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Chained nuclei and python pattern in skeletal muscle cells as histological markers for electrical injury

Hiroki Tanaka[a], Katsuhiro Okuda[a], Seiji Ohtani[a,*], Masaru Asari[a], Kie Horioka[a], Shotaro Isozaki[b], Akira Hayakawa[c], Katsuhiro Ogawa[d], Shiono Hiroshi[a], Keiko Shimizu[a]

[a] Department of Legal Medicine, Asahikawa Medical University, Japan
[b] Department of Surgery, Hitachi Corporation Hospital, Japan
[c] Tokyo Medical Examiner's Office, Tokyo Metropolitan Government, Japan
[d] Department of Pathology, Asahikawa Medical University, Japan

ABSTRACT

Electrical injury is damage caused by an electrical current passing through the body. We have previously reported that irregular stripes crossing skeletal muscle fibers (python pattern) and multiple small nuclei arranged in the longitudinal direction of the muscle fibers (chained nuclear change) are uniquely observed by histopathological analysis in the skeletal muscle tissues of patients with electrical injury. However, it remains unclear whether these phenomena are caused by the electrical current itself or by the joule heat generated by the electric current passing through the body. To clarify the causes underlying these changes, we applied electric and heat injury to the exteriorized rat soleus muscle *in situ*. Although both the python pattern and chained nuclear change were induced by electric injury, only the python pattern was induced by heat injury. Furthermore, a chained nuclear change was induced in the soleus muscle cells by electric current flow in physiological saline at 40 °C *ex vivo*, but a python pattern was not observed. When the skeletal muscle was exposed to electrical injury in cardiac-arrested rats, a python pattern was induced within 5 h after cardiac arrest, but no chained nuclear change was observed. Therefore, a chained nuclear change is induced by an electrical current alone in tissues in vital condition, whereas a python pattern is caused by joule heat, which may occur shortly after death. The degree and distribution of these skeletal muscle changes may be useful histological markers for analyzing cases of electrical injury in forensic medicine.

1. Introduction

Electrical injury is caused by direct contact with any source of electricity, including the household use of an electric power source. Contact with electric sources often results in electric burn marks on the skin and severe damage to various organs located along the flow-line of the electric current. An electric current passing through the organs that are critical to sustaining life, such as the heart or central nervous system, often causes electrocution [1]. When an electric current passes through living organs, damage to the organs results from both the electric current itself and the joule heat caused by the high electrical resistance of the organs and tissues. In cases of electrocution, examining the electric current pathway is important for forensic diagnosis. The input and output regions of the electric current can easily be determined if electric burn marks on the skin are externally observed. In contrast, when the skin is wet (e.g., due to sweating) or completely immersed in liquid, the electric burn marks on the skin may be absent because of the low electrical resistance of the skin. Consequently, histopathological changes in the tissues along the electric current pathway may provide critical information about electrical injury in such cases.

We have previously reported that irregular stripes crossing muscle fibers (python pattern) and small nuclei arranged in the longitudinal direction of muscle fibers (chained nuclear change) are uniquely observed in the skeletal muscle cells of patients with electrical injury [2]. If these pathological changes reflect the vital reactions during electric injury, they would be useful markers for the forensic diagnosis of fatal electrocution. Nevertheless, it remains unclear whether these phenomena are caused by the electric current itself or by the joule heat generated by the electric current. In this study, we investigated the causal factors underlying the python pattern and chained nuclear change by using an animal model in which rat soleus muscle cells were exposed to electric or heat injury *in vivo* and *in vitro*. We found that the chained nuclear change is caused by the electric current, whereas the python pattern is caused by joule heat and that both are reactions to an

* Corresponding author at: Department of Legal Medicine, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido 078-8510, Japan.
E-mail address: smho@asahikawa-med.ac.jp (S. Ohtani).

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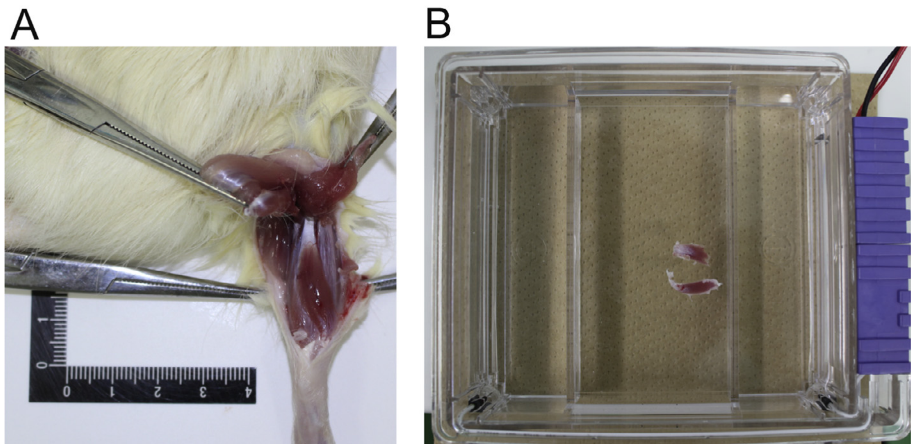

Fig. 1. A. Exteriorization of the rat soleus muscle. B. *Ex vivo* electrophoresis.

electric current passing through skeletal muscle tissues.

2. Materials and methods

2.1. Animal experiments

Male Wistar rats (8 weeks old) were purchased from Sankyo Labo Service Corporation (Tokyo, Japan). In order to know the direct effects of electric current and heat on the skeletal muscle, the rats were anesthetized with pentobarbital, and the soleus muscles were exteriorized. For electric current injury, conducting wires were placed in direct contact with the soleus muscles, and an alternating current (AC) of 100 V for 1 s was applied twice (Fig. 1A). For heat injury, a metallic bar heated to 100 °C was placed in direct contact with the soleus muscles for 1 s, and this was repeated twice (Fig. 1A). After the treatments, these rats were immediately sacrificed by pentobarbital injection, then the soleus muscle samples were taken. These experiments were also performed in rats subjected to cardiac arrest by potassium chloride injection. The soleus muscles of the cardiac-arrested rats were exposed to electric current or heat injury immediately and 0.5, 1, 2, 3, 4, 5 and 6 h after cardiac arrest. For this procedure, 3 rats were used in each experiment. These experiments were conducted according to the Asahikawa Medical University guidelines for the humane care of experimental animals.

2.2. Ex vivo exposure to electric current

The rat soleus muscles were removed from the body and were immediately placed in an electrophoresis tank containing 0.9% sodium chloride solution in Tris/Acetate/EDTA buffer (Fig. 1B). A constant voltage of 100 V current was then applied to the tissues for 15 min and 30 min. During the electric current flow, the temperature of the solution was maintained at 40 °C.

2.3. Histological analysis

All tissues were fixed in a phosphate-buffered formaldehyde (3.7%) solution and processed for paraffin embedding. The 3.5-μm-thick paraffin sections were stained with hematoxylin-eosin (H&E) and phosphotungstic acid and hematoxylin (PTAH). For the morphological analysis, we observed at least 100 myofibrils on the sections. Then, we scored the frequencies of the python pattern and chained nuclear change as follows: 3+, observed in more than 30% of myofibrils; 2+, observed in 20–30% of myofibrils; +, observed in 1–10% myofibrils; and −, not observed.

3. Results

3.1. Electric current injury in the soleus muscle in vivo

The python pattern, which consists of irregular stripes crossing the muscle fibers (Fig. 2A), and chained nuclear change, which is characterized by small-sized nuclei arranged in the long axis of the muscle fibers (Fig. 2B), were induced by electric current injury in the soleus muscle *in vivo* (Table 1). Analysis of the python patterns by PTAH staining revealed as myofibril hypercontraction band necrosis. Under anesthesia, approximately 20% of the muscle fibers in the FFPE specimens exhibited both the python pattern and chained nuclei change. These two histological patterns appeared close together. The python pattern was also induced in the soleus muscle in the cardiac-arrested rats during the 0–5 h after cardiac arrest (Table 1). However, although the chained nuclear change was observed immediately after cardiac arrest, it was not detected at 0.5 h or later in the cardiac-arrested rats (Table 1).

3.2. Heat injury in the soleus muscle in vivo

The python pattern was induced by heat injury in the soleus muscle *in vivo* (Table 1). This change was also observed in the tissues that received heat injury immediately and 0.5 h after cardiac arrest (Table 1). However, the chained nuclear change was not observed in the heat injury experiments in live and cardiac-arrested rats (Table 1).

3.3. Electric current flow in the soleus muscle ex vivo electrophoresis

The chained nuclei change was observed in 1–10% of the soleus muscle fibers after 100 V flow for 15 min and 30 min at 40 °C *in vitro* (Fig. 3A, B). When the electrophoresis was performed for 30 min, the chained nuclei changes showed a similar morphology presented in electric current experiments *in vivo* (Fig. 3A). On the other hand, when the electrophoresis was performed for 15 min, some of these showed the large spaces between each nucleus indicating the middle phase of complete formation of the chained nuclei changes (Fig. 3B). However, the python pattern was not observed in this condition, even though PTAH staining.

4. Discussion

In this study, we investigated the mechanism(s) underlying the python pattern and chained nuclear change in skeletal muscle cells after electric injury. Analysis of the python pattern by PTAH staining revealed substantial hypercontraction band necrosis. These morphological changes have been reported to be vital muscle reactions after

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