

Nuclear microscopy of normal and necrotic skeletal muscle fibers: an intracellular elemental microanalysis

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Abstract

The *in situ* total elemental composition and elemental concentrations present in mouse soleus (type I) and gastrocnemius (type IIA) muscle fibers were analyzed by using nuclear microscopy (NM). Elemental changes in necrotic fibers, induced by intramuscular injection with snake venom (*Pseustesichis australis*), were also studied 3 h post-injection. Nuclear microscopy is a new method based on nuclear technology that utilizes the interaction between a million-electron-volt nuclear particle beam and the muscle sample (in the case of the present study). Elemental analysis was done at the parts per million (ppm) level of sensitivity on unfixed, rapidly frozen and unstained single fast- and slow-twitch muscle fibers with imaging capabilities of μm spatial resolution and in multi-elemental mode. In total, 12 different intracellular elements were mapped, co-localized and analyzed in single normal and necrotic skeletal muscle fibers from mice. Elements such as potassium, sulfur, phosphorus, chlorine and sodium were found in concentrations from 1000 to 18 000 ppm. Unlike conventional electron-probe X-ray microanalysis, NM also detected and analyzed the trace elements such as magnesium, calcium, iron and zinc that were found in concentrations of 50 to 1000 ppm. Other elements — copper, manganese and rubidium — were also detected in concentrations of less than 50 ppm. The trace elements calcium, iron and zinc were more abundant in the soleus than the gastrocnemius (the level of iron was statistically significant). Calcium, sodium and chlorine were significantly elevated in venom-induced necrotic soleus muscle fibers. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Nuclear microscopy; Fast and slow muscle; Trace element analysis; PINI; Calcium; Necrotic fibers.

1. Introduction

Various elements in skeletal muscle play a role in normal biochemical processes of muscle function as well as in the pathological mechanisms of myonecrosis (Bowen, 1966; George, 1995; Gopalakrishnakone et al., 1997). Diseases like muscular dystrophy, hypokalemic periodic paralysis, myotonia congenita and malignant hyperpyrexia may be due to changes in intracellular and extracellular concentrations of ions such as Ca^{2+} , Na^+ , K^+ and Cl^- (George, 1995). The role of Ca^{2+} , Na^+ , K^+ and Mg^{2+} ions in the contraction and functioning of skeletal muscle has been reported extensively. Calcium is an important ion both in the physiology and the pathology of the cell. Calcium was also implicated in venom-induced myonecrosis (Gopalakrishnakone et al., 1997). The venom of *Pseustesichis australis* snake is rich in myotoxic phospholipase A_2 fractions and is capable of inducing skeletal muscle necrosis in both man

and experimental animals (Rowlands et al., 1969; Chen et al., 1993; Ponraj and Gopalakrishnakone, 1995; Gopalakrishnakone et al., 1997).

Mammalian twitch muscle fibers are mainly classified as slow-twitch fibers (type I — e.g., soleus) and fast-twitch fibers (type IIA — e.g., fast oxidative gastrocnemius; and type IIB — e.g., fast glycolytic extensor digitorum longus). These two fibers not only differ functionally, but also have different structural, biochemical as well as histochemical properties (Ruegg, 1992). The slow-twitch soleus muscle is non-fatiguing and appears red owing to its high myoglobin and mitochondrial contents. It is low in lactic dehydrogenase, creatine kinase, T-system and sarcoplasmic reticulum, but rich in actomyosin ATPase, succinic dehydrogenase and carbonic anhydrase III (Zheng et al., 1992). The fast-twitch gastrocnemius muscle is white because of its low myoglobin and mitochondrial levels, and fatigues easily. The susceptibility to myonecrosis also differs between slow and fast muscles, the soleus muscle being more susceptible than gastrocnemius to animal venoms and toxins (Duchen, 1970, 1971).

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