JOURNAL OF SURGICAL RESEARCH 188 (2014) 250–259

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Improved survival rate by temperature control at compression sites in rat model of crush syndrome

Takefumi Nakayama, MD,[a] *Masanori Fujita, MD, PhD,*[d,*] *Masayuki Ishihara, PhD,*[b,e] *Miya Ishihara, PhD,*[b,e] *Sho Ogata, MD, PhD,*[c] *Yoritsuna Yamamoto, MD, PhD,*[d] *Masafumi Shimizu, MD,*[f] *Tadaaki Maehara, MD, PhD,*[a] *Yasuhiro Kanatani, MD, PhD,*[g] *and Shoichi Tachibana, MD, PhD*[d]

[a] *Department of Surgery, National Defense Medical College, Tokorozawa, Saitama, Japan*
[b] *Department of Medical Engineering, National Defense Medical College, Tokorozawa, Saitama, Japan*
[c] *Department of Pathology and Laboratory Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan*
[d] *Division of Environmental Medicine, National Defense Medical College Research Institute, National Defense Medical College, Tokorozawa, Saitama, Japan*
[e] *Division of Biomedical Engineering, National Defense Medical College Research Institute, National Defense Medical College, Tokorozawa, Saitama, Japan*
[f] *Tokorozawa Meisei Hospital, Tokorozawa, Saitama, Japan*
[g] *Department of Health Crisis Management, National Institute of Public Health, Wako, Saitama, Japan*

ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form
7 December 2013
Accepted 13 December 2013
Available online 18 December 2013

Keywords:
Crush syndrome
Rat model
Compression
Warming
Hyperkalemia
Reperfusion injury

ABSTRACT

Background: Crush syndrome (CS) has been reported in disasters, terrorist incidents, and accidents, and the clinical and pathologic picture has gradually been clarified. Few lethal and reproducible animal models of CS with use of a quantitative load are available. A new model is needed to investigate pathologic and therapeutic aspects of this injury.

Materials and methods: Using a device built from commercially available components, both hindlimbs of anesthetized rats were respectively compressed for 6 h using 3.6-kg blocks. The effects of trunk warming alone without compressed hindlimbs (Group A), non-warming at room temperature (Group B), whole-body warming including compressed hindlimbs (Group C), or warming of compressed hindlimbs alone (Group D) during compression were examined. Survival rates were compared and hematological and histologic analyses were performed at specific time points after compression release.

Results: Limb or whole-body warming significantly worsened the survival of rats. We found a much lower survival rate of 0%–10% in animals, in which the hindlimbs were warmed during compression (Groups C and D) at 12 h after compression release, compared with 90%–100% in animals without warming of the hindlimbs (Groups A and B). Groups C and D showed significantly enhanced hyperkalemia at ≥4 h after compression release and all blood samples from dead cases showed hyperkalemia (>10 mEq/L).

* *Corresponding author*. Division of Environmental Medicine, National Defense Medical College Research Institute, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. Tel.: +81 4 2995 1626; fax: +81 4 2991 1612.
E-mail addresses: bxb01424@nifty.com, fujitama@ndmc.ac.jp (M. Fujita).
0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2013.12.012

Conclusions: We developed a new lethal and reproducible rat CS model with a quantitative load. This study found that warming of compressed limbs worsened the survival rate and significantly enhanced hyperkalemia, apparently leading to cardiac arrest.

1. Introduction

Crush syndrome (CS) was first reported clinically by Bywaters and Beall [1] in 1941 during World War II, based on the observation that victims pinned down among rubble in aerial bombing showed crush wounds and developed severe acute renal failure within a week. CS has subsequently been reported in disasters [2–4], terrorist incidents, [5] and accidents [5], and the clinical and pathologic picture has gradually been clarified [5–7]. In Japan, after the Great Hanshin-Awaji Earthquake in 1995, CS was diagnosed in 372 of 6107 hospitalized cases, and the mortality rate was 13.4% [2]. In the great Turkish earthquake in 1999, CS was diagnosed among 639 of 5302 hospitalized cases, and the mortality rate was 13.6% [3,4]. CS occurs after a prolonged period of compression of part of the body, followed by reperfusion injury with release of compression [5–7]. The high concentration of myoglobin released from necrotic muscle cells causes hyperglobulinemia, which progresses to acute renal failure with renal tubular necrosis [5,7]. Furthermore, simultaneous release of a high concentration of potassium causes hyperkalemia and may finally cause cardiac arrest [8].

The main form of therapy for CS is massive provision of fluid with hemodialysis immediately after the release of compression [9–11]. However, ensuring early rescue and treatment of victims is difficult during a disaster [2,3,9]. Thus, despite the increased knowledge and improved treatment, the outcomes of CS remain poor [2–4].

Several animal models of CS injury have been reported [12–15] mainly using the hindlimbs as the site of injury. Duncan and Blalock [12] used a special apparatus consisting of two boards in a dog model, whereas Akimau *et al.* [13] used a specially manufactured apparatus for a rat model (survival rate, 41.7%). Bywaters and Popjak [14] used rubber tourniquets for rabbits and Murata *et al.* [15] used similar tourniquets in a rat model (survival rate, 25%). All of these studies used a special apparatus to create the animal CS model, and thus limited the reproducibility of conditions and findings from the model.

In surgery, systemic warming is recommended to prevent hypothermia and surgical site infection [16,17] and relieve pain [17,18]. Warming is cost-effective and useful to maintain blood flow, tissue oxygenation, and euglycemia [17], and low back warming alleviates systemic inflammation [19] and breathing workload [20] and improves renal function [21,22]. In contrast, hypothermia or cooling is recommended to alleviate ischemic–reperfusion injury [23]. Furthermore, icing the compressed sites after compression release have retarded proliferation and differentiation of satellite cells at the early stage that might result in alleviation of ischemic–perfusion injury [24]. The tolerance time of skeletal muscle was reported to be 2.25 h at 34°C and 5 h at 26°C [25]. The ischemic tolerance time depends on ambient temperature. In this study, we happened to find that the warming of compressed hindlimbs substantially turned down the survival rates and increased blood potassium concentrations in the rat CS injury model. Therefore, the aim of this study was to establish lethal and non-lethal rat models of CS with controlling temperature of compressed hindlimbs.

2. Materials and methods

2.1. Experimental system components

We developed a new device (Fig. 1) to create a crush injury model. A pole 20 mm in diameter and 300 mm in height (PO-20-300; Sigma Koki, Tokyo, Japan) was vertically fixed to a magnetic base (MB-CB-PB; Sigma Koki) with a rod stand (RS-20-60; Sigma Koki). The magnetic base is firmly attached to a flat board (FB-545-50; Sigma Koki). A rectangular wooden board (Agathis, 250 × 80 × 10 mm) was fastened by three-pronged clamps (small double-leaf clamp, No. 91-2447-4; Sansyo, Tokyo, Japan). The clamp was fixed to the pole with a cross clamp connector (CCHN-20-12; Sigma Koki) at a right angle. The board and clamp move smoothly up and down the pole. The total weight of the loading part (including the board and clamp) is 340 g. Three interlocking blocks (Earthen Bricks, 198 × 98 × 30 mm and 1.2 kg each; Tokyo Electric Power Environmental Engineering, Tokyo, Japan) were loaded on the board and placed on the each hindlimb with the edge of the board on the thigh just caudal to the inguinal ligaments. Loads were placed flat using a level gauge (G-Director Level, ED-20GDLMR; Ebisu, Niigata, Japan). Using this experimental setup, a quantitative load could be used for limb compression.

2.2. Anesthesia and surgical procedure

All animal experiments were carried out according to the protocol approved by the Animal Experimentation Committee at the National Defense Medical College (Tokorozawa, Saitama, Japan). Ambient temperature was kept at 25 ± 2°C during the experiments. Male Sprague–Dawley rats weighing 290–320 g (9 wk old) were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan) and given *ad libitum* access to food and water. Animals were lightly sedated in an ether-filled anesthesia box. After sedation, intraperitoneal anesthesia was performed using a 27-gauge injection needle. The anesthetic agent was a 5 mg/mL solution of pentobarbital sodium (Somnopentyl, 64.8 mg/mL; Kyoritsu Seiyaku, Tokyo, Japan) in physiological saline, injected at an initial dose of 50 mg/kg body weight. For blood sampling, a catheter (Atom Indwelling Feeding Tube for Infant 3 Fr; Atom Medical, Tokyo, Japan) was indwelled and fixed *via* the left intracarotid artery through a midline neck incision before the experiments were conducted. The catheter was filled with heparinized saline. The heparinized saline solution (10 U/mL) was made from