USE OF 2,4-DINITROPHENOL FOR IMMUNOSUPPRESSIVE ACTION ON THE RECIPIENT DURING IMPLANTATION OF FETAL ORGANS IN MICE

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The immunodepressive properties of 2,4-dinitrophenol (DNP) were known as long ago as in the 1950s [14], and the compound was classed as an immunodepressant somewhat later [10, 12]. However, no practical use for the compound in transplant surgery has been found in the past because of the negative results of its uncontrolled application in various diseases [1, 2, 11, 13, 15]. The renewal of interest in DNP as an immunodepressant was caused by the description of its ability to significantly prolong the life of allogeneic transplants of fetal organs in mice following intraperitoneal injection in microdoses [6].

The aim of this investigation was to study the possibility and conditions of use of noninvasive methods of DNP administration to the recipient during allografting of organs and tissues. In particular, considering the ability of DNP to pass through the skin [3], we investigated a noninvasive method of percutaneous application of the compound. The aims of the investigation were: 1) to determine the effect of DNP on the time of rejection of allografts of different organs; 2) to study the response of the recipient of, in particular, lymphoid and other organs; 3) to establish the optimal program of DNP administration (dose, frequency, time).

METHODS

Experiments were carried out on 116 albino mice of both sexes weighing 20-30 g and 10 male albino rats weighing 200 g. The model of transplantation was the technique of implantation of fetal organs subcutaneously into the ear, developed previously [5, 6]. As implants we used the small intestine (FSI) in 45 mice and the pancreas (FP) in 28 mice, taken from fetuses on the 16th-20th day of intrauterine development. As the control (intact animals, mock operations, and their exposure to DNP), and also for cytologic experiments, 43 mice were used. Percutaneous application of DNP were given in the region of implantation by means of a glass spatula in a dose of 0.1 ml/10 g body weight. Solutions of DNP were used in concentrations of 10^{-3} to 10^{-6} M. In experiments on 10 rats, a hepatoma-25 was transplanted subcutaneously into the flank by the standard method, and DNP was injected intraperitoneally in a concentration of 10^{-3} M.

The state of the implants was assessed on the basis of the results of macroscopic and biochemical morphometry of the implants themselves and of the blood vessels of their bed, and also on the basis of the results of histologic investigation (staining with hematoxylin and eosin, azure and eosin). The state of the implant was judged by its size (diameter, thickness, area) and external appearance (the presence of hemorrhages, crusts, and scars). Changes in the parameters (diameter, length, tortuosity) of the arterioles and venules of its bed were monitored [7]. Examination of the histologic sections took account of the structural integrity of the organs and the degree of lymphoid infiltration and replacement by connective tissue. The recipient's state was judged from the clinical data, the results of biochemical analysis of the urine and blood by rapid methods (using strips of the "Labstix" and "Dextrostix" type) and also the

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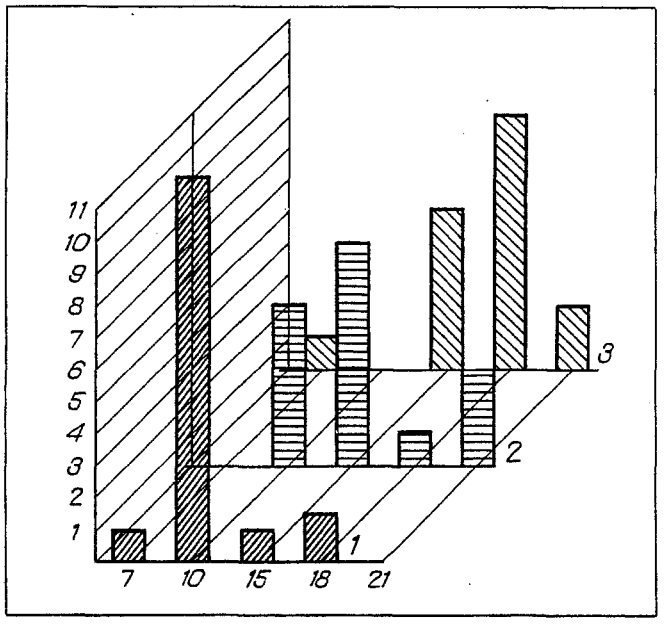

Fig. 1. Number of cases of total rejection of implants of fetal organs at various times after operation, depending on concentration of DNA solution injected into recipient. Abscissa) time (in days); ordinate) number of cases of rejection of implants. 1) Control; 2) DNP (10^{-5} M); 3) DNP (10^{-3} M).

results of hematologic analysis (a separate series, 27 animals), and histologic investigation of the internal organs (20 animals). To assess the effect of DNP at the cellular level, Hela-like tumor cells of the CaOv strain, whose viability was estimated on the basis of incorporation of ^{3}H-thymidine and ^{3}H-uridine into nucleic acids [4], were used.

RESULTS

Comparison of the effect of different concentrations of DNP solution on the rejection times of allogeneic implants of different organs showed that: 1) in all concentrations the peak of rejection is shifted to the right compared with the control, i.e., the use of DNP delays the process, and 2) delay of the process of rejection reaches a maximum when a 10^{-3} M solution is used (Fig. 1). Biomicroscopically the diameter of the blood vessels of the bed of the implant was smaller in the experiment than in the control, and histologically the infiltration zone around the implants also was smaller in the experiment than in the control, especially when a 10^{-3} M solution was used. With time, no "decomposition" of the implants was observed, but their gradual replacement by connective tissue. The general state of the recipients was not significantly disturbed, and no changes were found in the skin in the region of DNP application. At autopsy at various times until 3 months after the beginning of the experiment the internal organs were unchanged macro- and microscopically, with the exception of the mesenteric lymph nodes and the thymus gland, which were reduced in mass and size after 21 or more days when DNP was used in 10^{-3} M solution. Histologically, reduced density of the white pulp of the spleen, a decrease in size and cell density of the follicles of the mesenteric lymph nodes, and reduced density of the tissue of the thymus were noted histologically. Hematologic studies carried out 2 months after the beginning of the experiment confirmed the presence of slight but significant leukopenia in animals receiving DNP compared with the intact control (Table 1). Under these circumstances the compound did not affect the hemoglobin concentration. Thus percutaneous applications of DNP proved effective as a method of immunodepressive action, especially in a concentration of 10^{-3} M, with minimal risk of damage to the recipient.

implants were found compared with 1 month and nine animals in the control), it significantly delayed both the beginning and completion of rejection of FP implants (to 3 months or more). In this case no significant general or local changes were observed in the animals over a period of 6.5 months of observation. Nevertheless, the study of the possible carcinogenicity of percutaneous application of DNP appeared to be worth while.

The results of investigation of the action of DNP on nucleic acid synthesis in tumor cells of the CaOv strain showed that with DNP in concentrations of $7 \cdot 10^{-3}$ and 10^{-4} M, inhibition of DNA and RNA synthesis was observed, more especially DNA, and the degree of inhibition was inversely proportional to the concentration of DNP. A further reduction of the DNP concentration of 10^{-5} and 10^{-6} M showed that the compound no longer affected the viability of the cells.

Observation on rats with a transplanted hepatoma for up to 4 months showed that intraperitoneal injection of DNP (10^{-3} M) weekly for 2 months did not affect the rate or intensity of tumor growth: no significant difference was observed between control and experiment.

Thus administration of DNP to the recipient in the form of intraperitoneal injection [6] or percutaneous applications (the present experiments) leads to slowing of rejection of implants of fetal organs by 33-50%. This action can be reduced to delaying of the beginning of rejection, the prolongation of its development, and its conversion into a chronic process. The mechanism of this effect may be twofold: first, a mild immunodepressive action, as shown by the results of histologic and hematologic, and also of previous immunologic investigations, conducted by ourselves and other workers [8,

TABLE 1. Effect of DNP (10^{-3} M) on Leukocyte Count and Hemoglobin Concentration in Mouse Blood (M ± m)

Series	Number of animals	Number of leukocytes	Hemoglobin concentration, g/liter
Control I (intact animals)	5	5,2±0,5	147,0±7,8
Control II (intact animals + DNP)	3	2,4±0,1	143,0±10,5
Control III (mock operation + DNP)	4	3,3±0,6	141,0±8,7
Experiment I transplantation w/o DNP)	9	4,0±1,0	141,0±6,8
Experiment II (transplantation with DNP)	6	3,1±1,0	146,0±8,6

TABLE 2. Results of Measurement of Area of Implants (in mm^2) of Fetal Pancreas and Small Intestine with Local Application of DNP (10^{-3} M; M ± m)

Name of series	Number of animals	Parameters	Times of observation, days					
			7	14	18	21	28	60
FP without DNP	19	Area	10±4	6±3*	1±2	2,2	2,0	—
		Remnant/reject	0/3	—	2/13	5/13	4/14	1/18
FP + DNP	9	Area	12,11±4,4	12,1±3,5	4,1±0,4	1,0	0,9	0,7
		Remnant/reject	—	—	—	8/0	7/1	4/4
FSI without DNP	9	Area	12,3±3,6	7,5±4,7	3,7±5,8	0,4	—	—
		Remnant/reject	—	0/1	0/3	5/3	5/4	1/8
FSI + DNP	9	Area	12,6±7,3	8,7±2,2	2,7±3,0	0,9	0,8	0,8
		Remnant/reject	—	—	0/1	6/2	6/2	6/2

Notes. *) In this case measurement from eight of the animals studied; area — total area of implant; remnant — number of cases in which remnant of implant 1 mm^2 in area was preserved; reject — cases of total rejection.

10, 12, 14], and second, constriction of blood vessels around the implants, which may also bring about the reduction of lymphoid infiltration already mentioned. Percutaneous applications of DNP have many advantages: they constitute less risk for the animals on account of their noninvasive nature, and they can be used sufficiently infrequently because of the property of DNP to accumulate in the tissues and to be eliminated from the body slowly [14], and they enable the maximal concentration of the preparation to be created locally, in the region of implantation, which accounts for the absence of general complications in the present series of experiments (compared with intraperitoneal injection). No local signs of irritation or allergy likewise were observed, and no carcinogenic effects of the compound were disc[illegible]d. This contradicts data in the literature [4-8] to some degree, but in our opinion it can be explained by the [illegible]ion of the doses used and the frequency of their administration, thus ruling out any accumulative action on the recipient. A concentration of 10^{-3} M proved most effective, but lower concentrations (10^{-4}, 10^{-5} M) may be used under certain conditions. Since the peak action of DNP is achieved after 2-3 weeks, as shown by the appearance of changes in the lymphoid organs, the compound should be given 2-3 weeks before implantation. As further experiments on rats and dogs showed, performing the operation after DNP administration is not dangerous. Another noteworthy fact is that DNP was more effective when used with FP than with implantation of other organs. This may perhaps be explained on the grounds that under certain conditions DNP can stimulate proliferative activity of FP cells [9]. In that case, the action of DNP on FP resembles the tropic action of pentagastrin on FSI [5], and the use of these agents, promoting regenerative processes in allografts, could be a promising step in the conduct of the postoperative period in transplant surgery.

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