Effect of Transplantation of Fetal Liver Tissue on Regenerative Activity of Hepatocytes in Normal Rats

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Regenerative activity of hepatocytes was studied in rats after transplantation of fetal liver tissue preexposed to low-intensity laser. Stimulation of the mitotic activity of hepatocytes by fetal liver tissue exposed to low-intensity laser is proven.

Key Words: *liver; laser; fetal tissue transplantation; regeneration*

Methods of pathogenetic therapy in modern medical practice are based on the synthesis of new medical technologies. It was shown that transplantation of a small amount of human fetal liver cells stimulated the regeneration, growth, and differentiation in the recipient liver [2,3,5,10].

Introduction of lasers in medical practice opened new vistas for selective modulation of human cell and tissue functions in many diseases. Low-intensity laser (LIL) exposure of different power modifies ionic channels on the cell membrane by modulating the properties of the membrane lipid bilayer. This exposure can modify subcellular structures (mitochondria), and receptor systems and stimulate cell functions [4,8,9, 11]. The study of the effects of transplanted fetal liver tissue on hepatocyte regeneration in the recipient after preexposure of the transplant to LIL attracts special interest.

We studied regenerative activity of recipient hepatocytes after transplantation of fetal liver tissue exposed to LIL.

MATERIALS AND METHODS

Experiments were carried out on 80 adult male Wistar rats (200 g) kept under standard vivarium conditions at 18-20°C on mixed rations with free access to water. The animals were divided into 3 groups: 1) intact animals; 2) healthy animals in whom hepatocyte regeneration was stimulated by transplantation of liver tissue from 20-day rat embryos, corresponding by morphological structure to 5-month human embryos [2]; and 3) healthy animals receiving fetal hepatocytes from 20-day rat embryos immediately after exposure to LIL. The exposure was carried out at laser power density of 75 mW/cm^2 for 2 min. The experiments were carried out during one season in all groups. Allotransplant was prepared directly before injection. Suspension of liver fragments in normal saline was prepared in a Potter homogenizer. The number of nuclear cells was 3-5×10^5/ml suspension [2]. Viability of fetal cells was evaluated in a Goryaev chamber using erythrosine exclusion test [7]. Each animal received single transplantation (via intraperitoneal injection) of 1 ml of fetal hepatocyte suspension. In accordance with the requirements to studies of the effects of biopreparations on experimental animals the allotransplant dose 30-fold surpassed the clinical dose. For evaluation of the early effects of fetal hepatocyte transplantation, the animals were sacrificed 24 h after transplantation.

Material for microscopy was fixed in 10% formalin, dehydrated, and embedded in paraffin. Histological sections were stained with hematoxylin and eosin, by Van Gieson method, and using periodic acid-Schiff (PAS) reaction. Morphometric study was carried out using DiaMorph-Cyto computer image analysis system. Diameters of cells and their nuclei and the number of hepatocyte mitoses (in percent) per arbitrary unit of area were evaluated. The counts of T and B

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lymphocytes were evaluated per 1 mm^2 liver section by the indirect Coons' method using MAb (Ortho). Hepatocytes in apoptosis were detected using specific antibodies to nuclear DNA (RD system).

RESULTS

Microscopy showed a typical pattern of liver tissue in all groups of animals. Morphometric analysis showed that the number of dividing hepatocytes increased in group 2 and especially in group 3 (Fig. 1). Most cells were in the interphase period. This phase is most sensitive to liver stimulation.

The percentage of hepatocytes in the mitotic period reached the maximum in animals intraperitoneally injected with fetal hepatocytes pre-exposed to LIL (group 3).

The nucleus/plasma ratio in liver cells reflecting their mitotic activity was shifted towards the nucleus most of all in group 3 animals. The maximum number of binuclear hepatocytes was observed in the same group.

The number of T lymphocytes and apoptosis index increased in group 2 and especially in group 3 (Fig. 2). The increase in T lymphocyte count in liver tissue could be due to the fact that this cell population serves as the messenger of regeneration information and can realize mitotic processes during regeneration. For example, A. G. Babaeva demonstrated induction of proliferative activity in organs of intact animals by lymphocytes from operated donors [1].

On the other hand, the increase in apoptosis index (1.78 in group 1, 4 in group 2, and 6.35 in group 3) can be regarded as a compensatory process aimed at the maintenance of the number of hepatocytes under conditions of stimulation of their proliferative activity by transplantation of fetal liver tissue (containing growth factors stimulating hepatocyte proliferation [6]).

Hence, transplantation of fetal liver tissue exposed to LIL to normal rats markedly stimulated mitotic activity of hepatocytes during the early period after transplantation, which is seen from the increase in the relative number of mitoses in these cells.

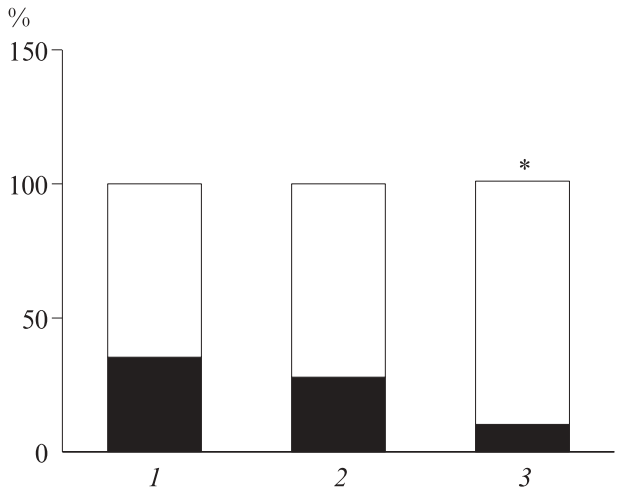

Fig. 1. Mitotic cycle of hepatocytes (in percent). Light bars: mitosis; dark bars: interphase. *1*) control; *2*) fetal tissues; *3*) fetal tissues+low-intensity laser (LIL). Here and in Fig. 2: **p*<0.05 compared to the control.

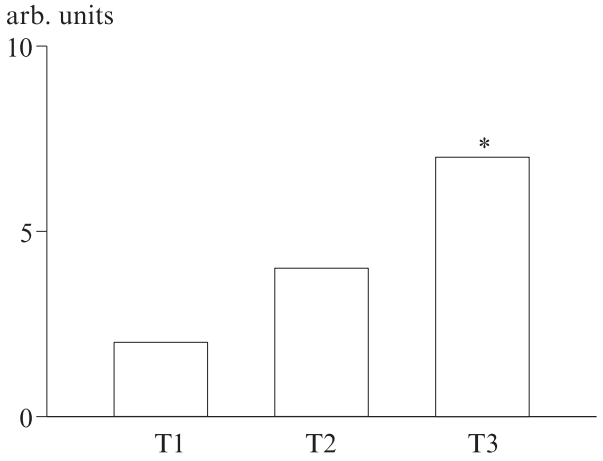

Fig. 2. Content of T lymphocytes in liver tissue. T1: control; T2: fetal tissue; T3: fetal tissue+LIL.

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