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EFFECT OF TACTIVIN ON THYMUS MORPHOLOGY IN EXPERIMENTAL LOWER LIMB TRAUMA IN MICE (THE ANTISTRESSOR EFFECT OF TACTIVIN)

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KEY WORDS: thymus; stress; tactivin; morphometry

In recent years the range of diseases and pathological states accompanied by immune disturbances, for the optimal treatment of which the use of various immunomodulators is indicated, has widened [2]. Changes characteristic of the stress reaction, and due to corticosteroids, develop under these circumstances in the thymus [8, 11]. There are indications in the literature that thymic hormones and, in particular, thymosins can counteract the development of a stress-induced immunodeficiency state [10]. Accordingly, we decided to study the possibility of using tactivin, one of the most active Soviet immunomodulators [1], on a model of experimental trauma, in which a stress-reaction (accidental involution) of the thymus also develops [9], with the aim of speeding up recovery of the organ after trauma.

EXPERIMENTAL METHOD

Experiments were carried out on mature female (CBA × C57BL/6)F_1 mice weighing 17.5-19.0 g. A closed fracture was produced manually in the lower third of the left femur of the experimental mice. Tactivin was injected subcutaneously in a sessional dose of 1.0 μg per mouse 1 day before the fracture or immediately thereafter (the mice were killed 24 h after the fracture) or during the 3 days after the fracture (mice were killed 5, 10, and 15 days after the fracture). Animals subjected to similar trauma, but not receiving tactivin, together with intact mice served as the control. Five animals were used at each point. The thymus was removed, weighed, and fixed in Bouin's fluid. Morphometric analysis was carried out on 4-μ-paraffin sections stained with azure II-eosin, in three different zones: subcapsular, cortical, and medullary. The relative areas occupied by the

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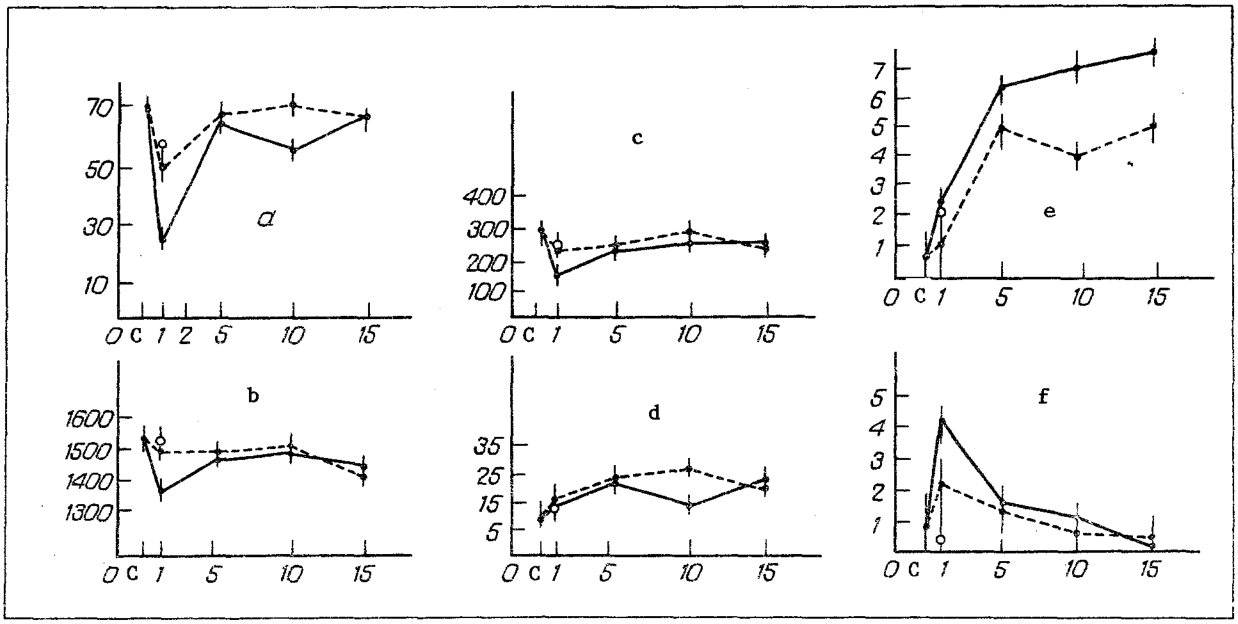

Fig. 1. Dynamics of change in weight and morphometric parametezs of thymus after experimental trauma and injection of tactivin. Abscissa: time after fracture of femur (in days); ordinate: a) dynamics of weight of thymus (in mg), b) cell density of thymus, c) number of blast cells, d) number of mitoses, e) number of pycnotic cells, f) of plasma cells (b-f given in conventional units). Vertical lines indicate limits of individual variations; C) control.

cortex and medulla by nonparenchymatous tissue (vessels, cysts, adipose and connective tissue) were determined used a 20× objective and 10× ocular. The density of the thymocytes (Tc), and the absolute and relative numbers of blast cells (Bl), mitotic figures (MF), pycnotic (Py) and plasma cells (PC) and reticular cells (RC) were counted in these same zones under a magnification of 900×, in 40 small squares of an Avtandilov grid. The numerical results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney nonparametric U test.

EXPERIMENTAL RESULTS

The weight of the thymus was reduced by half 24 h after the lower limb fracture (Fig. 1a). On morphological examination the area of the cortex was reduced, the area of the medulla increased, and solitary cysts and diffusely arranged PC appeared, sometimes forming concentrations around the vessels; the vessels themselves were dilated and congested. On morphometric analysis the density of the cells was significantly reduced (Fig. 1b) mainly in the cortex, and mainly on account of a considerable decrease in the number of B1 (Fig. 1c). Meanwhile there was a significant increase in the number of Py (Fig. 1e) and PC (Fig. 1f); the number of MI remained at the upper limit of normal (Fig. 1d). These changes are evidence of the development of accidental involution of the thymus.

Injection of tactivin before or immediately after the fracture did not change in principle the changes just described caused by fracture of the femur in the thymus. However, these changes were less marked: the weight of the thymus, the density of its cells, and the number of Bl were reduced by a lesser degree and the increase in the number of Py and PC also was less marked (Fig. 1).

Thus injection of 1.0 μg tactivin before or immediately after a fracture of the femur reduces the severity of the changes in the thymus due to the fracture and appears to inhibit the development of a stress-reaction of the thymus (accidental involution).

Several parameters of the thymus were found to have recovered 5 days after the fracture: its weight was increased and close to the control values (Fig. 1a), the ratio between the areas of the cortex and medulla was restored to normal, but solitary cysts, laminated structures consisting of epithelial cells, and dilated and congested blood vessels were still found. On morphometric analysis the density of the cells was increased compared with the previous time, but did not reach its initial control level, the absolute and relative numbers of Bl were increased, the number of MF and Py was increased mainly in the cortex, and the number of PC was sharply reduced (Fig. 1b-f). Against the background of tactivin administration similar changes in the structure and morphometric parameters of the thymus were observed at this time, but they were somewhat closer to the initial level in the control than in the experimental mice not receiving tactivin. It must be emphasized that at this time also, injection of tactivin in a sessional dose of 1.0 μg for 3 days after fracture of the femur leads to normalization of the structure of the thymus.

On the 10th and 15th days after fracture of the femur the mass, structure, and morphometric parameters of the thymus were close to the control values, and variations observed corresponded fully to the fluctuating character of function of the gland, possibly with a 5-day cycle. As will be clear from Fig. 1, all the parameters studied except Py were indistinguishable from the initial level, evidence of virtual restoration of the structure of the gland.

After administration of tactivin similar changes were observed in the thymus except those in MI and Py, the number of which was increased and reduced respectively on the 10th day compared with the experimental animals not receiving tactivin (Fig. 1d, e). On the 15th day the number of MI decreased and was indistinguishable from the control, but the number of Py was less than in the group of experimental mice not receiving tactivin. Thus on the 10th and 15th days after the fracture and a 3-day course of tactivin some degree of activation of the gland was observed, as shown by the ratio of MI and Py.

The results are evidence that an experimental fracture of the femur in mice is accompanied by a mild degree of involution of the thymus, in agreement with data in the literature [9]. Parallel with the decrease in its weight, changes also were observed in the relative areas of the cortex and medulla of the thymus, together with reduction of the cell density due mainly to a decrease in the number of Bl, and an increase in the number of Py and PC, i.e., accidental involution, which has been described in various pathological states including AIDS, emotional stress, and pregnancy [5, 11], develops. This phenomenon is based on exposure of the immune system and, in particular, of the thymus to a raised glucocorticoid level. Glucocorticoids are known to have multiple effects on Tc, including inhibition of DNA synthesis in them, thus preventing the cells from entering the S-phase and mitosis [6].

Meanwhile there are indications in the literature that thymosins have a protective effect on Tc against the lytic effect of glucocorticoids on these cells [7, 10, 12, 13].

The results are evidence that injection of tactivin before or immediately after experimental fracture of the lower limb in mice has a protective effect on the development of the stress reaction in the thymus (it delays or reduces the severity of accidental involution on the lst day after fracture) and helps to accelerate the process of regeneration of the thymus in the future (on the 5th-15th days). At the same time the process of regeneration of bone tissue in the region of the fracture is accelerated.

Our results are in agreement with and confirm previous observations on the protective effect of tactivin against involution of the thymus caused by administration of glucocorticoids [5] or by aging [3], and also data in the literature on the stimulating effect of thymic factors on regeneration of the thymus [4].

The antistressor effect of tactivin and of thymic factors in general may be based on a number of factors: the protective effect of thymic factors on Tc and against glucocorticoids due to a change in specific binding of glucocorticoids by Tc receptors or due to an increase in the number of glucocorticoid-resistant Tc, i.e., due to intensification of Tc maturation [13], and activation of reticuloendothelial stromal cells, secreting various thymic factors [4] or a decrease in the sensitivity of Tc to glucocorticoids [12].

All the considerations metioned above, together with the experimental data, lead to the conclusion that tactivin has a protective, antistressor effect on the thymus, and that it can be recommended for use in various states giving rise to a stress reaction of the gland, with the aim of diminishing its accidental involution and accelerating its regeneration.

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EXPERIMENTAL MORPHOLOGICAL EVALUATION OF WELDING OF LYMPHOVENOUS AND VENOVENOUS ANASTOMOSES BY CO_2 LASER WITH FLEXIBLE LIGHT GUIDE

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Microvascular surgery is a rapidly developing field. Microvascular anastomoses are nowadays usually formed by means of vascular suture. When anastomoses of very small diameter are created, a frequent complication is stenosis followed by thrombosis of the anastomoses due to hyperplasia of the intima at the site of insertion of the foreign body (the suture material). One way of improving the technique of anastomosis is by using the laser beam to weld the edges of the vessels. An experimental and clinical investigation of various sources of laser radiation showed that they were effective for ensuring vascular union [1-7]. An important limitation of the use of the CO_2 laser has hitherto been the absence of any suitable system for transmitting and aiming the radiation and maintaining constant power density. A moveable mirror and ball light guide is not suitable because it does not guarantee the maintenance of a constant distance to the tissue, and correspondingly, a constant power density. Circular welding with aiming of the laser beam through a microscope is unsuitable because of the impossibility of completely rotating the vessels.

The aim of this investigation was to develop a method of creating lympho- and venovenous anastomoses with the aid of radiation from a CO_2-laser, conducted along a flexible light guide.

EXPERIMENTAL METHOD

Experiments were carried out on 13 male and female dogs weighing from 13 to 24 kg. To form lymphovenous and venovenous anastomoses, vessels of both the superficial and the deep lymphatic and venous systems were used. The diameter of the anastomosed vessels was from 2.5 to 3.5 mm for veins and from 0.6 to 1.2 mm for lymphatics. The operations were performed under sterile conditions in operating theaters, under general anesthesia. After an adequate depth of anesthesia had been achieved, 2 ml of methylene blue was injected into the soft tissues of the dog's hind limb, after which the skin covering the aponeurosis was divided through a longitudinal incision along the medial surface of the thigh, below Poupart's ligament, and the

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