heterologous Valve Prosthesis Tissues on Their Physicomechanical Properties (M ± m)

Parameter	Native tissue	Preserved tissue			
		0,25 % GA	0,625 % GA	0,625 % GA + formaldehyde	1 % GA
Number of tissue specimens	46	56	74	58	43
Reserves of deformability, %	5,54±0,24	4,04±0,19*	3,99±0,13*	3,94±0,15*	4,33±0,14
Conventional reserves of tensile strength, kg/cm^2	55,9±3,5	72,9±4,6*	76,2±4,6*	72,3±3,7*	64,9±6,7

Legend. Asterisk indicates values of parameters for preserved valves which differ statistically significantly from values for unpreserved (native) valves.

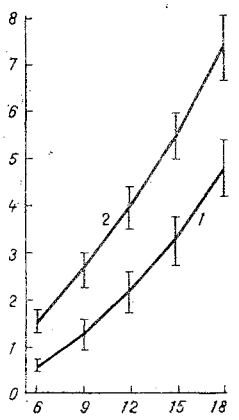

Fig. 2. Effect of preservation of heterologous aortic valve prostheses with 0.625% GA solution on pressure gradient recorded during working of valves on constant flow bench with different rates of flow of fluid. Abscissa, flow rate (liters/min); ordinate, pressure gradient (in mm Hg). 1) Parameters of valve function before preservation, 2) the same after treatment with GA.

EXPERIMENTAL RESULTS

The largest quantity of preservative defusing into the tissue was found with 0.625% solutions, in which 45 and 34% more GA penetrated into the tissue respectively than in 1 and 0.25% solutions ($P < 0.001$). The greatest absorption of preservative was observed on the first day (Fig. 1), when the GA concentration in a solution initially of 0.25% fell to 0.001%, which is unacceptable from the standpoint of reliability of sterilization [3, 6]. Toward the end of the first week diffusion of GA into the tissue had virtually ceased. To maintain a concentration of 0.625% of the GA solution, the solution had to be changed three times during the first week.

The results confirm those obtained in other investigations [9]. The increase of sorption of GA in a 0.625% solution was evidently connected with an increase in the concentration gradient of the aldehyde [4]. This was not observed in the 1% solution, evidently due to rapid blocking of the surface layers of the tissue. From this standpoint a 0.625% solution must be considered to be optimal.

After sensitization of the guinea pigs with homogenates of the native valve tissues, injection of the reacting dose of homologous antigen caused anaphylactic shock in 93% of the guinea pigs, and caused death of 26.6% of the animals (Table 1). The sensitizing activity of the preserved tissues was considerably depressed: 0.25 and 0.625% GA solutions prevented anaphylaxis in 40% of guinea pigs and reduced its intensity in the other animals. A combination of treatment with 0.625% GA solution followed by storage of the tissue in 4% formaldehyde

solution reduced the antigenic activity of the tissue by an even greater degree ($P < 0.05$). Meanwhile, a 1% GA solution reduced the sensitizing activity of the tissue by a much lesser degree than solutions with lower concentrations ($P < 0.05$).

In the immunodiffusion test with homologous antiserum, saline extracts of native tissues gave 6-8 precipitation bands in dilutions of 1:128-1:256. Preservation of the material for 24 h led to a substantial decrease in antigenic activity, and on more prolonged treatment with GA the extracts were inert, even during interaction with native antiserum.

The experiments thus showed that immune activity of tissues of heterologous valve prostheses can be substantially reduced by preservation with aldehydes. It can be tentatively suggested that this effect is due to a decrease in the quantity of water-soluble proteins and to blocking of antigenic determinants [6, 11]. GA solutions with low concentrations had higher activity of this kind, especially when combined with formaldehyde treatment.

It will be clear from Table 2 that preservation of the tissues led to an increase in conventional reserves of tensile strength and to a decrease in deformability. This effect was most marked when 0.625% GA solution was used and was least and not significant after preservation with the 1% solution.

Function testing showed that the cusps of native valves exerted virtually no resistance to fluid flowing at the minimal rate of 6 liters/min. Preservation, on the other hand, had three effects: first, it led to a significant increase in resistance of the cusps to the flow (Fig. 2): at flow rates of approximately 18 liters/min the resistance of the preserved valves was 41-77% greater than that of native valves ($P < 0.001$). Second, subsequent treatment with formaldehyde had no significant effect on the hydrodynamic parameters of prostheses preserved beforehand in GA. Third, in 1% GA solution the tissues preserved their plasticity to a greater degree. This was evidently due to penetration of a smaller quantity of the aldehyde from this solution into the tissue.

It can be concluded from these results that optimal preservation of heterologous valve prostheses is given by the use of a 0.625% solution of GA for 4 weeks, with three changes of solution. The use of this concentration and the technology of treatment result in maximal absorption of aldehyde by the tissue and the greatest decrease in antigenicity and increase in tissue tensile strength. Subsequent treatment of the tissue with formaldehyde solution increases the biological inertia of the material and guarantees its sterility.

LITERATURE CITED

1. L. S. Barbarash and V. N. Sergeev, Byull. Éksp. Biol. Med., No. 11, 554 (1978).
2. L. S. Barbarash et al., Grudn. Khir., No. 2, 35 (1984).
3. S. M. Krasovskaya, "Experimental validation of the use of a new type of heterologous valve prostheses," Author's Abstract of Candidate's Dissertation, Moscow (1981).
4. A. N. Mikhailov, Collagen of Skin and Principles of Its Processing [in Russian], Moscow (1971).
5. A. N. Sukasyan et al., Sb. Nauchn. Tr. Mosk. Nauchn.-Issled. Inst. Vaktsin Syvorotok, 26, 110 (1977).
6. W. W. Angell and J. D. Angell, Prog. Cardiovasc. Dis., 23, 141 (1980).
7. A. Carpentier et al., J. Thorac. Cardiovasc. Surg., 83, 27 (1982).
8. L. N. Cohn and J. S. Collins, in: Tissue Heart Valves, London (1979), p. 173.
9. P. M. Hardy et al., J. Chem. Soc., Chem. Commun., No. 5, 958 (1976).
10. E. Imamura et al., Thorac. Cardiovasc. Surg., 83, 610 (1982).
11. E. A. Woodroof, in: Tissue Heart Valves, London (1979), p. 347.