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Ultrastructural Changes of Rat Cardiac Myocytes in a Time-Dependent Manner After Traumatic Brain Injury

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We suggest an ultrastructural scoring system to evaluate the degree of damage in a time-dependent manner in cardiac myocytes after traumatic brain injury (TBI).

Forty Wistar-Albino female rats weighing 170–200 g were randomly allocated into five groups. Group 1 was the control and Group 2 was the sham-operated group. Group 3, Group 4 and Group 5 were trauma groups. Weight-drop technique was used for achieving TBI. Lipid peroxidation was estimated by thiobarbituric acid test. An electron microscopic scoring model was used to grade the subcellular changes.

Results of heart injury score (HIS) showed that the 24-h trauma group had statistically significant levels in nuclear damage compared with the other groups ($p < 0.05$). Sarcoplasmic reticulum and mitochondria scores of all trauma groups were significantly different from the control and sham groups ($p < 0.05$). The results showed that lipid per oxidation levels were statistically significant different between the control and all trauma groups ($p < 0.05$).

The electron microscopic scoring model worked well in depicting the traumatic changes, which were supported by lipid peroxidation levels. Traumatic brain injury produced obvious gradual damage on the ultrastructure of the cardiac myocytes and this damage was more significant in the 24-h trauma group.

Key words: Heart, histology, rat, reactive oxygen species

Abbreviations: *, Perinuclear edema: s, dilated SR: o, large edematous areas in the cytoplasm of the myocytes: N, increased heterochromatine in nucleus: old arrow, mitochondrial swelling: TEM, transmission electron microscopy: magnification ×7500, bar represents 1 μm.

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Introduction

From autopsy studies of patients dying at various periods after sudden increases in intracranial pressure, it appears that focal myocardial damage requires at least 6 h to develop, and 8–12% of patients dying from acute cerebral lesions show such cardiac damage (1). Approximately 10% to 20% of subjects with traumatic brain injury (TBI) who have no evidence of cardiac contusion or thorax trauma have myocardial dysfunction severe enough to exclude the heart from organ donation (2). On the other hand, up to 23% of the potential donor hearts are rejected owing to hemodynamic instability of the brain-dead donor (3). However, the relative importance of brain injury and death-related cardiac dysfunction on post-transplantation cardiac function and the reversibility of the observed changes remain controversial.

We previously reported for the first time that electron microscopy can be used to score time-dependent ultrastructural changes in tracheobronchial epithelia after TBI (4). Although a variety of ultrastructural pathological features of TBI on cardiac myocytes have been described previously, the effects of isolated TBI on cardiac myocytes' ultrastructure in a time-dependent manner has not been performed. In the present study, we evaluated whether isolated experimental TBI causes the time-dependent ultrastructural changes in cardiac myocytes, and propose an electron microscopic scoring model for the myocardial injury.

Materials and Methods

Surgical procedure

The local Institutional Animal Care Committee approved protocols used in this study. Male Sprague-Dawley rats, weighing 170–200 g, were placed in prone position. The surgical procedure was performed under general anesthesia induced by intramuscular 10 mg/kg of xylasine (Bayer, Istanbul, Turkey) and 60 mg/kg of ketamine hydrochloride (Parke Davis, Istanbul, Turkey). Following midline longitudinal incision, the scalp was dissected

over the cranium and retracted laterally. Coronal and sagittal sutures were observed. Right frontoparietal craniectomies were carried out lateral to the sagittal suture by a dental drill system. The dura was exposed and left intact. One-forty g/cm impact trauma was produced by the method of Allen (5). Rats were injured by a stainless steel rod (weighing 140 g, 5 mm in diameter) weight dropped vertically through a calibrated tube from a height of 10 cm onto the exposed dura. Scalp was sutured with silk sutures. Body temperature was continuously monitored during the whole procedure with a rectal thermometer and maintained at 37 °C using a heating pad and an overhead lamp. Rats were neither intubated nor ventilated between brain injury and heart sampling.

Sample obtaining from the heart

Two, 8, and 24 h after trauma for trauma groups and 24 h after sham operation for sham group, rats were re-anesthetized with the a combination of ketamine and xylasine. Rats were placed supine on an operating table. Midline sternotomy was performed. The systemic circulation was perfused with 0.9% NaCl. Samples for lipid peroxidation and electron microscopy were obtained from the left ventricle. Then, the rats were killed by decapitation under general anesthesia. Heart samples were collected in randomly numbered containers and given to the blinded observers. After evaluating the numbered tissues, the results were collected in the appropriate group lists.

Experimental groups

The rats were randomly allocated into five groups:

- Group 1: control group (n = 8): tissue samples were obtained immediately after midline sternotomy and no head surgery was performed.
- Group 2: sham-operated group (n = 8): scalp was closed after craniotomy and no trauma was given. Tissue samples were obtained 24 h after surgical interventions.
- Group 3: 2-h trauma group (n = 8): TBI of 140 g/cm was produced. Tissue samples were obtained 2 h after trauma.
- Group 4: 8-h trauma group (n = 8): TBI of 140 g/cm was produced. Tissue samples were obtained 8 h after trauma.
- Group 5: 24-h trauma group (n = 8): TBI of 140 g/cm was produced. Tissue samples were obtained 24 h after trauma.

Transmission electron microscopy

The specimens were fixed in 2.5% gluteraldehyde for 24 h, washed in phosphate buffer (pH: 7.4), postfixed in 1% osmium tetroxide in phosphate buffer (pH: 7.4) and dehydrated in increasing concentrations of alcohol. Then the tissues were washed with propylene oxide and embedded in epoxy-resin embedding media. Semi-thin sections approximately 2 μm in thickness and ultrathin sections approximately 60 nm in thickness were cut with a glass knife on a LKB-Nova (Nova, Bromma, Sweden) ultramicrotome. Semi-thin sections were stained with methylene blue and examined by a Nikon Optiphot (Nikon, Tokyo, Japan) light microscope. Ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate and examined with a Joel JEM 1200 EX (Joel, Tokyo, Japan) TEM. Five sections were examined per animal. In every section 50 fields were selected by the electron microscopist randomly blinded. Ultrastructural data were evaluated based on each subcellular change and the data were collected and estimated as heart injury score (HIS) (Table 1). This model was modified from the scoring model used in our previous study (4).

Lipid peroxidation assay

The samples were thoroughly cleansed of blood and were immediately frozen and stored in a – 20 °C freezer for assays of malondialdehyde. The level of lipid peroxidation in the heart muscle was determined using the method of Mihara and Uchiyama (6). Tissues were homogenized in 10 volumes (w/v) of cold phosphate buffer (pH 7.4). Half a milliliter of homogenate was mixed with 3 mL of 1% H3PO4. After the addition of 1 mL of 0.67% thiobarbituric acid, the mixture was heated in boiling water for 45 min. The color was extracted into n-butanol, and the absorption at 532 nm was measured. Using tetramethoxypropane as the standard, tissue lipid peroxidation levels were calculated as nanomole per gram of wet tissue.

Table 1: Heart injury scoring system for quantitative evaluation of ultrastructural findings

Category	Score
Nucleus	
Normal	0
Irregular chromatine distribution	1
Increased heterochromatine	2
Degenerated nucleus	3
Mitochondria	
Normal	0
Mitochondrion with prominent cristae	1
Mitochondria with edema	2
Collection of amorphous material	3
Sarcoplamic reticulum	
Normal	0
Dilated in some regions	1
Presence of vacuoles	2
Largely degenerated areas	3

Statistical analysis

All data collected from the experiment were coded, recorded, and analyzed by using SPSS 10.0.1 for Windows. ANOVA for parametric data and Kruskal-Wallis variance test for nonparametric data were used for comparing differences among groups. When analysis of variance showed a significant difference, the posthoc multiple comparison test was applied to demonstrate the differences in the groups. Between each test, the data were expressed as the mean value ± standard (SD) and $p < 0.05$ was accepted as statistically significant.

Results

In the TEM examination of the cardiac myocytes of the left ventricle in the control and sham groups, no ultrastructural pathology was observed (Figure 1). In the 2-h trauma group, a perinuclear edema was observed in the cardiac myocytes. Additionally, cardiac myocytes had dilated sarcoplasmic reticulum and swollen mitochondria were observed in this group (Figure 2). In the 8-h trauma group, in the muscle tissue of the left ventricle wall, a more prominent perinuclear edema was seen. Additionally, cardiac myocytes had dilated sarcoplasmic reticulum and swollen mitochondria were seen in this group (Figure 3). The most striking findings were observed in the 24-h trauma group. Large edematous areas were present in the cytoplasm of the cardiac myocytes and, additionally, myocytes had dilated sarcoplasmic reticulum. The presence of swollen mitochondria was another ultrastrustural finding. Interestingly, in some of the nuclei of the cardiac myocytes, heterochromatine was increased (Figure 4).

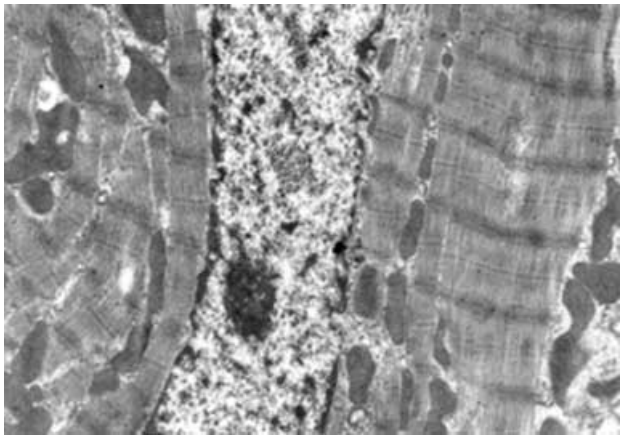

Figure 1: Transmission electron microscopy (TEM) showing no ultrastructural pathologic findings in the control group (original magnification ×7500).

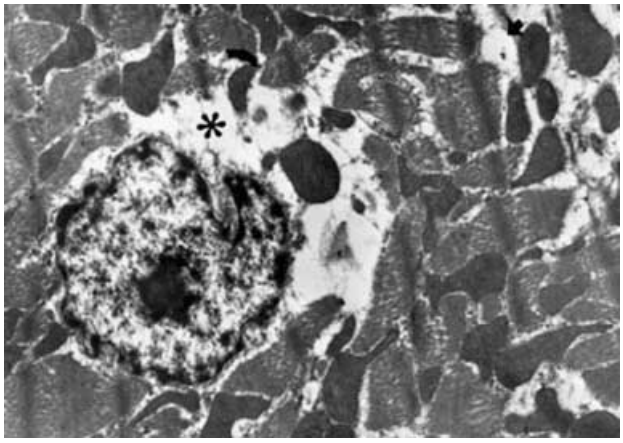

Figure 2: Transmission electron microscopy (TEM) showing, in the 2-h trauma group, a perinuclear edema in the myocytes, dilated sarcoplasmic reticulum and swollen mitochondria (original magnification ×7500).

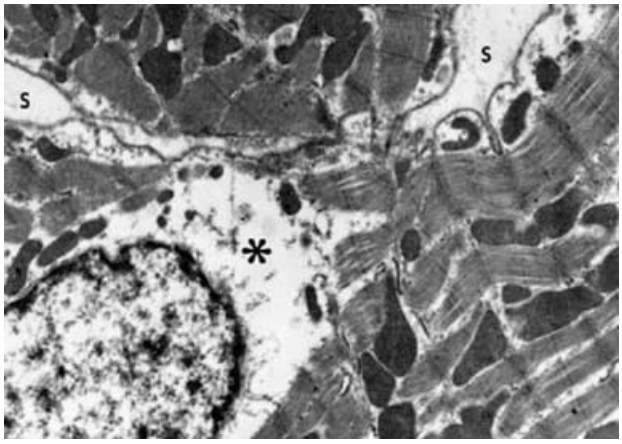

Figure 3: Transmission electron microscopy (TEM) showing, in the 8-h trauma group, a more prominent perinuclear edema, dilated sarcoplasmic reticulum and swollen mitochondria (original magnification ×7500).

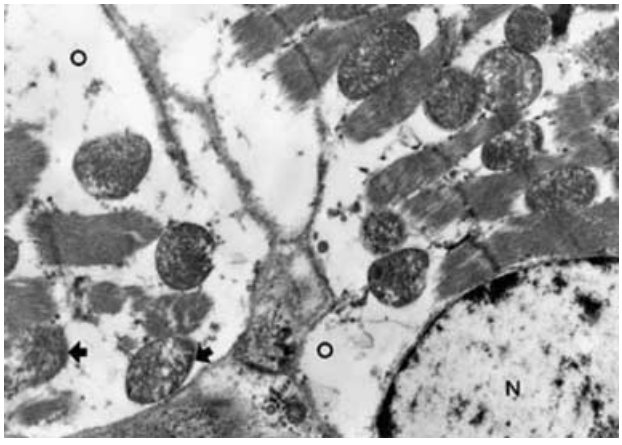

Figure 4: Transmission electron microscopy (TEM) showing, in the 24-h trauma group, large edematous areas in the cytoplasm of the myocytes, dilated sarcoplasmic reticulum and swollen mitochondria. In some of the nuclei of the myocytes heterochromatine was increased (original magnification ×7500).

Results of HIS showed that the 24-h trauma group had statistically significant levels in nuclear damage compared with the other groups ($p < 0.05$). Sarcoplasmic reticulum score of the trauma groups was significantly different from the control and sham groups ($p < 0.05$). Traumatic brain injury produced obvious damage to the ultrastructure of the sarcoplasmic reticulum as early as 2 h after trauma. Mitochondria scores of all trauma groups were significantly different from those of the control and sham groups ($p < 0.05$). Interestingly, the 24-h trauma group scores were statistically different from all the other groups ($p < 0.05$) (Figure 5).

These results show that although there was no statistically significant differences in the lipid per oxidation levels between the control and sham groups ($p > 0.05$), lipid per oxidation levels were statistically significant different between the control and all trauma groups ($p < 0.05$). Isolated head trauma increased heart tissue lipid peroxidation levels significantly in a time-dependent manner (Table 2).

Discussion

Cardiac injury occurring after TBI has been recognized for more than a century. In spite of the aforementioned pathophysiological mechanisms of the head trauma, there is not a single mechanism to be accepted. A combination of more than one mechanism seems to occur with a high probability. Acute myocardial dysfunction accompanying brain injury is characterized by marked alterations

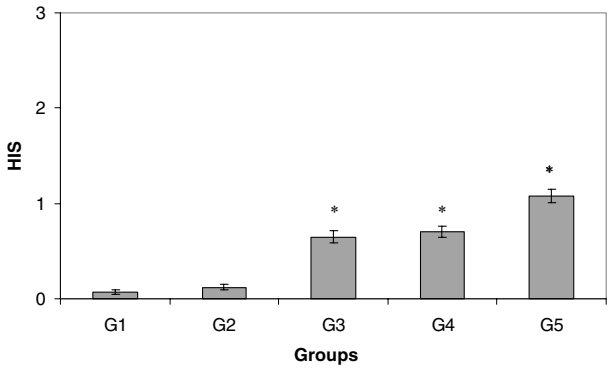

Figure 5: Effect of trauma on myocytes. Note that the ultrastructural scores in the control and sham groups are similar. *Significant difference from control group. Mean value of the ultrastructural scoring of the heart is presented as bars and SE of the mean is presented as vertical lines. Trauma produced obvious gradual damage on ultrastructure of the myocytes in a time-dependent manner.

Table 2: Heart tissue lipid peroxidation levels in five groups

Groups	TBARS (nmol/g of wet tissue)
G1 (Control)	80.71 ± 7.04
G2 (Sham)	83.00 ± 5.42
G3 (2-h trauma)	88.00 ± 8.79*
G4 (8-h trauma)	96.57 ± 1.72*
G5 (24-h trauma)	109.29 ± 7.99*

TBARS = thiobarbituric acid reactive substances.
*Statistically significant levels ($p < 0.05$).
TBARS' content of the heart is expressed as nmol TBARS/g of wet tissue, means ± SD.

in beta-adrenergic signal transduction as well as changes in the contractile apparatus (7). The myocardial ultrastructural damage after TBI thought to have been caused by catecholamines is described in clinical and experimental situations. This is described variously as myocytolysis, contraction band necrosis, focal coagulative necrosis, subendocardial petechial hemorrhage, edema formation, and interstitial mononuclear cell infiltration (8–10).

Bruinsma et al. showed that the presence or absence of histological changes was unrelated to the hemodynamic status after the induction of brain death in 6/9 hemodynamically unstable cats and 5/6 hemodynamically stable cats, which exhibited myocardial lesions. Also, no relationships were demonstrated between the acute increase of myocardial workload, the occurrence of hemodynamic deterioration, and myocardial histological changes after induction of brain death (11). On the other hand, sympathetic hyperactivity during central nervous system events leads to cardiovascular instability and neurogenic pulmonary edema (12,13). Massive sympathetic discharge following TBI causes both pulmonary and systemic vasoconstrictions. This causes an increase in both pulmonary blood pressure and left atrial pressure, causing an increase in pulmonary capillary pressure. The resultant pulmonary capillary damage causes alteration in capillary permeability.

The catecholamines, however, are important under stress conditions, such as brain injury, but may have damaging effects owing to the generation of reactive oxygen species (ROS) and formation of oxidation products (14). Oxidative stress has been considered as one of the basic events involved in cell and tissue damage. Importance of preventing and reducing the free-oxygen radical-induced tissue damage must be considered in various clinical conditions. After brain injury by ischemic or hemorrhagic stroke or trauma, the production of (ROS) may increase. Reactive oxygen species-mediated lipid peroxidation is one of the major mechanisms of secondary damage in TBI (15). Radicals can cause damage to cardinal cellular components such as lipids, proteins, and nucleic acids, leading to subsequent cell death by modes of necrosis or apoptosis (16).

Generation of ROS and peroxidation of lipids are extremely fast reactions, which are generally measured by their end-products, mostly thiobarbituric acid reactive substances (TBARs). Enhancement of TBARs is thought to be a marker of cell damage, which indicates an increased production of ROS and lipid peroxidation. Furthermore, in our study the follow-up period was only 24 h, but TBI increased heart tissue lipid peroxidation levels gradually. TBI may induce lipid peroxidation and may be the direct cause of large edema formation in the cytoplasm of the myocytes. Lipid peroxidation levels were found to have statistically significant differences among the control and all trauma groups ($p < 0.05$). The question of why, in our study, TBI increased ultrastructural changes and heart tissue lipid peroxidation levels significantly in a time-dependent manner cannot be answered at the present time. Pathophysiology of myocyte injury after TBI must be well understood, including the free-oxygen radical pathway. These findings are very important in building a base of support for the efficacy of pharmalogical agents in protection of myocytes after TBI.

In the present study, a grading system was used for quantitative evaluation of ultrastructural findings in cardiac myocytes. The deteriorating changes were scored as 0, 1, 2, and 3, with differing explanations according to the organelles' nucleus, mitochondria and sarcoplasmic reticulum. Our findings indicate that TBI produced by the weight-drop technique resulted in obvious, gradual damage on ultrastructure of the cardiac myocytes. Extension of these studies to humans suggests that a detailed assessment of the ultrastructure of the cardiac myocytes should be performed in victims of severe TBI.

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