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Original communication

Cardiac histopathological and immunohistochemical changes due to electric injury in rats

Nagwa M. Ghandour, MD Assistant Professor [a], Abeer E. Refaiy, MD Assistant Professor [b], Ghada Ali Omran, PhD Lecturer [a,*]

[a] Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Assiut University, Assiut City 71524, Egypt
[b] Department of Pathology, Faculty of Medicine, Assiut University, Egypt

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ABSTRACT

It has been a puzzling forensic task to determine the cause of death as a result of electric shock in the absence of recognizable skin marks or definite postmortem morphological findings. In forensic pathology, while classical macroscopic and microscopic morphology remain core procedures to investigate deaths, a variety of subsidiary measures has been developed and incorporated to detail that pathology. C-fos, one of a small group of genes called primary response genes and its protein product, fos, are crucial elements of complex signaling mechanisms believed to be responsible for cell response to stimulation. It has been found that c-fos plays a significant role in myocardial lesions, and has close relation to injury repair of the molecule. The aim of this study was to detect the histopathological findings in the myocardium after fatal and non-fatal electrical injury in rats and to investigate the potential role of c-fos expression using immunohistochemistry to distinguish antemortem from postmortem electrocution. Forty adult female rats were implemented and randomly divided into four groups (A, B, C and D). Group (A) rats were subjected to instantaneous antemortem electricity and their hearts were collected either immediately (A_1) or after an hour (A_2) before being subjected to cervical dislocation. Group (B) rats were electrically injured instantaneously postmortem, hearts were collected immediately (B_1) or an hour later (B_2) while Group (C) rats were electrified up to death, and their hearts were also gathered either immediately (C_1) or after an hour (C_2) from electrocution. Lastly, another group of rats served as a control group (Group D). Subgroup (D_1): rats were clamped but not electrified, before death and another group of rats were clamped but not electrified, after being killed by cervical dislocation. Sections from the hearts of all groups were fixed in formalin and routinely processed. The c-fos oncogene expression was evaluated in all groups by immunohistochemistry. Significant histopathological findings were detected in groups A and C. Few c-fos oncogene protein positive cardiomyocyte nuclei were seen in rats of groups (A_1) and (B_1). Additionally, increased expression in rats of groups C_1, C_2 and A_2 were observed. On the other hand, no c-fos protein expression was seen either in the control (groups D_1 and D_2) or in group B_2. Significant differences ($p < 0.001$) in c-fos expression were observed among rats of groups with antemortem electric injury (A_1, A_2) and those of postmortem injury (B_1 and B_2). Thus, in addition to classical histopathological methods, c-fos can be regarded as a target in identifying electrical injury, and can be used as an indicator to distinguish antemortem from postmortem electric shock.

1. Introduction

It is a challenging forensic task to determine the cause of death in an electrocuted victim without detectable current marks on the skin.[1] In order to find an effective way for diagnosis of these cases, forensic pathologists have been making lot of efforts to resolve this problem.[2] It is a well-known fact that electricity can cause death or any degree of damage to various organs and systems according to the type, voltage and intensity of the electrical current and the location of damage. The electrical shock may strike the victim's central nervous system, the cardiovascular system, the skeletal muscular tissue, the lungs, the skin and other internal organs.[3] Cardiac arrest can also be induced by a number of mechanisms with little or no tissue damage.[4] The principal cause of death was described by Michiue and associates in 2009[5] as cardiac failure due to ventricular fibrillation caused by a direct effect of the electric current.

* Corresponding author. Tel.: +20 88 2413295.
E-mail addresses: ghada_ali@hotmail.com, ghada_ali@yahoo.com (G.A. Omran).

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In forensic pathology, while classical morphology remains a core procedure to investigate deaths, a spectrum of ancillary procedures has been developed and incorporated to detail the pathology.[6] C-fos, one of a small group of genes called primary response genes and its protein product, fos, are integral components of complex signaling mechanisms believed to be responsible for cell response to stimulation. The effects of many types of stimulation including drug-induced seizures, activation of receptors, growth factors, neuroactive drugs, electrical stimulation, and physiological states have been studied.[6] The expression of c-fos is known to be increased in particular diseases and pathophysiological processes, indicating that it may play a role in the pathogenesis of some diseases. In rat models of myocardial stunning (MS), the expression of fos protein increased apparently, i.e. c-fos plays a significant role in myocardial lesion, and has close relation to injury repair of the molecule.[7]

The aim of this study was to evaluate the effect of fatal and non-fatal electric injury in rats, to characterize the pattern of the structural myocardial changes after electric injury, to study the immunohistochemical expression of c-fos in heart and to evaluate if it could be used as an indicator to distinguish antemortem from postmortem electricity.

2. Materials and methods

2.1. Animal groups and experimental design

The experimental procedures were carried out after ethical approval according to the National Institute of Health Guidelines for Animal Care.[8] A total of 40 healthy female Wistar Albino rats of 4–5 months old (with average weight 200 ± 50 g) were recruited. The animals were maintained under temperature 22 °C, a 12 h light/dark cycle, ad libitum availability of pellet food and water. The experimental groups were randomly divided into four groups and dealt with as follow: rats subjected to antemortem electricity (group A), rats subjected to postmortem electricity (group B), the third group was exposed to electricity up to death (group C), lastly the control group (group D). The rats were subjected to electric current according to the method described by Wang et al.[9] Two metal clamps were connected to a pole of 220 V alternating current. One clamp was connected to rats left hind limbs and other to right forelimbs. All the animals in whole groups were anaesthetized via ether inhalation before being electrified and/or cervically dislocated. The rats within the control group were only anaesthetized before killing but not electrified.

Group (A): Ten rats were subjected to instantaneous (for 5 s) antemortem electricity. This group was divided randomly into two subgroups. Group (A_1): five rats were subjected to cervical dislocation and the hearts were collected *immediately*. Group (A_2): five rats *were left alive for* 1 h from electrical injury and then subjected to cervical dislocation before hearts collection

Group (B): Ten rats were electrically injured instantaneously (for 5 s) postmortem, after death by cervical dislocation. This group was divided randomly into two subgroups. Group (B_1): hearts were collected *immediately* in 5 rats. Group (B_2): hearts were collected after 1 h from electrical injury in the other 5 rats.

Group (C): Ten rats were electrified up to death, also divided randomly into two subgroups; each subgroup consisted of 5 rats. Group (C_1): hearts were collected *immediately*. Group (C_2): hearts were collected *after* 1 h after electrocution.

Group D (the control group): Ten rats were divided randomly into two subgroups; each subgroup consisted of 5 rats. Group (D_1): rats were clamped (for 10 s), but not electrified, *before* death by cervical dislocation. Group (D_2): were clamped (for 10 s), but not electrified, *after* being killed by cervical dislocation.

2.2. Histopathological and immunohistochemical examination

Sections from collected hearts were fixed in formalin and routinely processed. Five micrometer (μm) sections were cut and stained with Hematoxylin-Eosin (H&E). The tissue sections were observed under light microscope (Olympus, Tokyo, Japan) for detection of histopathological changes. Immunohistochemistry (IHC) was performed according to manufacturer's protocol and as previously described by Zhang et al.[8] Tissue sections (4-μm thick) of formalin-fixed, paraffin-embedded specimens were deparaffinized, rehydrated, and transferred to phosphate buffered saline (PBS; pH 7.6). The slides were rinsed twice with PBS, and then endogenous peroxidase was blocked by the hydrogen peroxide for 5 min. Antigen retrieval was done by boiling the slides in citrate buffer (pH 6) for 12 min. Then the slides were washed three times with PBS before being incubated overnight with c-fos rabbit polyclonal primary antibody (Cat No E4460 Spring Bioscience Ca USA) at a dilution of 1:50. The slides were then rinsed three times with PBS and incubated for 10 min at room temperature with the biotinylated goat antipolyvalent antibody (Thermo Scientific, Fremont, USA). After that, they were rinsed with PBS for three times and incubated for 10 min with streptavidin peroxidase (Thermo Scientific, Fremont, USA) at room temperature. Washing with PBS, and diaminibenzidine were applied for 5 min. Thereafter, the slides were rinsed in distilled water (DW), counterstained with Mayer's Hematoxylin, dehydrated and then mounted. Positive control for c-fos antibody was sections from skin as c-fos is expressed in nuclei of epidermis of skin and also in epithelium of sweet glands. Negative control slides were performed by omitting the primary antibody. A distinct brown nuclear staining was scored positive.

2.3. Interpretation of the histopathological findings

The term myofibre break-up included the following histological patterns as was described by Finechi et al.,[3]: (1) bundles of distended myocardial cells alternating with hyper-contracted cells. (2) Myocardial nuclei in the hyper-contracted cells have a "square" aspect rather than the ovoid morphology seen in distended myocytes. (3) hyper-contracted myocytes alternated with hyper-distended cells that are often divided by a widened disc. The above mentioned histopathologic changes were scored blindly for all rats belonging to electrocuted and control groups.

2.4. Interpretation of the immunohistochemical expression of c-fos

A brown nucleus indicated positive expression of c-fos oncogene protein in cardiomyocytes. Brown–yellow particles in the cytoplasm indicated positive expression of c-fos oncogene mRNA. The number of positive nuclei of five high-power fields was calculated under light microscope after Zhang et al.,[7] Counting was undertaken in 50 fields and the average was calculated.

2.5. Statistical analysis

All data were expressed as mean value ± standard deviation (SD). To analyze significant differences between groups one-way ANOVA followed by Tukey's post hoc test for multiple comparisons were employed. A probability level (p) < 0.05 was considered significant.