The effect of electric countershock on cardiac troponin T (cTnT) and heart-type fatty acid-binding protein (h-FABP)

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Abstract: Electric countershock may cause injury to skeletal muscle with the elevations of cardiac troponin T (cTnT) and heart-type fatty acid-binding protein (h-FABP), and subsequently may affect the results of whole blood panel tests. A total of 27 patients with atrial flutter (n = 2) or atrial fibrillation (n = 25) were enrolled. Patients underwent electric countershock and blood sampling for cTnT and h-FABP at baseline and at various time points (immediately, 3, 6, and 24 hr after procedure). Whole blood panel tests for cTnT and h-FABP were also performed at the respective time points. Mean h-FABP was elevated 2.3 fold after electric countershock (P < 0.05), while there was no change in cTnT. The positive rates with the whole blood panel test was significantly higher for h-FABP than for cTnT at each time point (maximum diversity h-FABP: 55.6% v.s. cTnT: 0% at 3-hr after EC, P < 0.01). Electric countershock did not result in elevation of cTnT despite a rise in h-FABP. These data suggest that myocardial damage following electric countershock was minimal and that elevation of h-FABP may result from skeletal muscle damage. Thus, cTnT may be a more clinically useful for diagnostic indicator of myocardial damage. Furthermore, the whole blood panel test for cTnT has superiority to that for h-FABP following resuscitation, as levels are not affected by electric countershock.

Key words: ① cardiac troponin T, ② electric countershock, ③ heart-type fatty acid-binding protein, ④ myocardial damage

Introduction

Electric countershock has been used to treat both atrial and ventricular arrhythmias. The procedure may cause injury to the chest wall skeletal muscle, resulting in the release of various marker enzymes into the systemic circulation. Creatine kinase (CK) has been used in combination with the standard 12-lead electrocardiogram as a conventional diagnostic marker for myocardial damage. However, CK is also abundant in skeletal muscles and can be elevated in the presence of skeletal muscle damage from direct current trans-thoracic shock1,2 or injury, thereby potentially interfering with the accurate diagnosis of myocardial infarction. This is particularly true in the case of cardiopulmonary arrest on arrival (CPAOA), in which aggressive resuscitative efforts are likely to lead to skeletal muscle damage3,4.

Recently, the use of new cardiac markers, such as cardiac troponin T (cTnT) and heart-type fatty acid-binding protein (h-FABP), have therefore been increasingly employed for diagnostic purposes. cTnT is a cardiac regulatory protein found in the myocardium5,6,7, where h-FABP is also abundant8,9. In addition, rapid whole blood panel tests for h-FABP10,11, and cTnT11,12 make these markers attractive and convenient for determination of myocardial damage in the emergent setting. Onset of h-FABP elevation is more immediate than those of CK and cTnT13,14, h-FABP was more sensitive than cTnT within 2-hr onset of ischemia15. Despite these advantages, the impact of electrical countershock on release kinetics of h-FABP and also cTnT remains largely unknown. There is a possibility that skeletal muscle injury could result in elevation of cTnT13 and h-FABP14 and subsequently affect the utility of whole blood panel tests. Thus, the

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Table 1  Baseline characteristics of the patient population

<table>
<thead>
<tr>
<th>Patients</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>67 ± 9 (45 ~ 83)*</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>16/11</td>
</tr>
<tr>
<td>Dominant disease</td>
<td>Atrial flutter 2 (7.4)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (51.9)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>Brain infarction</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Prothrombin time international normalized ratio</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>Serum creatinine (mg·dl⁻¹)</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
</tr>
<tr>
<td>Left atrial dimension (mm)</td>
<td>42.6 ± 6.4</td>
</tr>
<tr>
<td>Left ventricular end-diastolic dimension (mm)</td>
<td>49.8 ± 7.2</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>60.7 ± 11.8</td>
</tr>
</tbody>
</table>

Data are presented as number (%) or mean ± SD, * mean ± SD (range).

aim of the present study was to determine whether electric countershock required for treatment of tachyarrhythmias resulted in an increase in serum cTnT and h-FABP as determined by quantitative and whole blood panel tests.

Methods

Patients
A total of 27 consecutive patients (16 men and 11 women; mean age, 67 years; range 45-83 years) presenting from January 2003 to February 2004 were recruited for this study at Gifu Prefectural Government Tajimi Hospital. Eligibility criteria for electric countershock were: ① atrial fibrillation or atrial flutter lasting longer than 48 hours; ② age > 18 years; and ③ patients without a left atrial thrombus. Underlying diseases in the study patients included atrial flutter (7.4%), atrial fibrillation (92.6%), valvular heart disease (63.0%), coronary heart disease (11.1%), cardiomyopathy (3.7%), hypertension (51.9%), hyperlipidemia (11.1%), and brain infarction (7.4%), as shown Table 1. Warfarin sodium administration was started in all patients at least 4 weeks before electric countershock. Prior to the procedure, transesophageal echocardiography was performed to confirm the lack of any thrombus in the left atrium. There was no clinical or echocardiographic evidence of acute myocardial ischemia or infarction within a 4-week period before or after electric countershock in any of the patients. Acute cardiovascular disorders, pulmonary embolism, myocarditis, renal failure, and skeletal muscle disease were not noted in any of the study patients during the observation period.

The study complied with the Declaration of Helsinki, and the protocol was approved by our institutional ethics committee with written informed consent obtained from all patients.

Clinical methods
Electric countershock was performed with an ordinary synchronized monophasic defibrillator with the patient under brief general anaesthesia with midazolam (0.1 mg·kg⁻¹), and pentazocine (15 mg) according to the routine practice in our department. Intramuscular injections were not administered during the observation period. Electrode pads were placed in the apex-anterior configuration. Hand-held electrode paddles were used to administer electric countershock according to the following energy protocol: initial selected energies were 50 Joules (J) for atrial flutter and 200 J for atrial fibrillation, with a stepwise increment of 50, 100, 150, 200, 300, up to 360 J, and 200, 300, up to 360 J, respectively. If sinus rhythm was not thereby restored, the electric countershock was regarded as unsuccessful, and the procedure was terminated. Electrocardiograms were obtained before and after the electric countershock.

Serial determinations of cTnT, h-FABP, CK, and CK MB isoenzyme (CK-MB) were performed with serum samples from all 27 subjects taken before and immediately after the procedure, and at 3, 6, and 24 hr thereafter. Assays for serum CK, and CK-MB activities were conducted immediately after obtaining blood samples. Other blood samples were stored at −70°C until assays for h-FABP and cTnT could be performed. In
addition to standard serum measurement, whole blood samples were collected in plastic tubes containing ethylenediamine tetra-acetic acid for cTnT and h-FABP panel test.

cTnT: cTnT was measured by an electrochemiluminescent immunoassay using a Modular Analytics ECLusys kit (Roche Diagnostics GmbH, Mannheim, Germany). The lower limit of detection was 0.01 ng·mL⁻¹, and the discrimination level used for myocardial injury was 0.1 ng·mL⁻¹. The intra-assay coefficient of variation was 1.6% at 1.45 ng·mL⁻¹.

h-FABP: Serum h-FABP concentration was quantified by direct sandwich-ELISA using a Markit M h-FABP kit (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). The discrimination level used for myocardial injury was 62 ng·mL⁻¹ and the intra-assay coefficient of variation was 3.0% at 8.8 ng·mL⁻¹.

CK: CK activity was measured by colorimetry using a Hitachi 917 automatic chemical analyzer (Hitachi High-Technologies Co., Inc., Tokyo, Japan). The coefficient of variation of within and between runs was 2.5% and 4.5% respectively. The upper limits of the CK reference ranges were 195 IU·L⁻¹ in males, and 180 IU·L⁻¹ in females.

CK-MB: CK-MB activity was determined by an immunoinhibition assay using CicaLiquid CK-MB inhibition reagent (Kanto Chemical Co., Tokyo, Japan). The upper limit of the CK-MB reference range was 25 IU·L⁻¹, and the intra-assay coefficient of variation was 4.0% at 21 IU·L⁻¹·37°C⁻¹.

Whole blood panel test for cTnT and h-FABP: A rapid test assay was employed for the whole blood panel test for cTnT and h-FABP. The test yields a positive result when serum cTnT is greater than 0.10 ng·mL⁻¹ (TROP T³ sensitive, Roche Diagnostics GmbH, Mannheim, Germany) and contains two monoclonal antibodies specific for cTnT, one gold-labelled, and the other biotinylated. A positive result is indicated by a red line produced by an accumulation of gold-labelled cTnT sandwich complexes over a 20-minute period. Excess gold-labelled antibodies accumulate at the control line, providing a visual signal that the test is valid.

The commercially available whole blood panel test for h-FABP (Rapicideck, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) is based on a dual monoclonal antibody sandwich method, using two distinct monoclonal antibodies and the gold-label method, as described previously. When two dark red bands appear on the cellulose nitrate test device within 15 minutes, the result is regarded as positive. The test yields a positive result when serum h-FABP is greater than 6.2 ng·mL⁻¹.

**Statistical analysis**

Statistical significance was tested with repeated analysis of variance (repeated ANOVA), followed by the Dunnett's multiple comparison test for serial change. Spearman's coefficients were calculated for ranked associations between cumulative energy and maximal value of observed cardiac markers. Differences in proportions for the whole blood panel test were evaluated by the chi-square test. A P value of less than 0.05 was regarded as statistically significant. All calculations were performed using the commercially available statistics software package, SPSS for Windows® version 10 (SPSS Inc., Chicago, USA).

**Results**

**Patient population**

Baseline characteristics of the patient population are summarized in Table 1. Prothrombin time-international normalized ratio on warfarin therapy was 1.9 ± 0.1. The means for the left atrial dimension and the left ventricular end-diastolic dimension were 42.6 ± 6.4 mm and 49.8 ± 7.2 mm, respectively. The mean left ventricular ejection fraction in the B-mode (biplane modified Simpson method) was 60.7 ± 11.8%.

The results of electric countershock are shown in Table 2. Normal sinus rhythm was restored in 21 of 27 (77.8%) patients. No adverse events (e.g., thromboembolism, haemorrhage, or rib fracture) occurred during the periprocedural period.

**cTnT and h-FABP**

Serial changes in cTnT and h-FABP are shown in Figure 1a. Baseline values of cTnT and h-FABP were 0.01 ± 0.0 ng·mL⁻¹ and 4.4 ± 0.4 ng·mL⁻¹, respectively. cTnT was not elevated above normal reference values during the observation period, whereas the mean value of h-FABP was significantly elevated to 10.2 ng·mL⁻¹ and 10.5 ng·mL⁻¹ with a 2.3 and 2.4-fold increase from the mean baseline value at 6-hr and at 24-hr, respectively (P < 0.05).

**CK and CK-MB**

Serial changes in CK and CK-MB are shown in Figure 1b. Baseline values were 69.5 ± 8.6 IU·L⁻¹ and 11.2 ± 0.7 IU·L⁻¹, respectively. Elevation of CK and CK-MB above normal limits was observed in 10 (37.0%) and 2 (7.4%) patients, respectively. The mean
CK value was significantly elevated to 5241 IU·l⁻¹ at 24-hr after electric countershock (P < 0.05) with a 7.5-fold increase from the mean baseline value. There was no statistically significant change in the CK-MB during the observation period.

**Correlations between cumulative delivered energy and maximal values**

As shown in Table 3, the maximal value for h-FABP positively correlated with the cumulative delivered energy (Spearman’s coefficient: rₛ = 0.633, P < 0.001). In contrast, no link was evident for cTnT (rₛ = -0.146, P = 0.47). Cumulative delivery energy correlated with maximal CK (rₛ = 0.628, P < 0.001) but not with CK-MB (rₛ = 0.227, P = 0.26). Number of shocks correlated with the maximal h-FABP (rₛ = 0.661, P < 0.001) and CK (rₛ = 0.636, P < 0.001) but not with cTnT or CK-MB (cTnT, rₛ = 0.22, P = 0.91; CK-MB, rₛ = 0.244, P = 0.23). Similar findings were obtained with regard to peak selected energy (h-FABP, rₛ = 0.510, P < 0.01; cTnT, rₛ = -0.317, P = 0.11; CK, rₛ = 0.450, P < 0.05; CK-MB, rₛ = 0.100, P = 0.63, respectively).

**Whole blood panel tests for h-FABP and cTnT**

Results of the whole blood panel test for cTnT and h-FABP are shown in Table 4. The positive rates (number of positive patients) for h-FABP before the procedure, immediately after the procedure, and at 3, 6, and 24-hr were 11.1% (n = 3), 33.3% (9), 55.6% (15), 51.9% (14), and 55.6% (15), respectively. The cTnT test was negative for all patients throughout the observation period. The positive number of whole blood panel test was significantly higher for h-FABP test than for cTnT at each time point (P < 0.01).

**Discussion**

cTnT is a myofibrillar regulatory protein that is considered a more specific indicator of irreversible cellular injury when compared with conventional cytosolic enzymes. The new criteria for the diagnosis of myocardial infarction published by the Joint Committee of European Society of Cardiology and American College of Cardiology advocate the use of cTnT as the most sensitive and specific marker for myocardial infarction. In the current study, the level of cTnT did not increase significantly after electric countershock, and no correlation was noted with cumulative electric countershock energy, consistent with previous reports that troponin levels are unaffected by electric countershock in patients with atrial fibrillation or atrial flutter.

h-FABP is soluble cytoplasmic protein that is abundant in the myocardium which is released into the bloodstream early in the course of myocardial damage. However, it is also contained in skeletal muscles, such as the pectoralis major muscles and intercostal muscles. In the present study, the values of h-FABP were significantly increased within 24 hours after electric countershock, correlating with cumulative electric countershock energy delivery, the number of shocks, and peak selected energy. Thus, the data suggest that elevation of h-FABP may result from skeletal muscle damage.

The present study also revealed elevation of CK and CK-MB in 37.0% and 7.4% of the patient population, respectively, in the CK case positively correlating with the total delivery of electric countershock energy, consistent with reports by other investigators. For example, Lund et al. reported significant elevation of CK (36%) and CK-MB (10%) in studied patients after electric countershock. Because the majority of CK is likely to be released from pectoral muscles as a consequence of electric countershock, false positive results could easily be generated even...
cTnT and h-FABP following electric countershock

Table 3  Correlation between electric countershock data and the maximal values of cardiac markers

<table>
<thead>
<tr>
<th></th>
<th>Cumulative delivered energy</th>
<th>Number of shocks</th>
<th>Peak selected energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$ ($P$ value)</td>
<td>$r_s$ ($P$ value)</td>
<td>$r_s$ ($P$ value)</td>
</tr>
<tr>
<td>Peak h-FABP</td>
<td>0.633 (&lt; 0.001)</td>
<td>0.661 (&lt; 0.001)</td>
<td>0.510 (&lt; 0.01)</td>
</tr>
<tr>
<td>Peak cTnT</td>
<td>-0.146 (0.47)</td>
<td>0.022 (0.91)</td>
<td>-0.317 (0.11)</td>
</tr>
<tr>
<td>Peak CK</td>
<td>0.628 (&lt; 0.001)</td>
<td>0.436 (&lt; 0.001)</td>
<td>0.450 (&lt; 0.05)</td>
</tr>
<tr>
<td>Peak CK-MB</td>
<td>0.227 (0.26)</td>
<td>0.244 (0.23)</td>
<td>0.100 (0.63)</td>
</tr>
</tbody>
</table>

A $r_s$ represents Spearman’s coefficients.

CK, creatine kinase; CK-MB, creatine kinase-MB sub fraction; cTnT, cardiac troponin T; h-FABP, heart-type fatty acid-binding protein.

Table 4  Results of whole blood panel tests before and after electric countershock

<table>
<thead>
<tr>
<th></th>
<th>Before EC</th>
<th>Immediately after EC</th>
<th>3-hr after EC</th>
<th>6-hr after EC</th>
<th>24-hr after EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
</tr>
<tr>
<td>h-FABP</td>
<td>11.1% (3)</td>
<td>33.3% (9) *</td>
<td>55.6% (15) *</td>
<td>51.9% (14) *</td>
<td>55.6% (15) *</td>
</tr>
</tbody>
</table>

Data are presented as positive rate (number). The cutoff levels used for cTnT and h-FABP were 0.1 ng·ml$^{-1}$ and 6.2 ng·ml$^{-1}$, respectively. * indicates $P < 0.05$ comparing with positive rate of cTnT.

EC, electric countershock; cTnT, cardiac troponin T; h-FABP, heart-type fatty acid-binding protein.

when low amounts of energy are delivered. CK-MB is also presented in skeletal muscle, but only accounts for between 0 and 8% of total CK activity. Although two patients (7.4%) in the current study had elevations in CK-MB after electric countershock, the small increase may have originated from damage to skeletal muscle.

In the emergency department, accurate and immediate diagnosis as to the etiology of CPAOA after successful primary resuscitation is critical for prognostic and treatment considerations. Commercially available whole blood panel tests for detection of cTnT and h-FABP are commonly used in the emergency department for diagnosis of myocardial infarction. In the present study, whole blood panel test for cTnT was negative in all subjects during the observation period. In contrast, more than half of subjects showed positive whole blood panel test for h-FABP at 3, 6, and 24-hr after electric countershock, pointing to a high likelihood of false positive results.

Study limitations

The present study possesses several limitations. First, the patient population was relatively small. However, the sample size was still sufficient to demonstrate statistically significant changes in several cardiac markers. In the context, it should also be noted that changes in cTnT level and other conventional cardiac markers were consistent with earlier reports.

Second, subjects with severe congestive heart failure were not included. Previous reports have demonstrated that patients with advanced congestive heart failure have higher levels of cTnT. These complicated patients may have subtle ongoing myocardial injury that might make them more susceptible to damage by electric countershock energy.

Finally, findings from subjects with atrial arrhythmias undergoing elective procedure in the present study cannot be extrapolated to patients undergoing emergent treatment for ventricular arrhythmias. It is known that the sarcolemmal membrane become fragile under the condition of cardiac arrest which imposes ischemia on cardiomyocytes. Cardiac markers are possibly released into bloodstream through the disrupted membrane upon electric countershock under ischemic condition. Unlike patients undergoing resuscitation, cardiomyocytes in the current subjects do not appear to have fragile sarcolemmal membrane. Therefore, the release kinetics of cardiac markers might be somewhat different from that in those who underwent resuscitation. However, the results of the present study allow us to generalize to those patients, as demonstrated in earlier reports. Additionally, previous study has shown that there was no significant elevation in cTnT after electric countershock for ventricular arrhythmia, which is consistent with the results of the present study of atrial arrhythmias.

Conclusions

The present study demonstrated an elevation in h-FABP at 6 and 24-hr after electric countershock that correlated with cumulative delivery of energy, the number of shocks, and peak selected energy. In contrast, cTnT did not exhibit any increase above the reference range and the whole blood panel test for cTnT was negative. The data indicate that electric countershock results in elevations of h-FABP and CK, but not cTnT, likely attributable to skeletal muscle.
damage. Therefore, cTnT appears to be clinically more useful as diagnostic indicator for myocardial damage. Whole blood panel test for cTnT appears to be superior to that for h-FABP in the context of resuscitation, as it is not affected by electric countershock.

References