Does Cyclophosphamide Enhance Myocardial Depression Induced by Volatile Anesthetics in Isolated Hearts?

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Abstract

Cyclophosphamide is the most commonly used alkylating agent for anti cancer chemotherapy and immunosuppression. Halothane and sevoflurane are volatile anesthetics and halothane has been demonstrated to enhance cyclophosphamide toxicity. However, the interaction between sevoflurane and cyclophosphamide has not yet been investigated. We evaluated the effects of cyclophosphamide on myocardial depression by sevoflurane and halothane in isolated rat hearts.

Seventy-two isolated rat hearts were placed in a Langendorff perfusion system. The heart rate was maintained at 300 bpm. Perfusion pressure was maintained at 60 mmHg. The left ventricular end-diastolic pressure was kept constant at 5 mmHg throughout the study. Halothane and sevoflurane were equilibrated for 20 minutes in the solution through a calibrated vaporizer. After stabilization, the heart was perfused with a solution equilibrated with 1 minimum alveolar concentration (MAC) of halothane or sevoflurane in combination with cyclophosphamide at a concentration of 0, 20, 200 or 2000 μM for 10 minutes. Systolic left ventricular pressure (SLVP) and the maximum left ventricular rate of pressure development (LV max dP/dt) were examined. At baseline measurement, there were no significant differences in SLVP and LV max dP/dt for the three groups.

In combination with cyclophosphamide at the concentration of 20 μM, halothane, not sevoflurane, reduced SLVP and LV max dP/dt. When the concentration of cyclophosphamide rose to a therapeutic level, halothane increased myocardial depression with cyclophosphamide, but sevoflurane did not. Halothane produced lower SLVP and LV max dP/dt than sevoflurane at only high concentrations of cyclophosphamide. These findings lead to the hypothesis that halothane markedly induces myocardial depression when the concentrations of cyclophosphamide rise to toxic levels.

In conclusion, Halothane increased myocardial depression more than sevoflurane did when co-administrated with cyclophosphamide. Our findings suggest that sevoflurane is an adequate anesthetic for patients undergoing cyclophosphamide therapy.

Key Words:
Cyclophosphamide, Halothane, Sevoflurane, Contractility, Isolated heart
Introduction

Cyclophosphamide is a useful anti-cancer chemotherapeutic alkylating agent for a variety of cancers, and is also used prior to transplantation as an immunosuppressant\(^1\). Nowadays, many patients with cancer or immune diseases who undergo surgery receive cyclophosphamide even though high doses of cyclophosphamide can cause serious cardiotoxicity\(^2\). Volatile anesthetics cause also myocardial depression during surgery\(^3\), giving rise to the clinical question of interaction between cyclophosphamide and volatile anesthetics during surgery. Halothane is a volatile anesthetic and has been demonstrated to enhance the toxicity of cyclophosphamide, but the mechanism of the interaction is not fully understood\(^4\)\(^\text{–}^6\). Sevoflurane is also a volatile anesthetic and is frequently used for general anesthesia in Japan\(^7\). However, it has not been clarified whether sevoflurane enhances the toxicity of cyclophosphamide.

The present study was designed to compare the effect of cyclophosphamide on myocardial depression induced by volatile anesthetics in isolated rat hearts using the Langendorff method.

Materials and Methods

The present experiment was performed in accordance with the US NIH Guide for the Care and Use of Laboratory Animals. Seventy-two male Wistar rats weighing 280–320 g were fed standard diets and allowed to acclimatize in a quiet environment for one week prior to the experiment. The rats were anesthetized with an intraperitoneal injection of 25 mg/kg pentobarbital with 200 IU of heparin. Then, the heart was quickly excised and attached to the Langendorff perfusion system. The solution was saturated with a mixture of 95% oxygen and 5% carbon dioxide, and warmed at 37 °C. The concentrations of the solutions were (in mM): NaCl 108, KCl 4.75, MgSO\(_4\) 1.19, KH\(_2\)PO\(_4\) 1.19, CaCl\(_2\) 2H\(_2\)O 2.54, Glucose 10, and NaHCO\(_3\) 22.5. Immediately after the heart was attached to the Langendorff perfusion system, a latex balloon filled with saline was inserted into the left ventricle. The isolated heart was perfused at a constant pressure of 60 mmHg. Two platinum needle electrodes were placed on the pericardial surface of the right atrium and right ventricle and connected to an electronic stimulator (SEN-1101, SS-10J, Nihon Koden, Japan). The heart rate (HR) was set at 300 beats per minute. Left ventricular end-diastolic pressure was maintained constant at 5 mmHg. Systolic left ventricular end-diastolic pressure (SLVP) was monitored continuously with a pressure transducer connected to a latex balloon by employing a polygraph (RM-6000, Nihon Koden, Japan). Left ventricular rate of pressure development (LV max dP/dt) was measured from the electric derivation of left ventricular pressure.

The hemodynamic states were stabilized for 20 minutes, and then the baseline of the hemodynamic state was measured. After performing baseline measurement, the heart was perfused with a solution that was equilibrated with 1 MAC of halothane or sevoflurane, without anesthetics in combination with cyclophosphamide at a concentration of 0, 20, 200 or 2000 μM for 10 minutes, and SLVP and LV max dP/dt were measured and recorded.

Twenty-four rats each were allocated randomly to three groups as follows: The control group was perfused with cyclophosphamide only. The halothane group was perfused with halothane and cyclophosphamide. The sevoflurane group was perfused with sevoflurane and cyclophosphamide. Within a group, rats were randomly assigned to receive cyclophosphamide at a concentration of 0, 20, 200 or 2000 μM. The MAC values of halothane and sevoflurane in rats were calculated as 1.0% and 1.4%, respectively\(^8\). Halothane or sevoflurane was equilibrated in the solution through a calibrated vaporizer. The concentrations of the anesthetic gas were monitored continuously using an anesthetic gas analyzer (CAPNOMAC ULITINA, Datex-Ohmeda, Finland). In preliminary studies, two solutions dissolved for 20 minutes and 30 minutes were collected from the Langendorff system. The anesthetic concentration of these solutions was determined by gas chromatography (JMS-AX505HA, Nihon Denshi, Japan).

Data were expressed as mean ± standard error (SEM). The data within groups were analyzed by two-way
ANOVA for repeated measurements. The data for the different groups were analyzed by one-way ANOVA, followed by Dunnett's test for comparison. Values where $P<0.05$ were considered statistically significant.

### Results

1. Anesthetic concentrations of perfused solution (preliminary study) (Table 1)

There were no significant differences between the

### Table 1. Anesthetic Concentration of Solution

<table>
<thead>
<tr>
<th>Anesthetic concentration</th>
<th>20 minutes</th>
<th>30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatile anesthetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>47±1</td>
<td>46±1</td>
</tr>
<tr>
<td>Halothane</td>
<td>42±3</td>
<td>44±2</td>
</tr>
</tbody>
</table>

20 minutes solution was dissolved for 20 minutes through calibrated vaporizer. 30 minutes solution was dissolved for 30 minutes through calibrated vaporizer.

### Table 2. Myocardial Effect of Cyclophosphamide (control)

<table>
<thead>
<tr>
<th>Concentration [μM]</th>
<th>0</th>
<th>20</th>
<th>200</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLVP [mmHg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109±4</td>
<td>101±2</td>
<td>109±7</td>
<td>109±3</td>
</tr>
<tr>
<td>After</td>
<td>107±6</td>
<td>97±4</td>
<td>105±9</td>
<td>101±4*</td>
</tr>
<tr>
<td>LV max dP/dt [mmHg/sec]</td>
<td>2455±167</td>
<td>2397±176</td>
<td>2807±242</td>
<td>2517±130</td>
</tr>
<tr>
<td>After</td>
<td>2397±75</td>
<td>2283±117</td>
<td>2808±214</td>
<td>2383±162*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. SLVP: systolic left ventricular pressure. LV max dP/dt: left ventricular maximum rate pressure development. #p<0.05 vs baseline measurements.

### Table 3. Myocardial Effect of Cyclophosphamide with Halothane

<table>
<thead>
<tr>
<th>Concentration [μM]</th>
<th>0</th>
<th>20</th>
<th>200</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLVP [mmHg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>113±5</td>
<td>108±5</td>
<td>114±5</td>
<td>110±4</td>
</tr>
<tr>
<td>After</td>
<td>103±5*</td>
<td>98±8*</td>
<td>101±4*</td>
<td>85±5*</td>
</tr>
<tr>
<td>LV max dP/dt [mmHg/sec]</td>
<td>2467±154</td>
<td>2450±124</td>
<td>2493±154</td>
<td>2333±120</td>
</tr>
<tr>
<td>After</td>
<td>2233±112*</td>
<td>2150±106*</td>
<td>2183±106*</td>
<td>1900±115*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. SLVP: systolic left ventricular pressure. LV max dP/dt: left ventricular maximum rate pressure development. Baseline: baseline measurements. After: 10 minutes after cyclophosphamide administration. #p<0.05 vs baseline measurements.
two solutions dissolved for 20 minutes and 30 minutes by sevoflurane or halothane. These results document that the solution was equilibrated completely, as the solution was dissolved for over 20 minutes.

2. Baseline hemodynamic measurement (Tables 2, 3, 4)

At baseline measurement, there were no significant differences in SLVP and LV max dP/dt for the three groups.

3. Effect of cyclophosphamide on myocardial function (Table 2)

Cyclophosphamide reduced SLVP (109 ± 3mmHg to 101 ± 4mmHg), and reduced LV max dP/dt (2517 ± 130mmHg/sec to 2383 ± 162mmHg/sec) significantly at the concentration of 2000 µM (P<0.05).

4. Effect of halothane and sevoflurane on myocardial function (Tables 3, 4)
Without cyclophosphamide, halothane reduced SLVP (113 ± 5mmHg to 103 ± 5mmHg) and reduced LV max dP/dt (2467 ± 15 mmHg/sec to 2233 ± 112 mmHg/sec) significantly, but sevoflurane caused no significant changes in SLVP and LV max dP/dt.

5. Effect of cyclophosphamide in combination with halothane and sevoflurane on myocardial function (Figs. 1, 2)

In combination with cyclophosphamide at the concentration of 2000 µM, Halothane reduced SLVP (77 ± 3%) and LV max dP/dt (81 ± 3%). Sevoflurane reduced SLVP (87 ± 2%) and LV max dP/dt (89 ± 2%). Halothane reduced more significantly than sevoflurane at high concentrations of cyclophosphamide (P<0.05).

Discussion

The present study was designed to investigate the effect of cyclophosphamide on myocardial depression induced by volatile anesthetics in rats. Our result demonstrates that halothane increased myocardial depression with cyclophosphamide more than sevoflurane did, because halothane reduced SLVP and LV max dP/dt in combination with cyclophosphamide more than sevoflurane did.

Effect of cyclophosphamide on myocardial function

Cyclophosphamide causes myocardial depression dose-dependently [9]. In our study, cyclophosphamide induced myocardial depression at a concentration of 2000 µM. A blood concentration was reported as 19.1 ± 6.7 (mean ± SEM) µM when female patients with breast cancer were given cyclophosphamide (50 mg/day) [10]. The concentration of cyclophosphamide in our study was 100 times higher than a clinical concentration. There have been several reports on the mechanisms of cyclophosphamid-induced myocardial depression. Hanaki et al. suggested cyclophosphamide-induced myocardial depression is associated with autonomic nervous disturbance [11]. Because cyclophosphamide inhibited the denerveted hearts in our study, we speculate that cyclophosphamide-induced myocardial depression may not be related to autonomic nervous disturbance. Herman et al. reported that cyclophosphamide increased the level of histamine [12]. However, the effect of histamine on cyclophosphamide-induced myocardial depression was ruled out in our study, because the perfused solution did not include any substances that release histamine. Kumar et al. showed the histopathological feature of myocardium in rats treated with cyclophosphamide [13]. They observed multifocal interstitial myocardial hemorrhages, multifocal myocardial necrosis, and inflammatory reactions including pericarditis and valvulitis changes. Our study did not include histopathological examination of the hearts. However, because the heart was exposed to cyclophosphamide for only 10 minutes, this was an insufficient time to cause myocardium necrosis. Our data suggest that cyclophosphamide caused direct myocardial depression. Herman et al. observed that cyclophosphamide induces direct myocardial depression in isolated dog hearts [12]. This agrees with our data. On the other hand, Nasser showed cyclophosphamide-induced cardiotoxicity by increasing inner membrane permeability in calcium in cardiac mitochondria [13]. It may be assumed that direct myocardial depression induced by cyclophosphamide is involved in intracellular calcium. Further studies are required to clarify the mechanism of cyclophosphamide-induced myocardial depression.

Effect of halothane and sevoflurane on myocardial function

Sevoflurane caused less myocardial depression than halothane in the present study. These results are similar to several clinical studies. In echocardiographic studies, Holzman et al. showed sevoflurane decreased myocardial contractility less than halothane did [14], and Wodey et al. also reported that sevoflurane decreased cardiac output less than halothane did [15].

The main mechanism that halothane and sevoflurane cause myocardial depression is profound alteration of the main cellular components involved in intracellular calcium homeostasis [16]. The differences between myocardial depression of halothane and sevoflurane may be explained by their differential effects on calcium inward currents (Ica), sarcoplasmic reticulum (SR) function and myofilaments [17]. Sevoflurane and halothane at concentrations greater than 1% and 1.2% respectively, decrease Ica [18, 19]. Calcium channel types in cardiac muscle...
include the T-type channel and L-type channel. In ventricular cells, the entrance of calcium into the cell is primarily the current presumably though the L-type channel\(^20\). Kanaya et al. suggested halothane and sevoflurane reduce the inward calcium current by inhibition of the L-type channel\(^21\). Sevoflurane and halothane reduce intracellular calcium of the myocardium. Davies et al. demonstrated that halothane caused a depression of Ca\(^{2+}\) sensitivity in myofilaments\(^22\). They also showed that sevoflurane did not affect Ca\(^{2+}\) sensitivity in myofilaments. Furthermore, they indicated that halothane reduced functional release from SR, but sevoflurane did not.

In this study, we determined the dose of anesthetics based on MAC value. However, MAC is a unit for anesthetic potency of volatile anesthetics. The differences of action between myocardial and central nervous system may cause the difference of myocardial depression between sevoflurane and halothane. Hence, it may be appropriate to use equimolar rather than equianesthetic concentrations to compare the myocardial effect between sevoflurane and halothane.

**Effect of cyclophosphamide in combination with halothane and sevoflurane on myocardial function**

We demonstrated that halothane increased myocardial depression induced by cyclophosphamide more than sevoflurane did. Animal studies demonstrated that halothane enhances the toxicity of cyclophosphamide\(^4-6\). For example, Bruce et al. reported that halothane increases the lethality of cyclophosphamide in mice\(^5\), and Rosenow et al. reported the same result in rabbits\(^6\).

However, in a clinical study, Lee et al. could not identify the cardiotoxicity of cyclophosphamide under halothane anesthesia\(^23\). On the other hand, it has not been evaluated whether sevoflurane enhances myocardial depression by cyclophosphamide.

In combination with cyclophosphamide at the concentration of 20 \(\mu\text{M}\), halothane, not sevoflurane, reduced SLVP and LV max dP/dt in the present study (Tables 3, 4). When the concentration of cyclophosphamide rose to a therapeutic level, halothane increased myocardial depression induced by cyclophosphamide, but sevoflurane did not. Halothane produced lower SLVP and LV max dP/dt than sevoflurane did at only high concentrations of cyclophosphamide (Figs. 1, 2). These findings lead to the hypothesis that halothane markedly induces myocardial depression when the concentrations of cyclophosphamide rise to toxic levels. Our study did not clarify the mechanism of interaction between cyclophosphamide and volatile anesthetics (halothane or sevoflurane). However, we speculate that a decrease of intracellular calcium may play an important role in the interaction between these drugs. Further investigations are required to determine the mechanisms of interaction between cyclophosphamide and volatile anesthetics.

**Conclusion**

Halothane induced myocardial depression with cyclophosphamide more than did sevoflurane. Our findings suggest that sevoflurane is an adequate anesthetic for patients undergoing cyclophosphamide therapy. We also recommend avoiding halothane anesthesia for those patients during surgery.

**Acknowledgment**

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**Reference**


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シクロホスファミドは、揮発性吸入麻酔薬の心抑制効果を増強するか？

西木戸 修 諭田 武志

抄録
【目的】シクロホスファミドは、抗癌剤や免疫抑制剤として頻用されるため、術前に投与されることが多い。シクロホスファミドの副作用の1つに心抑制があげられるが、術中に使用する揮発性吸入麻酔薬との相互作用は不明である。この実験ではラットの摘出心を用い、シクロホスファミドによる揮発性吸入麻酔の心抑制増強作用について検討した。
【方法】9週齢のWistarラットの摘出心（12群、各群6匹）をLangendorff法によりKrebs液で灌流させた。循環動態安定後Baselineの測定を行い、実験を開始した。1.0MACのハロタン（1.0％）またはセボフルラン（1.4％）を灌流液に添加器を用いて吹送した。シクロホスファミドはそれぞれ20μM、200μM、2000μMの濃度になるように灌流液に溶解した。10分間灌流した後、左心室収縮期圧（SLVP）、左室最大压変化率（LV max dP/dt）を測定した。
【結果】Baselineの測定では、SLVP、LV max dP/dt に各群間に有意差は認めなかった。高濃度のシクロホスファミド（2000μM）の投与において、セボフルランよりハロタンの投与がSLVP、LV max dP/dtを有意に低下させた。
【考察】ハロタンはセボフルランよりシクロホスファミドの心抑制を増強させた。シクロホスファミドを投与されている患者的麻酔は、セボフルランが適当だと推察された。