IMMUNOHISTOCHEMICAL STUDY OF RAT LUMBRICAL MUSCLES AT DIFFERENT TIMES OF ISCHEMIA AFTER ALLOGRAFTING INTO ANTERIOR CHAMBER OF THE EYE

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KEY WORDS: ischemia; allografting; anterior chamber of the eye; lumbrical muscle; immunohistochemistry, myosins

Limb trauma is often accompanied by ischemic damage to tissues that differ in their sensitivity to ischemia. The tactics of subsequent surgical intervention is largely dependent on the severity of these changes and how reversible they are. The effect of ischemia on morphological and functional characteristics of skeletal muscle is being intensively studied [2, 4]. Skeletal muscle tissue is known to be relatively highly resistant to ischemia [3], but its powers of compensation are limited. Restoration of the blood flow after the development of irreversible ischemic damage in the muscle leads to the appearance of what is called the "inclusion" syndrome [1]. However, the critical times for the appearance of these irreversible changes are not yet clear. To discover these temporal parameters we used a model of allografting of a whole lumbrical muscle of a rat, subjected to ischemia of varied duration, into the anterior chamber of the eye (ACE).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 160-180 g. Each series of experiments involved five rats. The deeply anesthetized animals were decapitated and the hind limbs were amputated and kept at 21-23°C. The second or third lumbrical muscles were isolated 3, 5, 6, 7, 8, 9, 10, and 11 h after amputation and transplanted into ACE of a rat [6]. On the 3rd and 7th days of transplantation the host animals were deeply anesthetized with ether and decapitated, the graft was removed and transferred into freshly isolated liver, together with a normal lumbrical muscle. Succinate dehydrogenase (SDH) activity was determined histochemically in frozen sections 8 μ thick, and immunohistochemical staining (PAP method) with monoclonal antibodies (AB) to fast myosin heavy chains ("Sigma") was carried out [5]. Sections for morphological study were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

The rat lumbrical muscles on immunohistochemical staining had the appearance of mixed, predominantly fast, muscle fibers (MF). Determination of SDH activity in the muscle revealed fibers with high enzyme activity (C-type) and also intermediate B-fibers.

Both surviving MF and newly formed muscle tubes (MT) were found in the muscle graft exposed to 3, 5, or 6 h of ischemia, on the 3rd day of culture (Fig. 1a). After treatment with AB, fast and slow fibers were identified among MF, and formed two populations depending on their SDH activity: MF of types B and C. Meanwhile, the presence of fast myosin and high SDH activity, characteristic of MF of type C were demonstrated in MT. Incidentally, these histo- and immunohistochemical characteristics of MT were found in all series of our experiments.

After ischemia of the muscle for 7, 8, and 9 h, MT and single preserved MF were found in the grafts. Depending on their SDH activity, the MF corresponded to the C type and were not stained by AB to fast myosin. After longer ischemia (10-11 h) no definitive MF could be found (Fig. 1a), although MT were found in the graft.

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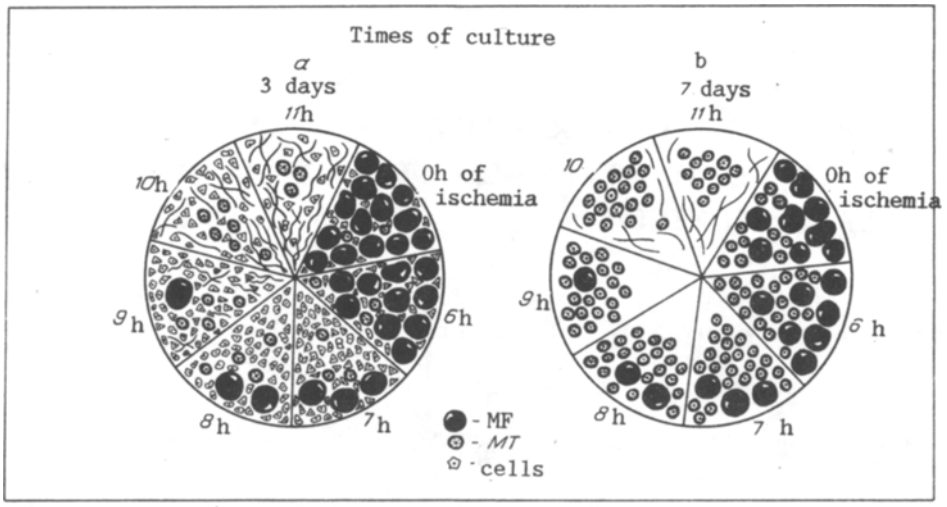

Fig. 1. Morphological characteristics of transplantation of rat lumbrical muscle: a) 3rd day, b) 7th day of culture in rat ACE after ischemia of varied duration.

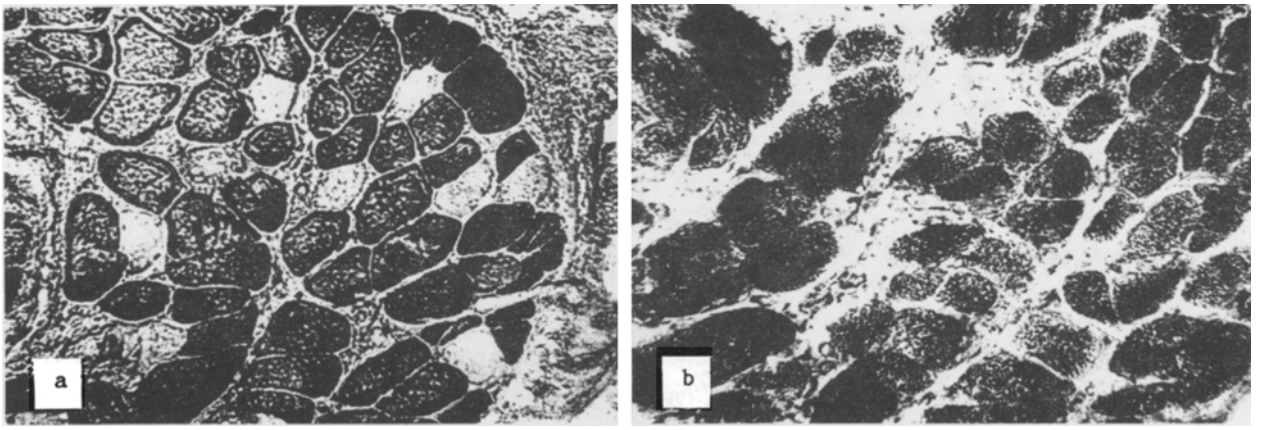

Fig. 2. Lumbrical muscles of rat after 6 h of ischemia on 7th day of culture: a) immunohistochemical staining with monoclonal AB to fast myosin heavy chains (pale MF are slow, dark MF are fast); b) SDH activity, fibers of B type.

On the 7th day of culture of the muscle (Fig. 1b), after 3, 5, and 6 h of ischemia the graft consisted of surviving MF and MT. Immunohistochemical staining revealed typical fast and slow fibers (Fig. 2a) just as in the 3-day graft and at the same periods of ischemia, but all had equal SDH activity, corresponding to the B type (Fig. 2b).

After 7-9 h of ischemia, MT and also solitary surviving MF were found in the 7-day graft (Fig. 1b). Depending on their SDH activity, the MF were identified as belonging to the B type, and staining with AB revealed no fast myosin in them. After 10-11 h of ischemia only MT were found in the graft.

It can be concluded from these results that after 6 h of ischemia the lumbrical muscle remained morphologically intact at both times of culture. If a muscle subjected to a longer period of ischemia (up to 9 h) was transplanted, only single surviving slow MF could be found in the preparation among MT. Hence it follows that irreversible damage probably takes place in the muscle in the interval between 6 and 9 h of ischemia, with the result that after a longer period of ischemia no MF capable of survival remain in the muscle. After death of the definitive MF, however, satellite cells forming MT are preserved in the graft.

Thus after 6 h of ischemia differentiated MF of the lumbrical muscle, transplanted into ACE, are still capable of recovery, but at the later stages of ischemia, regeneration of the muscle probably takes place through the activity of satellite cells.

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MORPHOLOGICAL EVIDENCE OF A POSSIBLE ROLE FOR CYTOCHROME P-450 IN THE DEVELOPMENT OF AUTOIMMUNITY IN THE LIVER

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In recent years there has been a marked increase in the number of patients with various forms of autoimmune pathology. Although significant progress has been achieved in the study of the pathogenesis of many autoimmune diseases [6], nevertheless the data on the etiology of these diseases are often extremely contradictory. In particular, great importance has been attached to the role of circulating immune complexes and autoantibodies in these processes [14]. As yet, however, no sufficiently convincing explanations of the causes of these widespread diseases have been established. Many previous investigations have shown that different pathological processes in the liver lead to a multicomponent disturbance of the integrity of its cells [13]. There is every reason to suppose that many acute diseases are transformed into chronic as a result of the addition of an autoimmune component, which thereafter plays an important role in the maintenance of inflammation. However, their probable trigger factors have not yet been explained. because we have no adequate model to reflect the mechanisms of onset of autoimmune diseases.

We accordingly decided to attempt to produce a model of autoimmune liver damage by injecting animals with paraquat, a herbicide which specifically activates lipid peroxidation (LPO) and which, because of this mechanism, induces cirrhosis of the liver [4].

EXPERIMENTAL METHOD

Mature male Wistar rats weighing 180-200 g were used. The animals received paraquat intraperitoneally in a dose of 60 mg/100 g body weight; control male rats received injections of sterile physiological saline. Liver samples from the

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