Experimental Study of the Relationship between Perfluoro-Octyl Bromide Emulsion and Norepinephrine Release in Reperfusion Arrhythmia: Isolated Guinea Pig Heart Model

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Background: Perfluoro-octyl bromide (PFOB), one of the perfluorochemical oxygen transporters, improved postischemic cardiac dysfunctions. Also norepinephrine (NE) is one of the important inducible factors on reperfusion arrhythmias (ventricular fibrillation [VF]). We used these methods to evaluate the relationship between PFOB emulsion and NE release on reperfusion arrhythmias.

Materials and Methods: The perfusion of isolated guinea pig hearts was employed: each of four groups of 6–7 hearts were used with Krebs-Henseleit solution (KHS) as control, and KHS with 5%, 15%, or 30% PFOB emulsion. The hearts were perfused in a constant pressure Langendorff model, stabilized for 30 min, followed by 30 min preischemia, then 30 min ischemia and 45 min reperfusion at normothermia.

Results: PFOB emulsion dose-dependently limited VF and inhibited NE release in reperfusion. Only 30% PFOB emulsion showed the significant improvement of VF (p = 0.05). In hemodynamic parameters, only 5% PFOB emulsion showed a significant decrease in reperfusion, but there was no difference in coronary flow rate (CFR). No differences among the four groups were demonstrated in cardiac oxygen metabolic parameters.

Conclusions: It was most likely that a high concentration of PFOB emulsion attenuated reperfusion arrhythmia by decreasing NE release. (Ann Thorac Cardiovasc Surg 2008; 14: 363–368)

Key words: perfluoro-octyl bromide, norepinephrine, reperfusion arrhythmia, ventricular fibrillation

Introduction

Ventricular arrhythmias, such as ventricular fibrillation (VF), elicits during the reperfusion of ischemic myocardium, leading to cardiac sudden death.15 Many investigators have thought norepinephrine (NE) to be a closely related and crucial factor of reperfusion arrhythmia in the experimental and clinical settings.11 NE release has been proposed mainly by two mechanisms: Ca2+-dependent exocytotic release and Ca2+-

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independent carrier-mediated release associated with the activation of NE transporter in the outward direction.\(^1,2\)

In protracted ischemia, intraneuronal acidosis caused by failure of the H\(^+\)/adenosine triphosphate (ATP) (H\(^+\)-ATP) pump due to ischemia activates the Na\(^+\)-H\(^+\) exchanger leading to accumulation of intracellular Na\(^+\). This phenomenon, combined with increased axoplasmic NE that causes a reversal of the NE transporter, was in an outward direction, eliciting a carrier-mediated NE release.\(^1\)

The carrier-mediated NE release strongly associates with reperfusion arrhythmias, especially VF (duration), in human, guinea pig, and rat hearts.\(^1,3–5\)

Perfluorochemical (PFC), one of the artificial oxygen transporters, is hydrocarbons in which most or all of the hydrogen atoms have been replaced with fluorine.\(^6,7\)

Perfluoro-octyl bromide (PFOB) emulsion was developed by a new base and emulsification technique from second-generation PFCs.\(^8\) We introduced a new emulsifying technology and improved the stability and oxygen transport capacity.\(^9\) Furthermore, this PFOB emulsion showed cardioprotective effects after 6 hours of heart cold storage in guinea pigs\(^10\) and beneficial effects during cardiopulmonary bypass with hemodilution.\(^11\)

Under myocardial ischemia reperfusion condition, some researchers reported that PFC showed the improvement of myocardium during transient ischemia in angioplasty\(^12,13\) and cardiac function after heart transplantation.\(^14,15\) However, there are few concerns about the relationship between PFOB emulsion and NE release in reperfusion arrhythmias.

The purpose of this study was to assess the relationship between PFOB emulsion and NE release in the reperfusion arrhythmias after protracted global ischemia in isolated guinea pig hearts.

Materials and Methods

Isolated heart perfusion

Twenty-six male Hartley guinea pigs (Sankyo Co., Ltd., Tokyo, Japan) weighing 400 to 500 g each were divided by four groups: Krebs-Henseleit solution (KHS), 5% PFOB emulsion, 15% PFOB emulsion, and 30% PFOB emulsion (n = 6–7 in each group). All animals have received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals,” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

The hearts were removed after cervical dislocation performed under light anesthesia with CO\(_2\) vapor. They were rapidly perfused at an aortic pressure of 29.6 ± 0.3 mmHg in a constant pressure Langendorff model. This model consisted of double reservoirs with filters, including a perfusion solution with or without drugs, pump, and heart chamber, with a KHS composed of NaCl 118.2 mmol/L, NaHCO\(_3\) 25 mmol/L, KH\(_2\)PO\(_4\) 1.0 mmol/L, KCl 4.83 mmol/L, MgSO\(_4\) 2.37 mmol/L, CaCl\(_2\) 2.5 mmol/L, and glucose 11.1 mmol/L, with or without PFOB emulsion (v/v; 5%, 15%, or 30%) with bovine serum albumin (BSA) saturated with 95% O\(_2\), 5% CO\(_2\) (pH 7.52 ± 0.02). BSA (3% for 15%, 30% PFOB emulsion, and 1% for 5% PFOB emulsion) was added to the perfusion buffer containing PFOB emulsion to prevent the formation of crystals with calcium. BSA concentration was determined by a preliminary study (data not shown). Also, in the preliminary study there were no significant effects on NE amount, reperfusion arrhythmias, and others (data not shown). Electrocardiograms were continuously recorded with surface electrodes from the right atrium and the left ventricle to measure the heart rate (HR) and to recognize arrhythmias. Cardiac temperature was measured by attaching a thermoprobe on the surface of the heart. The values of cardiac surface temperature were 37.2°C ± 0.1°C in preischemia, 36.9°C ± 0.1°C in ischemia, and 36.9°C ± 0.2°C in reperfusion, respectively. The cardiac pressure was determined from the left peak systolic pressure via the left ventricle, using a fluid-filled latex balloon connected to a transducer (Baxter International Inc., Deerfield, IL) for the measurement of left ventricular developed pressure (LVDP). The volume of the balloon was initially adjusted to achieve left ventricle diastolic pressure of 10 mmHg, and this volume was maintained throughout the experiments.

Experimental protocol

Initially, isolated guinea pig hearts were perfused to stabilize for 30 min with KHS, followed by 30 min perfusion as a preischemia with or without PFOB emulsion, 30 min ischemia induced by global stop perfusion, and 45 min reperfusion with or without PFOB emulsion. HR was evaluated at R-R intervals by electrocardiography. The coronary effluent was collected every 5 min for 30 min preischemia. In the first 10 min of reperfusion, sampling tubes were replaced every 2 min and during the final 35 min every 5 min. The volume of
coronary effluent collected in each period was measured for coronary flow rate (CFR) and analyzed for amount of NE. The CFRs are expressed in ml per min per wet heart weight (ml/min/g).

**Artificial blood substitute**

We used a previously reported PFOB emulsion. In brief, the PFOB emulsion (100 ml) used in this study was 28% PFOB with about a 210 nm average particle size as a base, 12% of perfluoroalcohol esters with oleic acid (FO-9982), 2.4% yolk lecithin, and 0.12% polyethylene glycol (PEG), 1, 2-distearoyl-sn-glycero-3-phosphatidylethanolamine-N-PEG (DSPE-50H).

**Evaluation of VF**

Arrhythmias were analyzed from continuously recorded electrocardiogram tracings, according to the guidelines defined by the Lambeth Conventions. VF was the most common and persistent type of reperfusion arrhythmia. Also the duration of VF showed the strong correlation with the amount of NE release in reperfusion. Thus, we used only VF as an index of reperfusion arrhythmia.

**NE measurements**

The amount of NE was assayed in the coronary perfusate by high-performance liquid chromatography coupled to electrochemical detection followed by a previously described method. Values are expressed in picomoles per gram of wet heart weight (pmol/g).

**Cardiac oxygen metabolic variances**

Oxygen concentration of perfusate was used as arterial PO2, and oxygen concentration of coronary effluent was used as venous PO2. O2 extraction was calculated as follows: perfusate O2–coronary effluent O2. Myocardial oxygen consumption (MVO2) was calculated as coronary flow volume/g × (perfusion PO2–coronary effluent PO2) × 24 µl O2/ml at 760 mmHg.

**Statistic analyses**

Experimental values were expressed as mean ± standard error of mean (SEM) for 6–7 hearts in each group (data normalized to 1 g of wet heart weight). Comparisons of more than two groups were performed by one-way analysis of variance (ANOVA), and the Bonferroni t test was used for post hoc analysis. Yates’ corrected χ2 test was used to analyze the differences in the incidence of VFs. A p value of <0.05 was considered statistically significant.

**Results**

**Duration and incidence of VF**

PFOB emulsion prevented the duration of VF in a dose-dependent manner; 30% (0 ± 0 sec) and 15% (44 ± 15.2 sec) PFOB emulsion significantly inhibited the duration of VF compared to KHS (281.6 ± 62.1 sec) and 5% PFC (292.3 ± 56.1 sec) (Fig. 1A). These VFs were an onset of reperfusion and naturally stopped. Furthermore, although 30% PFOB emulsion completely limited the incidence of VF (0%), 5% (100%), and 15% (83.3%). PFOB emulsion did not show the significant inhibition of the incidence of VF (Fig. 1B).

**NE release**

In Fig. 2, PFOB emulsion (5%, 594.4 ± 71.3 pmol/g; 15%, 349.58 ± 16.1 pmol/g; and 30%, 198.9 ± 24.8 pmol/g) showed dose-dependent inhibition of NE release compared to KHS (1,069.7 ± 177.3 pmol/g) during 45 min reperfusion, following 30 min normothermic global cardiac ischemia.
Table 1. HR, CFR, and LVDP in all experiments

<table>
<thead>
<tr>
<th></th>
<th>Preischemia</th>
<th>10 min reperfusion</th>
<th>45 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>KHS</td>
<td>233.3 ± 6.3</td>
<td>238.8 ± 18.7</td>
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<td></td>
<td>5% PFOB</td>
<td>223.5 ± 16.7</td>
<td>242.1 ± 22.3</td>
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<tr>
<td></td>
<td>15% PFOB</td>
<td>205.2 ± 13.6</td>
<td>200.2 ± 9.6</td>
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<td>30% PFOB</td>
<td>234.7 ± 15.5</td>
<td>213.3 ± 16.9</td>
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<tr>
<td><strong>CFR (ml/g/min)</strong></td>
<td>KHS</td>
<td>5.1 ± 0.7</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>5% PFOB</td>
<td>5.7 ± 0.6</td>
<td>2.9 ± 0.8</td>
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<td></td>
<td>15% PFOB</td>
<td>5.8 ± 0.5</td>
<td>4.8 ± 1.1</td>
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<td>30% PFOB</td>
<td>5.1 ± 0.7</td>
<td>3.4 ± 0.9</td>
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<tr>
<td><strong>LVDP (mmHg)</strong></td>
<td>KHS</td>
<td>49.1 ± 7.1</td>
<td>23.1 ± 4.3</td>
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<td></td>
<td>5% PFOB</td>
<td>37.9 ± 5.7</td>
<td>5.5 ± 2.9’</td>
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<tr>
<td></td>
<td>15% PFOB</td>
<td>40.7 ± 7.5</td>
<td>20.5 ± 3.9</td>
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<tr>
<td></td>
<td>30% PFOB</td>
<td>35.4 ± 3.5</td>
<td>36.0 ± 6.0</td>
</tr>
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</table>

Each value shows mean ± SEM (n = 6–7 in each group).  
*p <0.05 vs. KHS, derived by one-way ANOVA, and the Bonferroni t test was used for post hoc analysis.

HR, heart rate; CFR, coronary flow rate; LVDP, left ventricular developed pressure; KHS, Krebs-Henseleit solution; PFOB, perfluoro-octyl bromide; SEM, standard error of mean; ANOVA, analysis of variance.

Table 2. Oxygen extraction and MVO<sub>2</sub> in all experiments

<table>
<thead>
<tr>
<th></th>
<th>Preischemia</th>
<th>10 min reperfusion</th>
<th>45 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen extraction</strong></td>
<td>KHS</td>
<td>67.3 ± 1.6</td>
<td>56.2 ± 5.1</td>
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<tr>
<td></td>
<td>5% PFOB</td>
<td>71.3 ± 1.1</td>
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<td>15% PFOB</td>
<td>66.8 ± 2.2</td>
<td>65.6 ± 0.9</td>
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<td>30% PFOB</td>
<td>66.1 ± 2.0</td>
<td>63.1 ± 3.8</td>
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<tr>
<td><strong>MVO&lt;sub&gt;2&lt;/sub&gt;</strong></td>
<td>KHS</td>
<td>26.5 ± 3.6</td>
<td>24.3 ± 5</td>
</tr>
<tr>
<td></td>
<td>5% PFOB</td>
<td>30.3 ± 5.2</td>
<td>26.2 ± 4.7</td>
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<tr>
<td></td>
<td>15% PFOB</td>
<td>40.7 ± 6.5</td>
<td>30.0 ± 7.9</td>
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<tr>
<td></td>
<td>30% PFOB</td>
<td>55.8 ± 25.7</td>
<td>48.2 ± 27.7</td>
</tr>
</tbody>
</table>

Each value shows mean ± SEM (n = 6–7 in each group).  
*p <0.05 vs. KHS, derived by one-way ANOVA, and the Bonferroni t test was used for post hoc analysis.

MVO<sub>2</sub>, myocardial oxygen consumption; KHS, Krebs-Henseleit solution; PFOB, perfluoro-octyl bromide; SEM, standard error of mean; ANOVA, analysis of variance.

**Hemodynamics**

Only 5% PFOB emulsion showed significant decreases at 45 min reperfusion, compared to KHS in HR and LVDP (Table 1). However, we could detect no differences among the four experimental groups (Table 1).
Cardiac oxygen metabolic variances
As shown in Table 2, no significant differences were demonstrated among four experimental groups in O$_2$ extraction and MVO$_2$ in preischemia and reperfusion.

Discussion
In our study, the result of incidence showed a nondose-dependent manner because the number of each experimental group might be small. However, PFOB emulsion showed the inhibition of NE release and reperfusion arrhythmias in a dose-dependent manner (Figs. 1 and 2). In the protracted ischemia, NE release is carrier mediated, which has a strong correlation with reperfusion arrhythmia. To address the fashion of NE release in this study, we used the NE transporter inhibitor (desipramine hydrochloride; DMI, 10 nM). This compound significantly inhibited NE release ischemia and completely abolished reperfusion arrhythmias (data not shown), suggesting the carrier-mediated NE release in this study, supported by previous studies. Furthermore, PFC delivered sufficient oxygen to allow ATP production within submerged organs during ischemia. Collectively, PFOB emulsion might possibly prevent hypoxia with the inhibition of the failure of the H$^+$-ATP pump, leading to the attenuation of a carrier-mediated NE release associated with reperfusion arrhythmias. However, further studies are required to clarify the inhibitory mechanism of PFOB emulsion on reperfusion arrhythmias because PFCs have the potential to attenuate the production of reactive oxygen species, which induces reperfusion arrhythmias.

In hemodynamic parameters, high concentrations (15% and 30%) of PFOB emulsion did not change these parameters (Table 1). Some researchers demonstrated that PFC itself impairs cardiac contractile functions after hypothermic exposure. To the contrary, PFCs also attenuated the production of reactive oxygen species. At least there might have been a relationship between the concentration of PFCs and the production.

PFC emulsions have been evaluated as artificial oxygen carriers to reduce allogeneic blood transfusion and to improve tissue oxygenation in clinical setting. Also, it augmented acute hemodilution after trauma or surgery and treated the conditions, such as myocardial ischemia. The results of this study might reflect possible clinical benefits of new PFOB emulsion during cardiac surgery by method of preconditioning.

In conclusion, the preconditioning with high concentrations of PFOB emulsion might prevent the occurrence of reperfusion arrhythmias, at least in part, due to the inhibition of NE release.

Acknowledgment
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