# Envenomation of *Trimeresurus flavoviridis* Venom and its Components, Phospholipase A2 Isozymes

Motoo KITANO,[1*] Masato HIRANO,[1] Makoto YAHATA,[1]
Eri UMEMURA,[1] Aichi YOSHIDA,[2] Shosaku HATTORI,[3]
Naoko UEDA,[4] Daisuke TSURU,[5] Motonori OHNO[4]

## Abstract

The lesions caused by sublethal doses of *Trimeresurus flavoviridis (Tf)* venom injected into the anterior thigh muscle (quadriceps femoris muscle; QFM) of rats were studied with paraffin sections. A dose of 50 µg of *Tf* venom produced a large area of necrosis in QFM together with neighboring muscles. Phagocytosis of necrotic remnants was followed by marked regeneration of the muscle fibers. Myonecrosis was microscopically evident 30 minutes after the injection, and over the following 72 hours the necrotic muscles remained acellular and devoid of inflammatory reaction except at the very edge where liquefaction necrosis associated with inflammatory infiltrate was marked from the beginning. Blood vessels within and outside the necrotic foci were often involved in inflammatory changes and thrombosis, to some degree. However, marked hemorrhage was never noted in and around the foci. Phagocytosis of debris proceeded from the periphery, and after 24 hours the periphery of the necrotic foci was replaced by granulaion tissue containing many regenerating myoblastic cells. Abscesses developed in the vicinity of the injection site in several rats receiving the crude venom, but never after injection of its phospholipase A2 isozymes, BPI or BPII. Muscle necrosis after envenomation of *Tf* venom seems due primarily to direct action of the venom, though vascular thrombosis and ischemia may contribute. Interestingly, in the rats, the myonecrotic *Tf* venom itself may cause marked and extensive activation of muscle fiber regeneration, and the venom seems to be a great myotoxin but not a hemorrhagenic toxin.

Key words: *Trimeresurus flavoviridis (Tf)*, Envenomation, Snake venom, Myotoxin, Skeletal muscle, Quadriceps femoris muscle (QFM), Myonecrosis, Regeneration, Myoblast, Phospholipases A2 isozymes, Hemorrhage

## Introduction

Envenomation of *Trimeresurus flavoviridis (Tf)* snake venom causes two main symptoms, one local and the other systemic in humans. The local symptoms are severe pain, swelling, hemorrhage and necrosis in compliance with the bitten extremity, result in permanent rhage (HOMMA and TU, 1971).

We have examined the short term effects of the various components of *Tf* venom on rat skeletal muscle, quadriceps femoris muscle (QFM), with closer attention to impairment, necrosis and regeneration of muscle fibers, the latter occurring even at the earlier stages. Moreover, the rats have shown a strong resistance to hemorrhage by envenomation of *Tf* venom. Renewed interest in this venom has stemmed from our recent studies of its action on muscle and vascular wall of the rat and from its fractionation of the venom, which may shed light on the pathogenesis of local lesions caused by the snake bites.

## Materials and Methods

### ***Venom and its phospholipase A2 isozymes***

*Tf* venom was collected in Amami-Oshima island and lyophilized. Its phospholipase A2 (PLA2) isozymes, [Asp49] PLA2, BPI and BPII, the latter two being [Lys49] PLA2s, were separated by conventional chromatographies at Sojo University (LIU *et al*, 1990; KIHARA *et al.*, 1992). The crude venom and PLA2 isozymes were weighed and dissolved in sterile physiological saline immediately prior to use.

### ***Rats***

Young adult female and male rats (80-100g) of F344 (Charles River Japan, Inc.,), Dark-Agouti (Shizuoka Laboratory Animal Center), Wistar/Furth (maintained in our laboratory by sister-brother mating) strains were divided into two to five groups, each group consisting of 6 rats (total; 90) (Table 1).

### ***Experimental procedures***

The rats of the first four groups were anesthetized with pentobarbital sodium (Nembutal: Abbot Lab, U.S.A.). The crude venom and three PLA2 isozymes (50 μg each) in 100 μl physiological saline were injected into each rat of four groups at the upper two-thirds of the right QFM. The fifth group, the control rats, were similarly injected with 100 μl sterile physiological saline.

The animals were allowed to survive for the periods ranging from 30 minutes to 72 hours and were sacrificed under ethyl ether anesthesia. Both the right and left hindlimbs and visceral organs including the brain were immersed for several days in 10% buffered

| Of snake (Tf) | Duration | (F344$^{*}$, DA, WF : f+m$^{**}$) | Necrosis | Inflammatory infiltration | Hemorrhage | Edema | Muscular regeneration | Inflammatory infiltration | Changes in the vascular walls$^{***}$ | Hemorrhage | Edema | Others[#] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crude Venom | 30m | 6 | -~+ | - | -~+ | + | - | - | - | - | + | - |
| | 6h | 6 | + | + | -~+ | + | - | -~+ | - | - | + | - |
| | 24h | 6 | ++~+++ | +++ | -~+ | ++ | + | ++ | -~+(Th) | -~+ | ++ | - |
| | 48h | 6 | ++~+++ | +++ | -~+ | ++ | ++ | ++ | -~+(Th) | -~+ | ++ | + |
| | 72h | 6 | ++~+++ | ++ | -~+ | ++ | ++ | ++ | -~+(Th) | -~+ | ++ | + |
| | Total | 30 | | | | | | | | | | |
| [Asp49] PLA$_2$ | 24h | 6 | ++ | ++ | -~+ | ++ | + | ++ | -~+(Th) | - | ++ | - |
| | 48h | 6 | ++ | ++ | -~+ | ++ | + | ++ | - | - | ++ | - |
| | Total | 12 | | | | | | | | | | |
| BPI | 24h | 6 | ++ | +~++ | -~+ | ++ | + | + | -~+(Th) | - | ++ | - |
| | 48h | 6 | ++ | +~++ | -~+ | + | ++ | + | -~+ | - | + | - |
| | Total | 12 | | | | | | | | | | |
| BPII | 24h | 6 | ++ | +~++ | -~+ | + | ++ | + | -~+ | - | ++ | - |
| | 48h | 6 | ++ | +~++ | -~+ | + | +++ | + | + | - | ++ | - |
| | 72h | 6 | + | + | -~+ | + | +++ | + | - | - | ++ | - |
| | Total | 18 | | | | | | | | | | |
| Control (physiological saline) | 30m | 6 | - | - | - | - | - | - | - | - | - | - |
| | 24h | 6 | - | - | - | - | - | - | - | - | - | - |
| | 48h | 6 | - | - | - | - | - | - | - | - | - | - |
| | Total | 18 | | | | | | | | | | |

kinds of toxins, among three strains of rats and among female and male rats, so we preferred to describe here all together.

Macroscopically, there was a more or less marked necrotic change with a mild swelling of the muscle mass in the venom-injected right hind leg. A close examination of injected QFM revealed an absence of marked hemorrhage (Fig 1).

Histologically, the muscle tissue injected with physiologic saline solution had a typical histology of skeletal muscle with no abnormality in muscle fibers, nerves or blood vesseles.

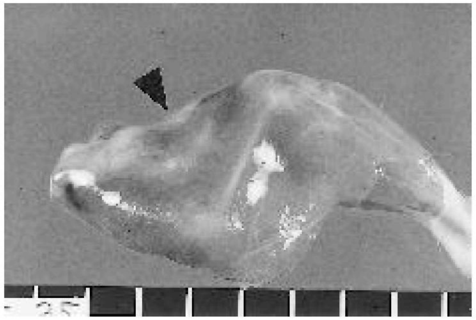

Fig. 1: A large necrotic lesion in the QFM of a F344 male rat 48 hours after the inoculation of the crude venom of *Tf*. Note the severe and extensive atrophy of the skeletal muscles of the anterior thigh due to necrotic changes without a marked hemorrhage.

There were groups of affected cells accompanied by the decrease of fine cytoplasmic structures to some degree, which were located in the periphery of the muscle as early as 30 minutes after inoculation. At this time edema without inflammatory cells were observed, and many of the skeletal muscle cells presented edematous swelling (Fig.2).

Necrosis of muscle fibers in QFM was indicated by nuclear pyknosis and fragmentation of the myofibrils into homogenous eosinophilic masses separated by empty-looking segments 6 hours after injection. There were many areas where necrotic cells predominated. Some of them appeared to be in the initial stages of cell degeneration, i.e. some muscle cells had only edematous swelling, whereas other cells were in a more advanced stage of damage with the formation of dense clumps of myofibril alternating with cellular spaces apparently devoid of myofibrillar material. The findings support the view that the earliest alterations seem to take place in the periphery of the necrotic focus. A very mild inflammatory infiltrate was observed at this time.