USE OF MATHEMATICAL METHODS OF EXPERIMENTAL PLANNING TO CHOOSE OPTIMAL CONDITIONS FOR PRESERVATION OF THE HEART BY WEAK ALDEHYDE SOLUTIONS

V. I. Tel'pukhov, V. A. Shepelev, and A. N. Lisenkov

UDC 615.361.12.014.41:519.24

KEY WORDS: aldehydes; ischemia; preservation of the heart; optimization; mathematical experimental planning.

In modern transplant surgery various combinations of methods of preservation, preserving solutions, and inhibitors of cell metabolism are used to protect the organ for grafting [9]. In recent years several workers have extensively used weak solutions of aldehydes as a preservative to protect organs and tissues from ischemic damage [1, 5].

The object of this investigation was to optimize the conditions and methods of preservation of the heart by the use of weak concentrations of aldehydes and different preserving solutions, by the use of mathematical experimental planning methods [3].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats of both sexes weighing 200-300 g. A combination of two methods was used to preserve the heart: *in vitro* and *in vivo*. For *in vivo* preservation, formaldehyde, acetaldehyde, and glutaraldehyde were used in various concentrations and volumes, whereas for preservation *in vitro* the same aldehydes were used but in a lower concentration. The aldehydes for preservation *in vitro* were made up in different basic preserving solutions. The donor (for preservation *in vivo*) was prepared by injection of one of the aldehydes into the animal's inferior vena cava at the rate of 1 ml/min, followed by removal of the heart. For preservation for 5 h, simple methods of keeping the isolated heart and solutions belonging to different groups were used [6-8]. Deconservation was carried out on a model of the isolated perfused rat heart [10]. To assess the viability of the heart the velocity of coronary perfusion and the beginning of appearance of fibrillations and contractions of the left ventricle were recorded. The strength of the cardiac contractions was judged indirectly from the pressure in the aorta.

The effect of the following seven factors was studied, each at three levels. For preservation *in vitro*: X_1) method of preservation of the heart [with no change of solution (0), change in solution every hour, i.e., 4 times (1); periodic washing of the coronary circulation and fractional perfusion (2); X_2) type of aldehyde (formaldehyde (0), acetaldehyde (1), glutaraldehyde (2); X_3) concentration of aldehydes [0.05% (0), 0.005% (1), 0.0005% (2)]; X_4) basic medium [Ringer's solution (0), medium 199 (10, Bretschneider's solution (2)]. For preservation *in vivo*: X_5) type of aldehyde [formaldehyde (0), acetaldehyde (1), glutaraldehyde (2)]; X_6) concentration of aldehyde [2.5% (0), 0.25% (1), 0.025% (2); X_7) quantity of aldehyde injected, in ml/100 g body weight [0.7 (0), 1.4 (1), 2.1 (2)].

In the experiments of series I the viability of the heart (Y) was determined by a method of expert assessment on a 5-point scale: 1) no contractions, 2) fibrillations, 3) separate contractions, 4) arrhythmic contractions, 5) complete restoration of rhythm. In series II the viability of the heart was judged on the basis of five parameters, the values of which also were estimated on a 5-point scale (Fig. 1): Y_1) volume velocity of perfusion (in mg/g): Y_1^1) 8-10 (5 points), Y_1^2) 6-8 (4 points), Y_1^3) 4-6 (3 points), Y_1^4) 2-4 (2 points), Y_1^5) 0-2 (1 point); Y_1) the time of appearance of fibrillations: Y_2^1) after 1 min (5 points), Y_2^2) after 2-4 min (4 points), Y_2^3) after 5-7 min (3 points), Y_2^4) after 8-10 min (2 points), Y_2^5) after

V. V. Kovanov's Academic Group, Brain Institute, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 106-109, May, 1982. Original article submitted July 9, 1981.

0007-4888/82/9305-0679$07.50

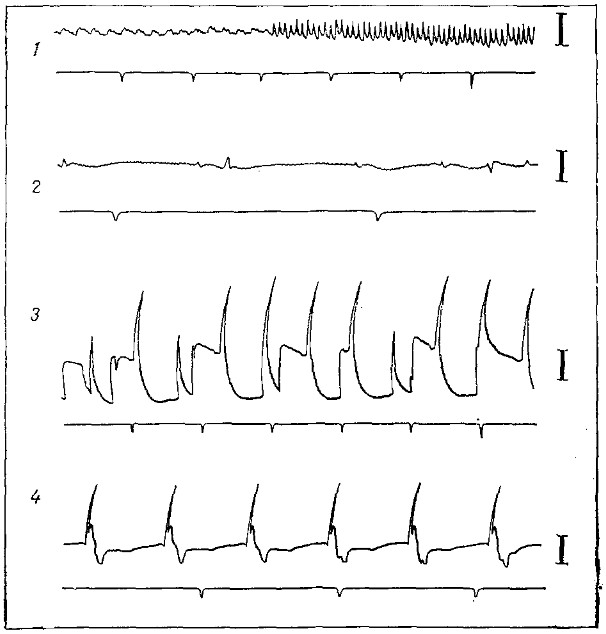

Fig. 1. Variants of restoration of cardiac activity based on expert assessment (Y_5). 1) Y_5^4, 2 point (before large-wave fibrillation), 2) Y_5^3: 3 points (before separate contractions), 3) Y_5^2: 4 points (before **xxx** arrhythmic contractions), 4) T_5^1: 5 points (before normal rhythm). Calibration, 1 mV. Scale division of time marker 1 sec.

TABLE 1. Plan $3^7//18$ and Results of Experiments (series I)

№	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y
1	0	0	0	0	0	0	0	1
2	0	1	1	2	1	1	1	5
3	0	2	2	1	2	2	2	2
4	1	0	1	1	1	2	0	2
5	1	1	2	0	2	0	1	1
6	1	2	0	2	0	1	2	3
7	2	0	2	2	1	0	2	4
8	2	1	0	1	2	1	0	2
9	2	2	1	0	0	2	1	3
10	0	0	2	1	0	1	1	1
11	0	1	0	0	1	2	2	1
12	0	2	1	2	2	0	0	4
13	1	0	0	2	2	2	1	3
14	1	1	1	1	0	0	2	1
15	1	2	2	0	1	1	0	3
16	2	0	1	0	2	1	2	2
17	2	1	2	2	0	2	0	4
18	2	2	0	1	1	0	1	2

11 min or more (1 point); Y_3) the time of appearance of concentrations: Y_3^1) after 1 min (5 points), Y_3^2) after 2-4 min (4 points), Y_3^3) after 5-7 min (3 points), Y_3^4) after 8-10 min (2 points), Y_3^5) after 11 min or more (1 point); Y_4) intra-aortic pressure (1 unit corresponds to 25 mm Hg): Y_4^1) 2-3 (5 points), Y_4^2) 1.5-2 (4 points), Y_4^3) 1-1.5 (3 points), Y_4^4) 0.5-1.0 (2 points), Y_4^5) 0.1-0.5 (1 point); Y_5) restoration of cardiac activity according to the ECG: Y_5^1) before normal rhythm (5 points), Y_5^2) before arrhythmic contractions (4 points), Y_5^3) before separate contractions (3 points), Y_5^4) before fibrillation (2 points), Y_5^5) no recovery of contractions (1 point).

In the second stage, to determine the optimal conditions of preservation more precisely and to obtain mathematical models describing the dependence of the parameters of contractility and the three principal quantitative factors (X_3 — concentration of aldehyde for preservation *in vitro*, X_6 — concentration of aldehyde for preservation *in vivo*, X_7 — quantity of aldehyde added), the second order plan $3^3//15$ cnsisting of 15 variants was realized in the proximity of the best variant shown above (Table 2).

TABLE 2. Plan $3^3//15$ and Results of Experiments: Values of Output Parameters and Their Point Ratings (series II)

No.	X_3	X_6	X_7	Y_1	Y_2	Y_3	Y_4	Y_5	Y total
1	−	−	0	2,86 (2)	6 (3)	12,3 (1)	0,4 (1)	3 (3)	2
2	+	−	0	2,97 (2)	1 (5)	3 (4)	1,7 (4)	2 (4)	4
3	−	+	0	2,21 (2)	2 (4)	8,7 (2)	0,7 (2)	3 (3)	3
4	+	+	0	1,47 (1)	2 (4)	5,0 (3)	1,3 (3)	3 (3)	3
5	0	−	−	6,33 (4)	1 (5)	1,0 (5)	2,2 (5)	1 (5)	5
6	0	+	−	4,87 (3)	1 (5)	2,3 (4)	2,2 (5)	1 (5)	4
7	0	−	+	4,03 (3)	1 (5)	1,7 (4)	1,7 (4)	2 (4)	4
8	0	+	+	6,0 (4)	1 (5)	1,0 (5)	2,2 (5)	1 (5)	5
9	−	0	−	2,9 (2)	1 (5)	2,7 (4)	1,5 (4)	2 (4)	4
10	+	0	−	1,96 (1)	2 (4)	5,3 (3)	1,0 (3)	3 (3)	3
11	−	0	+	2,29 (2)	1 (5)	5,7 (3)	1,3 (3)	3 (3)	3
12	+	0	+	4,1 (3)	1 (5)	1,7 (4)	1,2 (3)	2 (4)	4
13—15*	0	0	0	5,76 (3)	1 (5)	1,0 (5)	1,83 (4)	1 (5)	4

*Mean values of response given here were calculated from results of three parallel determinations in variants 13-15, in which the dispersions of reproducibility were: $S^2\{y_1\} = 0.239$, $S^2\{y_2\} - 0.467$, $S^2\{y_3\} = 0.845$, $S^2\{y_4\} = 0.134$, $S^2\{y_5\} = 0.197$, $S^2\{y_5\} = 0.197$, $S^2\{y_{total}\} = 0.13$.

According to the plan, each factor was varied at three levels and 15 variants of combinations of the levels of these factors were realized. In this paln $X_3 = C_3$, where C_3, the concentration of aldehyde, had the levels $X_3 \cdot \Delta X_3 = 0.005 \cdot 5^{\pm 1}$, which corresponded to concentrations C_3 of 0.025, 0.005, and 0.001%; $X_6 = C_6$, where C_6, the concentration of aldehyde, had levels $X_6 \cdot \Delta X_6 = 0.025 \cdot 5^{\pm 1}$, which corresponded to concentrations C_6 of 0.125, 0.025, and 0.005%; X_7, the quantity of aldehyde added, had levels $X_7 \pm \Delta X_7 = 0.7 \pm 0.2$ ml aldehyde/100 g body weight.

From the results of these experiments the following adequate equations were obtained for dependence of the recorded parameters of contractile activity, and also of the general parameter (Y), on the three basic factors, assessed in points, including only coefficients found to be significant by Student's t-test:

$$Y_1 = 1 - 0,199\,x_6 - 0,225\,x_3x_6 + 0,689\,x_3x_7 + 0,858\;x_6x_7 - 2,94_3^2 - 0,447x_6^2$$

$$Y_2 = 1 - 0,5\,x_3 - 0,375\,x_6 + 1,25\,x_3x_6 - 1,0\;x_3^2 + 0,75x_6^2 - 0,75x_7^2$$

$$Y_3 = 1 - 1,8\,x_3 + 1,4\,x_3x_6 - 1,65\,x_3x_7 + 4,3\;x_3^2 + 1,95x_6^2 - 1,45x_7^2$$

$$Y_4 = 1 - 0,325\,x_3 + 0,125\,x_7 + 0,25\,x_3x_6 - 0,575\,x_3x_7 - 0,175\,x_6x_7 + 1,35\;x_3^2 + 0,25x_6^2$$

$$Y_5 = 1 + 0,1625\,x_2 - 0,175\,x_3x_6 - 0,817x_3^2 + 0,232x_7^2$$

$$Y = 1 + 0,25\,x_3 - 0,5\,x_5x_6 + 0,5\,x_3x_7 + 0,5\,x_6x_7 - 3,4\,x_3^2 - 2,7\,x_6^2 - 2,4\,x_7^2$$

Analysis of these equations enables the influence of the most significant factors and their interactions on each parameter Y and also the general parameter of contractile activity to be discovered.

Coordinates of the special point — the optimum for the general parameter, corresponded to values of X_3 = 0.037% (aldehyde concentration for preservation *in vitro*), X_6 = 0.03% (aldehyde concentration for intravenous injection), X_7 = 0.65 ml (quantity of aldehyde given/100 g body weight) with Y = 5 points — the best contractile activity of the preserved heart.

The optimal conditions of preservation could thus be determined by multifactorial design methods with a small number of variants (plans $3^7//18$ and $3^3''15$).

The use of the conditions thus discovered thus provides the most effective method of protecting a test organ against a noxic damage and of recovering its contractile activity after preservation.

LITERATURE CITED

1. V. V. Kovanov, V. F. Parfent'eva, V. D. Rozvadovskii, et al., in: Proceedings of an All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Tbilisi (1966), pp. 86-88.
2. A. N. Lisenko, Mathematical Methods of Planning Multifactorial Medico-Biological Experiments [in Russian], Moscow (1979).
3. A. N. Lisenkov, Vestn. Akad. Med. Nauk SSSR, No. 5, 78 (1973).
4. E. V. Markova and A. N. Lisenkov, Combinatorial Plans in the Aims of Multifactorial Design [in Russian], Moscow (1979).
5. É. L. Muzykantskii, "Protection of the myocardium against ischemic damage by formaldehyde," Author's Abstract of Candidate's Dissertation, Moscow (1980).
6. N. A. Onishchenko, V. P. Rastorguev, É. M. Balakirev, et al., in: Transplantation of Organs under Clinical and Experimental Conditions and Artificial Organs [in Russian], Moscow (1978), pp. 95-97.
7. V. F. Portnoi, L. N. Cherkashchenko, A. V. Machulin, et al., in: Transplantation of Organs and Tissues [in Russian], Baku (1979), pp. 253-254.
8. I. V. Stupin, "Assessment of method of preserving the heart," Author's Abstract of Doctoral Dissertation, Moscow (1974).
9. V. I. Shumakov, E. Sh. Shteingol'd, and N. A. Onishchenko, Preservation of Organs [in Russian], Moscow (1975).
10. D. J. Hearse and E. B. Chain, Biochem. J., 128, 1125 (1972).