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Short Communication

Comparison of histological findings and the results of energy-dispersive X-ray spectrometry analysis in experimental electrical injury

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ABSTRACT

The findings of histological examination and the results of energy-dispersive X-ray spectrometry (EDX) analysis were compared to identify skin metallization in experimental electrical injury. Rats were divided into three experimental groups (n = 5, each group): control, current exposure for five seconds, and current exposure for ten seconds. A relatively high peak of copper, which was used as an electrical conductor, was detected in formalin-fixed skin samples of the two current exposure groups by EDX. There was a significant increase of the specific X-ray intensity in the two current exposure groups compared to the control group. On histological examination, epidermal nuclear elongation was observed in all samples of the two current exposure groups. However, deposition of metal was observed in two samples of each current exposure group. Metallization is an important finding for the diagnosis of electrocution. The present results suggest that EDX analysis is useful for the proof of metallization in electrocution, even where it is not identified on morphological examination.

1. Introduction

It is well known that autopsy findings in electrocution cases are nonspecific because most deaths by electricity are caused by cardiac arrhythmias or respiratory arrest [1]. Detection of current marks is an important finding for the diagnosis of electrocution [1,2], but they may not be recognizable by the naked eye in some cases [1,3–5]. Therefore, the diagnosis of fatal electrical injuries is usually based on histological findings, such as nuclear elongation and blister formation in the epidermis [1–7]. However, these findings are also observed in thermal injuries and are not specific to electrical injuries [1–7].

When the skin is exposed to electricity, electrolysis occurs, conductor ions may be embedded in the skin, and metallization can occur [1,2]. The metallization has diagnostic value in cases of electrocution because it is observed only in skin through which current has passed [2–5]. However, it is sometimes difficult to detect metallization by histological examination [3].

When the atom receives X-ray irradiation, the characteristic X-ray is emitted. Since the characteristic X-ray has a unique set of energies for each element, we can identify the element in the sample [8–11]. Energy dispersive X-ray spectrometry (EDX) analysis is a method to detect the characteristic X-ray and it is a simple and convenient method to detect various elements in various types of samples [8–11]. It has been reported that EDX is useful not only for the detection of metallic elements in paraffin-embedded tissue [12,13], but also in formalin-fixed tissue [14,15].

A comparison of histological findings and EDX analysis in experimental electrical injury is reported.

2. Material and methods

2.1. Animal experiments

All animal experiments were approved by the Kagawa University Animal Investigation Committee. Seven-week-old male Wistar rats (Charles River Laboratories Japan, Inc., Yokohama, Japan) (n = 15, weighing 200–215 g) were enrolled in this study. All rats were divided into three experimental groups (n = 5, each group): control; current exposure for five seconds (5-s); and current exposure for ten seconds (10-s). All experiments were performed under deep anesthesia (mixture of 0.375 mg/kg of medetomidine, 2 mg/kg of midazolam, and 2.5 mg/kg of butorphanol i.p.).

The conductor was made from 5-mm-diameter copper. For the two current exposure groups, after shaving the hair of the chest, the current-

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carrying equipment was put on the chest and electrified for five or ten seconds with 100 V. The rats were sacrificed following the current application. The skin of the chest was immediately collected and fixed with formalin. For the purpose of biosafety, we used formalin fixed tissue.

For the control group, after shaving the hair of the chest, the current-carrying equipment was just put on the chest for five seconds. Then, each rat was sacrificed by the excessive dose of anesthetic administration. The skin of the chest was collected and fixed with formalin.

The samples were divided into two sections each. One was used for EDX analysis, and the other was used for histological examination.

2.2. EDX analysis

A Rayny EDX-720 system (Shimadzu, Kyoto, Japan) was used for detection of elements from formalin-fixed tissues. Operating conditions for the EDX were as follows: target, Rh anode; operating voltage, 50 kV; X-ray path, air; detector, Si (Li); and measurement time, 100 s. For the examination by EDX, the formalin solution was wiped off each tissue, which was placed directly on a sample cup (Double open-end X-RA, Shimadzu, Kyoto, Japan) with polyester thin-film (Mylar film, Shimadzu) [12,13]. The sample cup was placed on the sample stage of the equipment without any further preparation. Confirmation of the characteristic X-ray was performed using the Kα lines of copper (8.04 eV). The X-ray intensity was taken as the average of three measurements.

2.3. Histological examination

Hematoxylin and eosin (HE) and rubeanic acid staining of the skin were performed in all experimental groups. Nuclear elongation and blister formation in the epidermis and deposition of copper were examined in all samples.

2.4. Statistical analysis

The X-ray intensities of copper of the three groups were compared. JMP® 11.0 (SAS Institute Inc., Cary, NC, USA) software was used with the Kruskal-Wallis test and the Steel-Dwass test. A p value < 0.05 was considered significant.

3. Results and discussion

The EDX spectra of skin samples of the three experimental groups are shown in Fig. 1. A relatively high peak of copper was detected in the skin samples of the two current exposure groups compared to the control group. The X-ray intensities (cps/μA) of copper of the skin samples in the control group, 5-s group, and 10-s group were 0.43 ± 0.05, 1.77 ± 0.62, and 2.20 ± 0.68 (mean ± SD), respectively. The X-ray intensities of copper of these skin samples were significantly increased in both the 5-s and 10-s groups compared to control ($p < 0.05$, respectively) (Fig. 2), but there was no significant difference between the 5-s and 10-s groups.

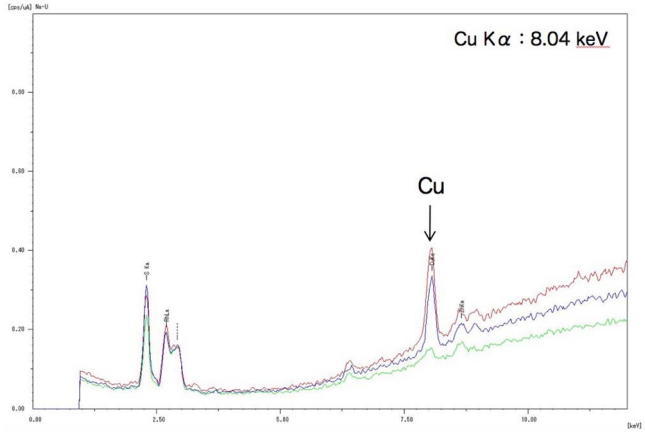

Fig. 1. EDX spectrum of the formalin-fixed tissues (green line, control group; blue line, current exposure for 5-s group; red line, current exposure for 10-s group).

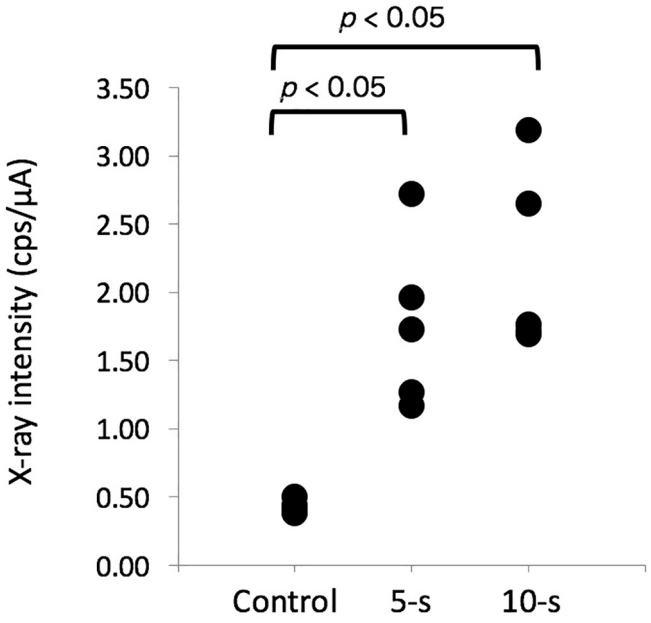

Fig. 2. The X-ray intensity of copper (Kα) of the formalin-fixed tissues of the three groups. There are significant differences between the 5-s and control groups ($p < 0.05$) and between the 10-s and control groups ($p < 0.05$).

The number of samples that showed elongation of cell nuclei and blister formation in the epidermis and deposition of copper by staining with rubeanic acid on histological examinations can be seen in Table 1. Elongation of cell nuclei in the epidermis was observed in all samples of the two current exposure groups, and blister formation of the epidermis was observed in two samples of the 5-s group and all samples of the 10-s group (Fig. 3c and e). Rubeanic acid staining was positive in two samples each of the two current exposure groups (Fig. 3d and f). No morphological changes were observed on both HE and rubeanic acid staining in the control group (Fig. 3a and b). The detection rate of metallization was 40% in each current exposure group.

In this study, a relatively high peak of copper that was used as a conductor in the two current exposure groups was detected by EDX analysis. The X-ray intensities of copper in the current exposure groups were significantly higher than in the control group. On histological examination, elongation of cell nuclei in the epidermis was observed in all skin samples of the two current exposure groups, but metallization was not observed in some skin samples of these groups.

Metallization is a specific finding in electrical injuries, and

Table 1
Summary of histological findings of the samples.

Group (total number of samples)	Number of samples		
	Nuclear elongation	Blister formation	Deposition of copper
Control (5)	0	0	0
Current exposure			
5-s (5)	5	2	2
10-s (5)	5	5	2