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Muscle fibre necrosis induced by intramuscular injection of drugs

David Manor and Menachem Sadeh
Department of Neurology, The Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Israel

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Summary. A number of amphiphilic and lipid-soluble drugs of heterogeneous pharmacological properties, when injected into rat anterior tibial muscles, induced acute muscle fibre necrosis. The myotoxic agents were: penicillin, cloxacillin, phenobarbital, haloperidol, diazepam, hydantoin, metoclopramide, pentazocine and chlorpromazine. The regenerative process, studied using the latter three medications, showed rapid regeneration, complete within 3 weeks. Injection of the water-soluble drugs aminophylline, tranexamic acid and vitamins B_6 and B_{12} produced no tissue damage. The pathogenesis of muscle fibre necrosis is suggested to involve direct damage to cell membranes by lipid soluble drugs.

Keywords: muscle fibre necrosis, muscle fibre regeneration, intramuscular drug injection

Muscle fibre necrosis after intramuscular injection of local anaesthetics is a well known phenomenon (Benoit & Belt 1970; Hall-Craggs 1974, 1980; Jirmanova & Thesleff 1972; Schultz & Lipton 1978; Seibel *et al.* 1978). Observations by electron microscope show hypercontraction of sarcomeres several minutes after injection, followed by degenerative changes in the mitochondria and the nuclei, distortion of the tubular system, and damage to the sarcolemma, much of which disappears within 3 h. The basal membrane remains intact. One to 3 days after injection, macrophages infiltrate into necrotic muscle fibres. The regenerative process begins after 3 days, leading to complete reconstitution of muscle fibres 3 weeks later. Nerves and blood vessels remain intact, or show minor changes. The rapid necrosis and regeneration of muscle fibres after local anaesthetic injection, make it a widely used method for investigating these processes (Ishiura *et al.* 1983; Jones 1982; Rifenberick *et al.* 1979; Steer & Mastaglia 1986; Wagner *et al.* 1976; 1978).

Chronic administration of high oral doses of several compounds with different therapeutic actions including psychotropic drugs, tricyclic antidepressants and antihistamines caused a myopathy with muscle fibre necrosis, longitudinal splitting and vacuolization (Drenckhahn & Lüllmann-Rauch 1979). It has been suggested that myotoxic drugs by virtue of their being cationic amphiphilic molecules, interact with sarcolemma and organelles causing necrosis (Drenckhahn & Lüllmann-Rauch 1979; Kuncl & Wiggins 1988). Repeated antibiotic injections into the quadriceps muscles of infants (Norman *et al.* 1970; Bergeson *et al.* 1982)

Correspondence: Dr M. Sadeh, Department of Neurology, The Chaim Sheba Medical Center, Tel Hashomer 52621, Israel.

and self-injection of pentazocine or meperidine by narcotic addicts (Aberfeld *et al.* 1968; Steiner *et al.* 1973; Levin & Engel 1975) produced fibrous contracture of the muscles.

In our study, we tried to find out whether amphiphilic drugs previously established as inducers of myopathy by oral administration as well as other amphiphilic agents, antibiotics and narcotics, cause muscle fibre necrosis after intramuscular injection, as do local anaesthetics. We examined the degeneration and regeneration of muscle fibres after intramuscular injection of a number of these drugs and several others that are not amphiphilic, such as water soluble vitamins.

Materials and methods

Drugs were injected into the anterior tibial muscles of 3-month-old Sprague–Dawley rats. Seventy-seven rats were used in the experiments. Drugs and concentrations are shown in Table 1. The concentrations are similar to those being used clinically for human injections. For diazepam and phenobarbital a lower concentration was also tried. A dose of 0.5 ml of each was injected bilaterally into the anterior tibial muscles of three rats. Under mild ether anaesthesia a 25 gauge needle was inserted along the tibial bone, and the medication was administered during the withdrawal of the needle. Another similar injection was performed more superficially. This amount and mode of administration have been commonly used in studies with intramuscular local anaesthetic injections (Hall-Craggs 1974; Jones 1982). Five rats were similarly injected with saline (0.5 ml into anterior tibial muscles) and served as controls.

Three days after injection the rats were killed by overdose of ether. The muscles were dissected, mounted on gum tragacanth and quickly frozen in isopentane, cooled by liquid nitrogen. Sections 10 μm thick were cut in a cryostat, stained with haematoxylin and eosin, and examined by light microscope. Three medications, chlorpromazine, metoclopramide and pentazocine, were bilaterally injected into six rats and the muscles were excised and examined 3 h and 1, 3, 5, 10 and 21 days later. Six rats injected bilaterally

Table 1. List of drugs injected into rat's anterior tibial muscle

Generic name	Trade name (manufacturer)	Concentration (mg/ml)
Phenobarbital	Luminal (Winthrop)	130
Phenobarbital	Luminal (Winthrop)	13
Diazepam	Assival (Assia)	1
Diazepam	Assival (Assia)	5
Chlorpromazine	Taroctil (Taro)	10
Pentazocine	Talwin (Winthrop)	10
Metoclopramide	Pramin (Rafa)	5
Haloperidol	Halidol (Abic)	1
Phenytoin	Dantoin (Teva)	10
Aminophylline	Aminophylline (Teva)	10
Benzylpenicillin G	Benzylpenicillin G	330000*
Cloxacillin	Orbenine (Teva)	50
Vitamin B_1	Bereon (Teva)	50
Vitamin B_6	Besextan (Teva)	10
Tranexamic acid	Hexacapron (Teva)	100

* U/ml.

Table 2. Effect of pharmacological agents on muscle fibre, observed 3 days after intramuscular injection

Myotoxic agents	Non-myotoxic agents
Metoclopramide	Tranexamic acid
Penicillin	
Chlorpromazine	Vitamin B_1
Cloxacillin	
Pentazocine	Vitamin B_6
Haloperidol	
Phenytoin	Aminophylline
Phenobarbital	
Diazepam	

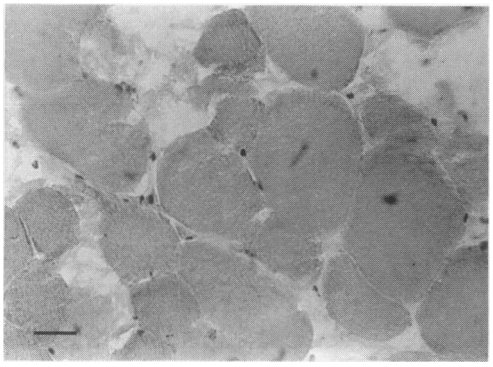

Fig. 2. Three hours after intramuscular metoclopramide injection, muscle fibres are rounded with non-homogeneous broken cytoplasm. Bar, 20 μm.

with saline served as controls. Sections from days 5, 10 and 21 were also stained with modified trichrome, PAS, Sudan black and with reactions for NADH-TR, ATPase at pH 9.4, and after preincubation at pH 4.6 and 4.3, and alkaline phosphatase.

Results

Results obtained 3 days after injection of various drugs are summarized in Table 2. Those that were myotoxic induced extensive muscle fibre necrosis with macrophage infiltration. The non-myotoxic drugs caused no damage, except for occasional minor injury along the needle track, similar to saline-injected control muscles (Fig. 1).

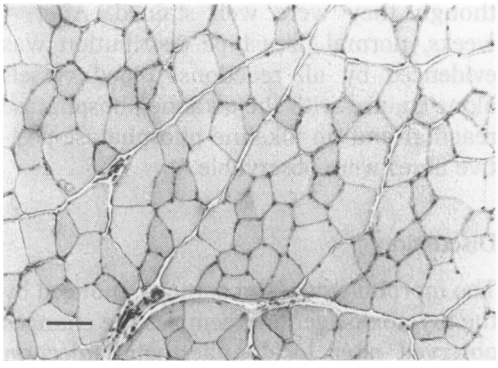

Fig. 1. Control muscle, 3 days after saline injection. Muscle fibres appear normally with no evidence for tissue damage. Bar, 50 μm.

The process of degeneration–regeneration, observed after intramuscular injection developed as follows. Three hours after injection of metoclopramide or pentazocine, muscle fibres showed extensive changes. Damaged fibres exhibited pyknotic nuclei and hyalinized cytoplasm, in which myofibrils were not discernible, and were more circular in outline than the hexagonal-appearing unaffected fibres (Fig. 2). In 24 h, affected fibres showed fragmented cytoplasm, and some appeared empty. At this time macrophages started to invade the fibres and phagocytose the remnants. At day 3 some fibres had become so completely disorganized that it was no longer possible to distinguish between myonuclei and infiltrat-

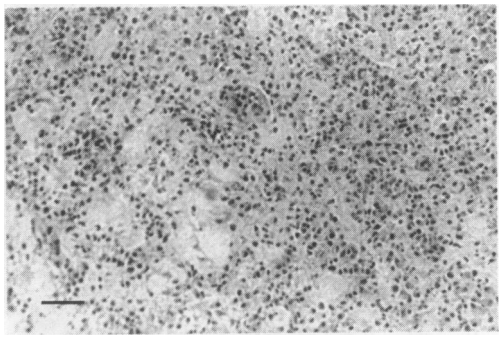

Fig. 3. Three days after injection, the section is crowded with numerous nuclei of infiltrating macrophages and myoblasts. Bar, 40 μm.

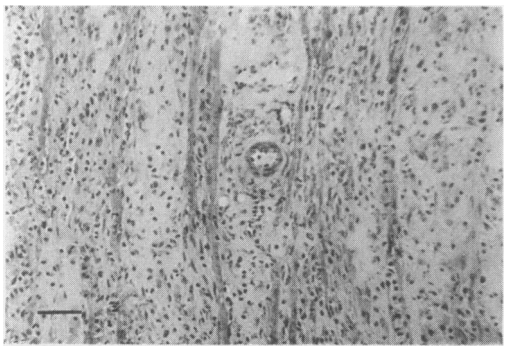

Fig. 4. Longitudinal section 3 days after injection, showing early myotube formation. Bar, 40 μm.

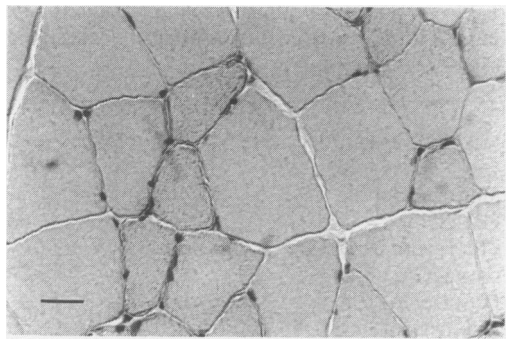

Fig. 6. Three weeks after injection, muscle has a mature appearance with peripheral nuclei. Bar, 20 μm.

ing cells (Fig. 3). In some areas, longitudinal sections showed newly formed myotubes with long chains of nuclei (Fig. 4). By day 5 numerous regenerating fibres characterized by a basophilic cytoplasm, vesicular nuclei and occasional prominent nucleolei appeared (Fig. 5). The regenerating fibres increased significantly in diameter at day 10, though many fibres still had centrally placed nuclei. Three weeks after injection the muscle appeared normal, except for some fibres with internal nuclei (Fig. 6). After intramuscular injection of chlorpromazine the same process of degeneration–regeneration occurred also but at a slower rate; 10 days later there were some necrotic fibres, invaded by macrophages. Repair was not complete, and after 3 weeks small areas of fibroblast proliferation and increased connective tissue were observed.

Histochemical studies of control tibialis anterior muscles showed an uneven distribution of fibre type throughout the cross-section. Ninety-six % of fibres were type 2 with a scattering of type 1 fibres; the latter were crowded in a small and deeper area. Five days after metoclopramide, pentazocine and chlorpromazine administration, no differentiation of fibre types could be observed with ATPase reactions and there was only a weak staining reaction for the NADH-TR. Ten days after injection the two fibre types could be clearly recognized with the ATPase reactions, but not with the NADH-TR, though they were well stained. After 3 weeks, normal fibre type distribution was evidenced by all reactions. Blood vessels alone stained with the alkaline phosphatase reaction and no alkaline phosphatase-positive fibres were observable.

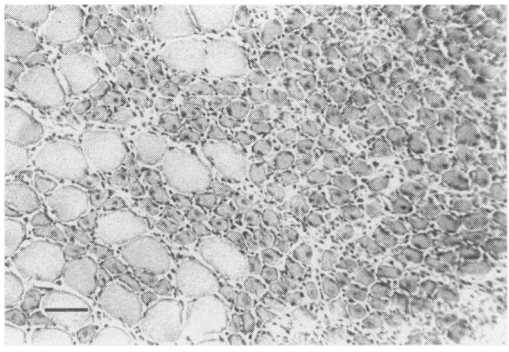

Fig. 5. Five days after injection, transition zone between regenerating fibres on the right and undamaged fibres on the left. Bar, 50 μm.

Discussion

The morphology of the damage, induced by the myotoxic agents, resembles the changes observed after local anaesthetic injection (Benoit & Belt 1970; Hall-Craggs 1974). Similar rapid regeneration, was demonstrated after the injection of three substances

used in our study. The complete recovery of the muscle after metoclopramide or pentazocine injection, suggests that these agents affect myocytes and spare other elements found in muscle tissue, i.e. basal membrane, satellite cells, blood vessels and nerves. The damage caused by chlorpromazine, on the other hand, may be less specific, affecting basal membrane or blood vessels and resulting in a mild degree of fibrosis.

It has been suggested that muscle fibre destruction by the local anaesthetic bupivacaine (Marcaine), is the consequence of its competition with calcium for special sites on the intracellular and surface membrane system; by increasing the concentration of calcium in the sarcoplasm, it causes the activation of proteolytic enzymes (Benoit *et al.* 1980; Kakulas & Raimond 1985; Nonaka *et al.* 1983). Other cells are less dependent on calcium-binding membranes and hence less sensitive to Marcaine (Schultz & Lipton 1978).

We have demonstrated that several drugs belonging to different pharmacological categories may cause injury similar to that caused by local anaesthetics. All the drugs found to be myotoxic are lipid-soluble and most of them are amphiphilic. All the non-myotoxic agents are water-soluble only. Thus the results of this study support the concept that amphiphilia and lipid-solubility are important for exhibiting the necrotizing effect, as previously suggested for orally administered drugs by Drenckhahn & Lüllmann-Rauch (1979) and Kuncl & Wiggins (1988). They proposed that cationic amphiphilic drugs interact with anionic groups of membrane and are adsorbed to the lipid–water interface. The pathogenetic mechanism of further membrane damage is unclear; however, it possibly eventually leads to a breach in its integrity. Membrane rupture, allowing calcium and other extracellular ions to enter the sarcoplasm, brings about muscle fibre necrosis. The large surface of the myocyte tubular system renders it much more susceptible to toxic agents than other cells.

Further investigation of the mechanism by which these pharmacological agents cause muscle fibre necrosis may elucidate some aspects of the pathogenesis of muscle damage in muscular dystrophies, in which the morphology is similar to that of drug-induced muscle fibre necrosis (Nonaka *et al.* 1983; Engel 1986).

Clinically, it seems that the local injury after a single intramuscular injection of the drugs studied, is fully reversible; however, frequent injections at the same site might cause permanent damage. In an experimental model, repeated injections of the local anaesthetic bupivacaine have produced chronic myopathy with fibrosis (Sadeh *et al.* 1984). In humans, repeated injections of antibiotics and narcotics have induced fibrous contractures (Norman *et al.* 1970; Bergeson *et al.* 1982; Aberfeld *et al.* 1968; Steiner *et al.* 1973; Levin & Engel 1975). Since narcotic-dependent persons often inject proximal muscles, this may masquerade as limb-girdle dystrophy (Choucair & Ziter 1984). It is worth noting, that there may be an elevation of serum creatinine kinase, following the injection of such a myotoxic agent.

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