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The effect of Mercurascan on some enzymes of striated muscle in acute and chronic stages of ischaemia

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The mercurial derivative of fluorescein—Mercurascan (MSC)-Bis-hydroxymercurifluorescein—has been shown both experimentally and clinically to be a substance with many actions.

Testimony to the wide applicability of MSC is *inter alia* [1, 2] its action on myocardial metabolism in the acute stage of experimental ischaemia in the dog [3, 4]. It has been shown that MSC facilitates normalisation of ionic balance in the ischaemic focus as well as metabolic processes in tissue damaged by ischaemia.

Experimental ischaemia of the anterior tibial muscle of the rat results in metabolic defects which remain in the chronic stage [5, 6]. The present work investigates the effect of MSC on the activities of some enzymes of the metabolic cycle during acute, subacute and chronic stages of experimental ischaemia of striated muscle.

MATERIALS AND METHODS

The technique of producing ischaemia of the anterior tibial muscle in the rat induced by interruption of the right common iliac artery has been described elsewhere [7]. In the present experiments we used Wistar strain rats of mean body wt 200 g. The effect of MSC was tested in the acute (one week after ligation), subacute (two weeks) and chronic stages (11 weeks) of ischaemia. In the first two stages of ischaemia MSC was administered in a dose of 0.5 mg/kg body wt i.v. into the tail vein once daily over the entire period. In the chronic animals the same dose was administered by the same route twice weekly for 11 weeks. Control, sham-operated animals received only saline injections. Twenty animals in each group were used.

After decapitation 1, 2 and 11 weeks, muscle was quickly dissected, superficial fascia was removed along with the terminal tendons. The samples were then dried on filter paper, weighed, and each muscle was homogenised (Ultra-turrax-Kunkel & Janke) as a 1% suspension in physiological saline, pH 7, at 0°. After 1 hour's extraction at 0–3°, the homogenate was centrifuged at 0°, 2,000 *g*, and the supernatant used to determine the activities of malate dehydrogenase, lactate dehydrogenase, ATP-ase, acid phosphatase and carboxyl esterase, along with total protein content. The determinations—routine for this laboratory—were as follows: acid phosphatase(ACP) (E.C. 1.3.2) was determined by a modification of the method of Kaplan & Narahara [8], ATP-ase (E.C. 3.6.1.4) by a modified Bang & Nowotny method [9], carboxyl esterase (carb. est.) (E.C. 3.1.1.1) by the method of Seligman *et al.* [10], malate and lactate dehydrogenase (MDH, LDH) (E.C. 1.1.1.37, E.C. 1.1.1.27) by the neotetrazolium technique using phenazine-methosulphate as a direct electron acceptor (of 11). All activities were corrected for protein content as determined by the method of Lowry *et al.* [12] and Student's *t*-test was used to evaluate statistical differences to the level of $P < 0.05$.

RESULTS AND DISCUSSION

MSC administration affected mainly the activity of MDH in ischaemic muscle in acute, subacute and chronic stages (Fig. 1). Whereas in ischaemic muscle of untreated animals MDH activity was significantly depressed after one week, as well as in chronic stage, in the MSC-treated animals MDH activities remained in the normal (= control animals) range for up to 11 weeks (Fig. 1).

MSC also resulted in the ischaemic muscle in an increase in the activity of carb. est. and a normalisation of levels

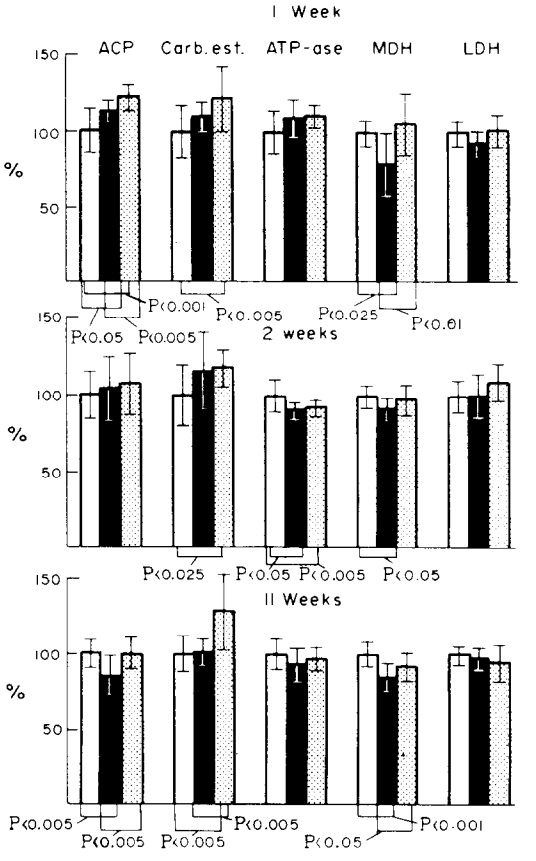

Fig. 1. The effect of MSC on Acid phosphatase (ACP), Carb. ester., ATP-ase, Malatdehydrogenase (MDH) and Lactatdehydrogenase (LDH) in the Anterior Tibial muscle, after 1, 2 and 11 weeks of ischaemia. White column—control muscle; Black column—ischaemic muscle without MSC; Hatched column—ischaemic muscle plus MSC. Average activity in the a.t.m. of control rats: ACP—1.6 μM phenol/100 μg prot./hr; Carb. ester.—2.13 μg β-naphtol/100 μg prot./hr; MDH—67.2 μg diform./100 μg prot./hr; LDH—340 μg diform./100 μg prot./hr; ATP-ase—1.20 μg P/100 μg prot./hr. Results are expressed as percentage differences, taking the mean activity of controls as 100 per cent (mean ± S.D.).

of ACP in the chronic stage of ischaemia (Fig. 1). The other enzymes investigated were not affected by MSC administration. The fact that after MSC injection there was no decrease in the activity of MDH—as a representative enzyme of the Krebs cycle—suggests a favourable metabolic effect of this substance. It is known that the Krebs cycle is one of the richest sources of macro-ergic phosphate bonds necessary not only for muscle work but also for many other metabolic processes, including protein synthesis. Normalisation of MDH activity in ischaemic muscle due to MSC administration indicates a renewal of energetic processes in the ischaemic muscle and is in agreement with previous findings on the effect of MSC in the acute stage of ischaemia of the myocardium [3, 4]. The effect of MSC on the activity of lysozomal enzyme-ACP and carb. est. can also be considered a biological advantage, since decreases in the latter in most cases are related to tissue damage. However, the limited scope of our experiment does not permit us to draw definitive conclusions.

Previous work has shown that in the chronic stage of ischaemia, when there is a return of blood flow through the muscle practically to normal levels, a number of enzyme activities remain altered [5, 6]. The possibility of influencing to the normalisation enzyme activities is for this reason an extremely important problem. Therefore, MSC can be considered to be a substance which facilitates reparation of metabolic processes rendered abnormal by ischaemia.

The mechanism of MSC action cannot yet be satisfactorily explained. On the basis of present knowledge the mechanisms should be related to its capacity for firm binding to damaged cells with a favourable effect on the mitochondrial membrane during myocardial transplantation in the dog [13]. According to Southard *et al.* [14] substances related to fluorimercuriacetate (FMA) have a suppressive-inductive action on mitochondrial function. This action has been explained by authors as an effect of FMA on endogenous mitochondrial ion transport. On a molecular basis, FMA can produce changes in the mitochondrial membrane in which some functions (e.g. oxidative phosphorylation, Ca^{2+} transport and phosphate accumulation) are suppressed, whereas others (e.g. ATP hydrolysis, K^+ and Mg^{2+} transport and configurational changes) are stimulated. These observations do not explain the metabolic function of MSC, but do indicate the complexities which will probably be involved.

The above results mainly demonstrate that the previously found favourable effect of MSC on the metabolism of the ischaemic myocardium in acute stage can be demonstrated also during acute and chronic stages of ischaemia of striated muscle.

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