A study of myonecrosis induced by the venom of the scorpion *Tityus serrulatus*

E. Luque, J.D. Martín, J. Peña, R. Roldan and R. Vaamonde
Department of Histology and Embryology, Faculty of Medicine, Córdoba, Spain

**Summary.** The pathogenesis of skeletal muscle necrosis produced by *Tityus Serrulatus* venom was studied by means of light microscopy and electron microscopy. Wistar rats were inoculated subcutaneously, at some distance from the muscles under study, with a sublethal dose of scorpion venom. Samples were taken of the tibialis anterior muscles of both rear legs, 2, 7 and 24 hours postinoculation. Light microscopy analysis after 2 hours revealed certain changes identified as «delta lesions», and also the presence of hyperconcentrated muscle cells. Electron microscopy confirmed these lesions and also enabled us to identify a degree of discontinuity in the plasma membrane with a persistence of the basal membrane. Hyperconcentrated fibers could still be observed 7 hours postinoculation. Histochemical analysis revealed high levels of calcium within the fibers. 24 hours after inoculation with the venom, numerous phagocytic cells were found in the degenerated fibers. Muscle cells were also found to have undergone alterations indicative of an ischemic process. The most characteristic finding 7 days postinoculation was the appearance of regenerative fibers. After thirty days the muscles regained their normal appearance. It is suggested that *Tityus Serrulatus* venom induces myonecrosis by means of a twofold action: direct action, which gives rise in the first place to a rupture of the plasma membrane, permitting a massive entry of calcium this being a key factor in the process of cell lesion and an assumed indirect action due to ischemia.

**Key words:** Myonecrosis - Scorpion venom - Skeletal muscle

**Introduction**

Skeletal muscle necrosis is the most common change occurring in various injuries such as the introduction of animal toxins. Myotoxic activity in venom has been widely reported in snakes (Harris, 1980; Ownby et al., 1982; Queiroz et al., 1984; Gutierrez et al., 1986), scorpions (Adam and Weiss, 1959; Del Pozo et al., 1966) and spiders (Tu, 1977; Ownby and Odell, 1983). The pathogenesis of these myonecrosis share certain common signs (Ownby and Odell, 1983).

*Tityus serrulatus* is the species responsible for most cases of scorpion sting in Brasil (Bucherl, 1953), where its venom has drastic effects, causing a large number of deaths (Bucherl, 1971). Scorpion venom produces histological changes in the cardiac muscle (Poon-King et al., 1963; Gueron et al., 1967; Kotnharai et al., 1976), and alterations to the smooth muscle have been reported by Daniel and Posey-Danifi (1983). Studies of modifications to the skeletal muscle are mostly of a physiological nature (Del Pozo, 1966; Parmas and Russell, 1967; Vital Brazil et al., 1973; Juimovich et al., 1982).

The purpose of this study is to analyse the pathogenesis of skeletal muscle necrosis induced by *Tityus serrulatus* venom, comparing it with myonecrosis induced by other animal venoms.

**Materials and methods**

**Experimental material**

The toxin used was the venom of the scorpion *Tityus serrulatus* (SIGMA), supplied in the form of lyophilised powder, and kept in a moisture-free atmosphere at 4°C until use. The experimental animals, 3-month-old Wistar rats weighing 200±10 g. received subcutaneous dorsal injections of 0.868 mg. venom (equivalent to the dose used by Bucherl in white mice), dissolved in 0.1 ml. of physiologic saline solution. After being anaesthetised with ether, rats were decapitated at intervals of 2h, 7h, 24h, 7d, and 30d after administration of the venom. They were divided into five groups corresponding to these five time-intervals and each consisting of 8 animals,
in addition to two control groups receiving 0.1 ml.
physiologic saline solution. The soleus muscles of both
rear legs were studied.

Light microscopy analysis

An initial sample of the proximal third of the muscle
was collected, cooled in liquid nitrogen (−160°C) and
subsequently subjected to ultrarapid freezing in isopentane,
for histological and histochemical examination. The
techniques used were: hematoxylin-eosin (Dubowitz
and Brooke, 1973), modified Gomori thichrome (Engel
and Cunningham, 1963), alizarin red (Pearse, 1972),
acridin orange (Sarnat, 1983), adenosin triphosphatase
ATPase at ph 9.4) (Dubowitz and Brooke, 1973) and
NADH-tetrazolium reductase (NADH-tr) (Dubowitz
and Brooke, 1973).

Electron microscopy analysis

A second sample from the middle third was collected
for analysis using electron microscopy. Samples were
fixed in 2.5% glutaraldehyde buffered at pH 7, and
subsequently postfixed in 1% osmium tetroxide and
embedded in araldite. Ultrathin sections were stained
with uranyl acetate and lead citrate, and observed
through a Phillips 400 microscope.

Quantification of histological changes

200 muscle fibres were counted in the central area of
each of the transverse sections of the muscles. The different
histological changes were classified quantitatively
according to the proportion of fibres in which they were
present: < 1% absence (-); 1-3% slight (+); 3-8%
moderate (++); > 8% severe (+++) (Table I).

Results

Control groups

No alterations were observed in rats injected with 0.1
ml. solution and sacrificed at the same times as
experimental animals.

Two hours post-inoculation

Focal lesions observed in many cells in peripheral
areas of the muscle fibre were subsequently identified by
electron microscopy as focal necroses (Fig. 1). Disruption
of the plasma membrane was observed in some fibres
with normal sarcoplasm, although the basal lamina
remained intact (Fig. 2).

Among the most characteristic morphological changes
was the presence of rounded, swollen, eosinophilic fibres,
which had a very dark appearance under histochemical
analysis. Both their morphology and their response to
histological and histochemical tests suggest that they may
be hypercontracted fibres, also known as «opaque» or
«hyaline» fibres (Cullen and Mastaglia, 1982). Since
hypercontraction seems to be caused by the presence of
calcium within the fibre, the alizarin red technique was used
to detect possible calcium deposits. The hypercontracted
fibres responded positively to this technique, staining
quite intensely (Fig. 3). Electron microscopy revealed
clumped sarcoplasm myofibrils, giving rise to increased
electron density (Fig. 4). This clumping also explains the
abnormal morphology of the myoichondria, some of
which showed very dense crests. Many small, randomly
located vesicles were observed in the affected areas, as a
result of the alterations of the sarcoplasmic reticulum.
The tubuli were found to have disappeared among the
myofibrillar mass, and myonuclei were compressed
against the plasma membrane.

In addition, although less frequently, fibres presented
a fragmentation of the sarcoplasm and increased
hematoxylin-eosin staining, together with a clear loss of
enzyme activity in histochemical reactions.

A small number of cells showed evident signs of
necrosis, with macrophage infiltration detected by
means of the analysis of acid phosphatase activity. No
regenerative fibres were observed.

Seven hours post-inoculation

No variation was observed in the number of
hypercontracted fibres. Fibres with fragmented
sarcoplasm, and others showing macrophage infiltration,
were still observed.

Some of the fibres studied were found to have clearly
defined peripheral intrasarcoplasmic areas showing
reduced staining with histochemical techniques (Fig. 5).
These areas showed no activity with ATPase = at ph 9.4
and NADH-tr. Electron microscopy revealed an alteration
of the myofibrillar pattern, with considerable Z line
streaming and a loss of recorded pattern. Significantly,
the transition from these to normal areas was quite
abrupt (Fig. 6). In some cases, megamitochondria were
observed in some areas of degeneration.

24 hours post-inoculation

After 24 hours, a notable decline was observed in the
number of hypercontracted fibres. Fibres began to
appear which, while similar in size and rounding to
hypercontracted fibres, differentiated in their response
to staining, presenting a pale sarcoplasm in response to
hematoxylin-eosin and modified Gomori trichrome, and
a decrease in, or total loss of, enzyme activity in response
to histochemical techniques. Some of these fibres
showed evidence of macrophage infiltration (Fig. 7). The
fact that this type of fibre was not observed in previous
groups, together with a notable fall in the number of
hypercontracted fibres in this group, suggests a
necrotic evolution of the hypercontracted fibres
described in previous groups. Ultrastructurally, these
fibres showed a homogeneous sarcoplasm in which it was
impossible to differentiate myofibrils; mitochondria
varied in size, with dark matrices and dense crests.
Nuclei were found to be pyknotic.
Fig. 1. 2 hours post-inoculation. Periphery of a muscle fibre showing clumped myofibrils which compress and deform mitochondria. A focal necrosis is visible in the centre. ×3,000
Fig. 2. 2 hours post-inoculation. Electron micrograph showing muscle fibre with discontinuity of the plasma membrane; the basal lamina remains intact. ×8,530
Myonecrosis induced by scorpion venom

Fig. 3. 2 hours post-inoculation. Two fibres with greater calcium content are visible in the centre. Alizarin red-S x400

<table>
<thead>
<tr>
<th></th>
<th>2 h.</th>
<th>7 h.</th>
<th>24 h.</th>
<th>7 d.</th>
<th>30 d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>«DELTA LESION»</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HYPERCONTRACTED FIBRE</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>NECROTIC MUSCLE FIBRE</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>INFILTRATED BY PHAGOCYTIC CELLS</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>ISCHEMIC MUSCLE FIBRE</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

< 1% absence (–); 1–3% slight (+); 3–8% moderate (+ +); > 8% severe (+ + +).
Fig. 4. 2 hours post-inoculation. Muscle fibre showing marked hypercontraction. Myofilaments form dense masses which compress mitochondria and myonucleus. ×3,000
Myonecrosis induced by scorpion venom

Some fibres were still found to have a fragmented sarcoplasm.

Some muscle fibres were found to contain peripheral areas lacking enzyme activity, similar to those described at 7 hours post-inoculation.

7 days post-inoculation

A considerable decrease was observed in the number of hypercontracted fibres. As in the previous group, some rounded and swollen fibres were observed, in which all histoenzymatic techniques revealed a pale sarcoplasm. These fibres were mostly found to be infiltrated by macrophages. Isolated cases were found of muscle fibres showing fragmentation of the sarcoplasm.

Although many fibres were normal in shape, others were smaller, with a basophilic sarcoplasm, centralized nuclei and increased oxidative enzyme activity. These stained strongly with ATPase and showed reddish spots with acid phosphatase. The use of a fluorescent microscope with the acridin orange technique revealed these to be regenerative fibres (Fig. 8).

Electron microscopy showed several activated satellite cells, characterised by an increased cytoplasm containing a large number of organelles and a few small, disorientated filaments.

30 days post-inoculation.

The samples in this group were of normal appearance, similar to those of the control groups. A few necrotic fibres were observed, together with some centralized nuclei.

Nervous structures showed no significant changes in morphology. Focal swelling was observed in the endothelial cells of the capillaries, together with destruction of membrane structures in the form of myeline figures (Fig. 9). The lumen of some bloodvessels was found to contain erythrocytes. These changes were infrequent, and observed at 2 and 7 hours post-inoculation. No variation was observed in vascular calibre by ultrastructural analysis, although this was not confirmed by morphometric studies.

The most significant clinical signs observed in the experimental animals were respiratory disorders and a conjunctival hemorrhage observed in all animals. Contrary to expectations, no hemorrhage was observed in the muscle. Clinical signs disappeared 3-4 hours after inoculation of the venom.
Fig. 6. 24 hours post-inoculation. Electron micrograph showing muscle fibre with intense destructuration of the upper area, and scattered streaming. Abrupt transition to normal area. ×3,900
Fig. 7. 24 hours post-inoculation. Muscle fibres with acid phosphatase activity and macrophage infiltration. Acid phosphatase × 400

Fig. 8. 7 days post-inoculation. Presumably regenerative muscle fibres showing orange fluorescence (arrow) due to high ribonucleic acid content. Acridin orange × 400
Fig. 9. 7 hours post-inoculation. Electron micrograph showing muscle capillary wall, with lumen occupied by erythrocytes. Visible cytoplasm swelling and myelina figures which are protruding towards the capillary lumen. ×8,530.
Discussion

In the pathogenesis of myonecrosis, the earliest signs in affected fibres seem to be related to the plasma membrane. 2 hours after inoculation of the toxin, many wedge-shaped lesions were observed in the muscle fibres. These have been called «delta lesions» and represent areas of cell degeneration located beneath portions of disrupted or discontinuous plasma membrane (Mokri and Engel, 1975).

Electron microscopy revealed that the plasma membrane was rapidly affected, even in fibres with normal sarcoplasm, discontinuity being found in the latter, although the basal lamina remained intact. Similar lesions have been reported for viper venom (Arroyo and Gutierrez, 1981; Arroyo and Cerdas, 1984; Gutierrez et al., 1984; Gutierrez et al., 1986) and for the venom of arachnids like the tarantula (Ownby and Odell, 1983). In all these cases, the plasma membrane was affected. This study does not enable us to determine the cause of the structural alteration of the membrane, but it is thought to be related to the phospholipase A2 reported by Venkaiah and Partasarathy (1983) existing in the venom of such arachnids.

The most important consequence of membrane alteration is the infiltration of calcium within cell. The increase of calcium within skeletal muscle fibres gives rise to hypercontraction and clumping of myofilaments (Gutierrez et al., 1984), as well as to the activation of calcium-dependent proteases and phospholipases responsible for increased cell degradation (Duncan, 1978; Trump et al., 1981). Due to the infiltration of calcium, many hypercontracted fibres were observed which either returned to normal or became necrotic according to the extent to which the sarcolemma was affected.

In studies of the action of viper venom on skeletal muscle, Arroyo and Cerdas (1983) and Gutierrez et al. (1984) report not only direct action of the toxin on the muscle fibres, but also an indirect effect due to ischemia. Alterations at 7 and 24 hours, consisting of intrasarcoplasmic areas showing no ATPase or oxidative activity, and focal areas of Z lines streaming, may correspond to what Karpati et al. (1974) describe as one of the models adopted by muscle cells in cases of ischemia.

It is well known that scorpion venom provokes significant alterations in vascular calibre, which may account for the ischaemic action. In this respect, Cheyml et al. (1976), studying the effect of 5 scorpion venoms on the vascular system, confirmed the existence of vasoconstriction due to the stimulating effect of the venom on the sympathetic ganglia, an action lasting from 30 minutes to 1 hour. Henriquez et al. (1978) report a strong suprarenal discharge due to the influence of the venom of the scorpion Tityus serrulatus. Malhotra et al. (1976) attribute renal damage produced by scorpion venom to arteriolar vasoconstriction.

The absence in our study of modifications in vascular calibre may be accounted for by the fact that, as Cheyml et al. (1976) have reported, vasoconstriction takes place between thirty minutes and 1 hour after inoculation of the toxin, whereas in the present study, experimentation began 2 hours after inoculation. Changes in the vascular endothelium were found to be minimal in this study, and no evidence of hemorrhage was found in the muscle, which suggests that the presence of ischemic fibres may be due to a functional disorder described by other authors.

Finally, this study shows that the venom of the scorpion Tityus serrulatus provokes myopathic changes in skeletal muscle when administered at some distance from it. It is believed that the venom affects muscle fibres in two ways: by direct action on the muscle fibre, and indirect action due to ischemia.

References


Myonecrosis induced by scorpion venom

myotoxin of Bothrops Asper. Experimental and Molecular Pathology 40, 367-379.


Accepted April 14, 1987