Postmortem Acidification of Blood/Organs Induces an Increase in Flecainide Concentration in Cardiac Blood and the Contribution of the Lungs to This Increase

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Abstract

Postmortem acidification of blood and the contribution of this phenomenon to increased flecainide concentrations in cardiac blood were evaluated in rabbits. Flecainide was administered intravenously, antemortem peripheral blood was collected 15 min after administration, then rabbits were sacrificed. Blood and organs were collected immediately or 24 h after death, or immediately or 24 h after performance of cardiac massage. Postmortem left/right cardiac blood and organs showed lower pH than antemortem blood, and flecainide concentrations in all postmortem blood samples were higher than those in antemortem blood. Increased flecainide concentrations in cardiac blood were enhanced by postmortem cardiac massage and postmortem interval. In perfusion experiments using rabbit lung and heart, even if the flecainide concentration in inflow was kept constant, outflow concentrations were 2- to 3-fold higher than in inflow when inflow pH changed from 7.4 to 5.5. In contrast, flecainide concentration in outflow decreased immediately and then remained low when pH of perfusate changed from 5.5 to 7.4. These results demonstrate that flecainide accumulates in the lungs before death, and this accumulated flecainide releases into blood following postmortem acidification of blood/organs.

Introduction

Concentrations of basic drugs in autopsy cases and sacrificed animals are known to be higher in the lungs than in blood. Moriya and Hashimoto (1) reported autopsy cases in which concentrations of basic drugs such as methamphetamine were much higher in the lungs than in cardiac blood. Likewise, Pounder et al. (2) described that levels of dothiepin, a basic drug, in lung were 143- to 147-fold higher than levels in cardiac blood in an animal experiment. Concentrations of basic drug in cardiac blood are also known to increase after death (1,3), and blood starts to acidify immediately after death (4–6).

Some authors have referred to the possibility of postmortem release of basic drugs from organs into blood following this acidification of blood (1,5) or to the influence of accumulated drug in lungs (1,2,7,8). Valuable information on the mechanisms underlying postmortem increases of drug concentrations in cardiac blood have already been acquired from many experiments, but the basic facts have remained unproven.

Flecainide is used as a pharmacotherapy for tachyarrhythmia. Although this agent is popular and widely used, cases of fatal poisoning have been reported (9,10). Determination of the concentration of flecainide in blood/organ is required for autopsy diagnosis in such cases. However, as a basic drug (pK\textsubscript{a} 9.36), flecainide accumulates in the lungs in the living body (11,12), and concentrations in cardiac blood increase markedly after death (13). This means that appropriate interpretation of flecainide concentrations in cardiac blood/organ as autopsy material is required in suspected cases of fatal poisoning.

The present study experimentally investigated the relationships between pH and accumulation/release of flecainide in/from the lungs and examined the mechanisms underlying postmortem increases to flecainide concentration in cardiac blood. Furthermore, the effects of postmortem cardiac massage on changes in flecainide concentration were also examined because patients found in cardiopulmonary arrest (CPA), including cases of fatal poisoning, often receive cardiopulmonary resuscitation (CPR), which causes artificial blood flow after initial stoppage of the bloodstream.

Materials and Methods

Reagents and animals

Flecainide was provided by Eisai (Tokyo, Japan) as flecainide acetate. Paroxetine hydrochloride was provided by Glaxo SmithKline (Tokyo, Japan) and was used for internal standard. Both acetonitrile (Sigma Aldrich, St. Louis, MO) and methanol (Wako Pure Chemical Industries, Osaka, Japan) used in this study were of high-performance liquid chromatography
(HPLC)-grade. Biochemical-grade N-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES; Wako Pure Chemical Industries, Osaka, Japan) and 2-(N-morpholino)ethanesulfonic acid (MES; Sigma Aldrich) were also used. Analytical grade was allowed for all other reagents. In animal experiments, male rabbits with a body weight of about 3.0 kg (Japanese White; Japan Lamb, Hiroshima, Japan) were used. Heparin sodium salt (Nacalai Tesque, Kyoto, Japan) and warfarin sodium salt (Wako Pure Chemical Industries) were purchased as chemical reagents.

Animal experiments on postmortem distribution of flecainide

All animal experiment protocols were approved by the Animal Care and Use Committee at Okayama University and were performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Research Council. Blood readily displays strong coagulation in rabbits after death, and blood clots interfere with drug extraction. Furthermore, coagulation of blood in vessels was considered likely to interrupt drug redistribution. Therefore, animal experiments were performed using rabbits treated with heparin and warfarin. Rabbits were provided with water containing 10 mg/L of warfarin sodium salt from two days before the experiment, and 1000 U/kg of heparin sodium salt was administered immediately before experiments by intravenous injection via the auricular vein. Flecainide was administrated to heparinized rabbits by intravenous injection via the auricular vein at a dose of 1 mg/kg body weight. Fifteen minutes after administration, antemortem peripheral blood was taken from the auricular vein of the contralateral ear. After sacrifice by CO₂, the rabbit was fixed in a supine position with the limbs affixed to the table with rope. Left/right cardiac blood as well as both lungs, the heart, and the liver were obtained immediately fixed to the table with rope. Left/right cardiac blood as well as (Scientific Industries, Bohemia, NY). The extraction column was activated with 3 mL of methanol twice and rinsed with distilled water. After sequential washing with 3 mL of 0.2 mol/L sodium carbonate solution, 3 mL of distilled water, and 0.8 mL of acetonitrile twice, flecainide was eluted by adding 1.6 mL of methanol. The eluent was evaporated, and the residue was resuspended in 0.15 mL of the HPLC-mobile phase. If the flecainide concentration exceeded the range of the calibration curve, extraction was performed again from the corresponding sample diluted 2-, 5- or 10-fold with distilled water.

Perfusion experiment

Isotonic 0.1 M TES solution (pH 7.4) was made using the following procedure. First, 22.93 g of TES and 4.48 g of NaCl were dissolved in about 900 mL of distilled water. The pH of this solution was adjusted to 7.4 using 5 N NaOH, then the volume of the solution was adjusted to 1000 mL by distilled water. Isotonic 0.1 M MES solution (pH 5.5) was made in the same manner as TES using 19.52 g of MES and 5.24 g of NaCl. Each solution was diluted with a 10-fold volume of Ringer’s solution, and constructed solutions were designated as 10-mM TES-buffered Ringer’s solution and 10 mM MES-buffered Ringer’s solution, respectively. Flecainide-containing 10-mM TES/MES-buffered Ringer’s solution (500 or 1000 µg/mL) was prepared using 1 L of TES/MES-buffered Ringer’s solution spiked with 50 or 100 µL of 10 g/L flecainide ethanol solution. Rabbits were sacrificed by CO₂, and both lungs were removed together with the heart after ligation of the inferior and superior venae cavae and aorta. Blood contained in organs was washed out using Ringer’s solution at a flow rate of 10 mL/min using a peristaltic pump from the right ventricle to the left atrium (Figure 1). After 20 min, the perfusate was changed to 10 mM TES-buffered Ringer’s solution and the flow rate was lowered to 1 mL/min. After 10 min, 500 or 1000 µg/mL flecainide-containing 10-mM TES-buffered Ringer’s solution, adjusted to pH 7.4 was infused until the concentration of flecainide in the outflow equaled that in the inflow to allow distribution of the drug into the organ. The perfusate was then changed to 10 mM MES-buffered Ringer’s solution with the same flecainide concentration. Outflow pH was monitored using a 6961-5C small sample amount flow-type electrode (Horiba, Tokyo, Japan). Outflow was collected every minute using a model 2110 fraction collector (Bio-Rad Laboratories, Hercules, CA), and the flecainide concentration was determined directly by HPLC using the absolute calibration method. In another perfusion experiment, pH was changed from 5.5 to 7.4 for perfusate in which flecainide concentration had been adjusted to 1000 µg/L.

HPLC condition

Flecainide concentrations were determined using a Prominance HPLC system equipped with a RF-10Axl fluorescence HPLC monitor (Shimadzu, Kyoto, Japan) according to the reported method (15). Briefly, a reversed-phase Symmetry C₁₈ column (Waters, Milford, MA) was used for separation at a column temperature of 40°C. The mobile phase comprising 10 mM potassium phosphate buffer (pH 3.0) and acetonitrile (70:30 v/v) was used at a flow rate of 1.0 mL/min. Excitation and emission wavelengths for the detector were set at 310 nm
and 375 nm, respectively. Retention time of flecainide and internal standard were 3.1 and 3.9 min, respectively. The required analytical time for one sample was 10 min for extracted samples or 6 min for samples from the perfusion experiment. All correlation coefficients of calibration curves for extracted samples in the ranges of 5–50 µg/L (for antemortem peripheral blood), 10–100 µg/L (for postmortem cardiac blood), and 100–1000 µg/L (for organs) were > 0.990. The limit of detection was 1.0 µg/L. Correlation coefficients of calibration for the absolute calibration method with perfusion samples in the range of 300–2500 µg/L was 0.999.

Results

Redistribution of flecainide and pH values

Ratios of flecainide concentration in postmortem blood or in organs to antemortem peripheral blood (P/A or O/A ratio) are summarized in Table I. Flecainide concentrations in all postmortem samples were higher than levels in antemortem blood, and an extremely high O/A ratio was observed for the lungs compared with other samples in all groups.

Differences in flecainide concentration for blood samples within groups were tested using the paired t-test. Levels of flecainide in all postmortem blood samples were significantly higher than those in antemortem blood with the exception of both left/right cardiac blood in Group I and right cardiac blood in Group IV (Figure 2). No significant differences in flecainide concentration were apparent between left and right cardiac blood in any groups according to paired t-testing. No significant differences in flecainide concentrations of blood samples were seen between any groups using unpaired t-testing.

Table II shows postmortem changes in blood pH. The pH of both left and right cardiac blood was significantly reduced in all groups, and pH values of the investigated blood samples were significantly higher in Group I than in the other three groups. The pH values of lung, myocardium, and liver were within the ranges of 6.6–7.1, 6.5–6.9, and 6.1–6.8, respectively. Those values tended to be higher in Group I than in other groups, but no significant differences were identified.

Perfusion experiment

The flecainide concentration in outflow through the lungs increased with falls in pH despite a constant inflow concentration, reaching about double the inflow concentration at 1000 µg/L at pH 5.8 and almost triple at 500 µg/L at pH 6.0. The pH value in outflow then stabilized, and flecainide concentration in outflow gradually started to fall to the level seen at the start of the experiment (i.e., inflow level) (Figure 3). Conversely, when perfusate pH increased, flecainide concentration in outflow quickly fell by about two-thirds and remained low (Figure 4).

Discussion

Postmortem changes to drug concentrations in cardiac blood represent an important issue in forensic toxicology as such changes can result in the misdiagnosis of cause of death (16). Some reports have identified increased drug concentrations in blood specimens collected above the diaphragm after death, including cardiac blood (7,13,16,17). Thus, determining drug concentrations in blood from various parts and evaluating obtained results is important. However, cardiac blood has frequently been used for the determination of drug concentrations.

Table I. Postmortem Distribution of Flecainide*

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 6)</th>
<th>Group II (n = 6)</th>
<th>Group III (n = 5)</th>
<th>Group IV (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Cardiac blood</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Left</td>
<td>2.6</td>
<td>1.94</td>
<td>2.8</td>
<td>0.74</td>
</tr>
<tr>
<td>Right</td>
<td>2.3</td>
<td>0.62</td>
<td>2.3</td>
<td>0.93</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>14.2</td>
<td>2.97</td>
<td>24.2</td>
<td>13.64</td>
</tr>
<tr>
<td>Right</td>
<td>18.2</td>
<td>3.50</td>
<td>26.3</td>
<td>18.12</td>
</tr>
<tr>
<td>Myocardium</td>
<td>4.5</td>
<td>2.56</td>
<td>10.3</td>
<td>11.62</td>
</tr>
<tr>
<td>Liver</td>
<td>17.2</td>
<td>17.81</td>
<td>6.6</td>
<td>3.39</td>
</tr>
</tbody>
</table>

* All values are given as the ratio of postmortem flecainide concentration in blood to antemortem peripheral blood (P/A ratio) or as the ratio of flecainide concentration in organs (tissues) to antemortem peripheral blood (O/A ratio) for samples collected immediately (Group I) and 24 h (Group II) or immediately (Group III) and 24 h (Group IV) after postmortem cardiac massage.
Postmortem changes in blood concentrations of drug have generally been thought to result from postmortem redistribution of drug from organs into blood together with passive diffusion from gastrointestinal residues containing high concentrations of drug (1,7,17–20). Some practical cases, however, have suggested that postmortem increases in blood concentrations of basic drugs, including flecainide, might occur without diffusion of the drug from gastrointestinal residues (1,13). In one case involving flecainide (therapeutic serum concentration, about 200 µg/L), concentrations of flecainide in postmortem cardiac blood were reported as 44.2 mg/L in the left heart and 13.8 mg/L in the right heart, although the concentration in antemortem peripheral blood collected 13 h before death was 2.5 mg/L (13). Therefore, we designed an animal experiment in which flecainide was administered intravenously to exclude the effects of passive diffusion and clarified the contribution of redistribution to postmortem increases in drug levels in cardiac blood. A 1 mg/kg dose of flecainide was used for rabbits in this study as the approved therapeutic dose of flecainide for human use is 50–100 mg per administration.

We focused on postmortem changes to blood pH and postmortem increases in flecainide concentration in cardiac blood. Sawyer et al. (6) showed that acidification of cardiac blood after death did not differ significantly between carbon dioxide asphyxiation and cervical dissociation, but carbon dioxide asphyxiation showed less variability in cardiac blood pH at all postmortem time points compared with cervical dissociation. Carbon dioxide asphyxiation was therefore adopted in this study. The pH of postmortem cardiac blood was obviously lowered (Table II), and postmortem flecainide concentration in cardiac blood was clearly increased (Table I; Figure 2). These two postmortem events were considered to be correlated. A causal relationship between them has been assumed (1), but each of the phenomena has only been reported individually (4,6,13) with no previous studies providing a simultaneous investigation. Flecainide is a basic drug, and the molecular form of flecainide may have a tendency to bond to various binding sites because blood is slightly basic in the living body. As another probable mechanism of accumulation of flecainide, this basic drug has been proposed to enter cells from blood by diffusion and to accumulate in acidic vacuoles such as lysosomes (21) because the protein-binding rate of flecainide in blood in the human living body is 40–60% (22–24), which seems relatively low. The lysosomal uptake of basic drugs depends on the difference in pH between the intra- and extralysosomal space (21,25). Accumulated flecainide should thus be released from tissues into blood following postmortem acidification of blood. Flecainide is also highly accumulated in the lung (Table I), and this accumulated flecainide must play some role in the postmortem increases seen for flecainide concentration in blood. The fact that flecainide concentration in left cardiac blood was comparatively higher than that in the right for all groups supports the possibility of the lung participating in postmortem increases to flecainide concentration in cardiac blood because pulmonary blood more easily moves into the left cardiac cham-

| Table II. Values of pH in Antemortem Peripheral Blood and Left/Right Cardiac Blood* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Group I (n = 6) | Group II (n = 6) | Group III (n = 5) | Group IV (n = 6) |
| Mean  | SD              | Mean  | SD              | Mean  | SD              | Mean  | SD              |
| Antemortem peripheral blood     |                |                |                |                |
| Mean  | 7.90†           | 0.0000  | 7.83†           | 0.082  | 7.88†           | 0.110  | 7.83†           | 0.175  |
| Postmortem cardiac blood        |                |                |                |                |
| Left                           | 7.10†          | 0.303  | 6.58†,§         | 0.076  | 6.60†,§         | 0.071  | 6.51†           | 0.166  |
| Right                          | 7.07†          | 0.225  | 6.53†,‡         | 0.151  | 6.69†,‡         | 0.124  | 6.45†           | 0.141  |

* Collected immediately (Group I) and 24 h (Group II) after death or immediately (Group III) and 24 h (Group IV) after postmortem cardiac massage.
† p < 0.005 compared with antemortem blood (paired t-test).
‡ p < 0.05.
§ p < 0.01.
# p < 0.005 compared with Group I (unpaired t-test).

Figure 2. Flecainide concentrations in postmortem cardiac blood and antemortem peripheral blood. Group I: samples collected immediately after death (n = 6); group II: samples collected 24 h after death (n = 6); group III: samples collected immediately after postmortem cardiac massage (n = 5); group IV: samples collected 24 h after postmortem cardiac massage (n = 6). (* p < 0.05 and paired t-test).
bers than the right. In an attempt to clarify the relationship between changing pH and flecainide behavior in the lung, we conducted perfusion experiments using rabbit lung to evaluate the effects of pH changes on perfusate and the contribution of the lung.

Flecainide concentration in outflow was increased when inflow pH was lowered even when the drug concentration in the inflow was kept constant in the perfusion experiment (Figure 3). These results show that the release of flecainide from the lung into pulmonary blood is caused by postmortem acidification of blood if flecainide is adequately distributed in the lung. The postmortem increase in flecainide concentration in cardiac blood thus appears largely attributable to the movement of released flecainide from the lungs into the cardiac chambers via pulmonary vessels.

Conversely, accumulation of flecainide in the lungs appeared to occur when the pH of perfusate rose as flecainide was lost from the outflow under these conditions (Figure 4). The flecainide concentration in outflow immediately fell and remained low throughout the observation period, contrasting with the behavior of flecainide after lowering the pH of the perfusate (Figure 3). The lung thus appears to keep accumulating flecainide in this period. This result might explain why concentrations of flecainide in the lung were higher than those in other organs (Table I) because blood pH in the pulmonary vein is higher than that in the pulmonary artery under normal conditions (26). Meanwhile, blood pH would be lowered in organs other than the lung when blood passes through tissues producing carbonic acid during cellular respiration.

The postmortem redistribution of drugs in cardiac blood is considered to be affected by drug distribution to organs and tissues before death. Myocardium is in direct contact with cardiac blood while the lung and heart are connected only via the pulmonary arteries and veins. The liver and heart are relatively closely connected via the hepatic veins and inferior vena cava. If a patient is found in CPA, CPR or cardiac massage are usually performed, both of which force blood flow through such organs and from the liver and lung into the right and left chambers of the heart, respectively. Artificial blood flow in the early postmortem period has been considered to affect drug redistribution, so the effects of blood movement during open-chest cardiac massage on changes in flecainide concentration in cardiac blood were evaluated. Significant postmortem increases to flecainide concentration in left and right cardiac blood were observed in Group III (with cardiac massage) but not in Group I (without cardiac massage) (Figure 2). Furthermore, large variability was observed, and no significant differences in flecainide concentrations in left and right blood were seen between Groups II and IV by unpaired t-testing, although mean levels tended to be higher in Group IV than in Group II. A few values showed significant differences within and between groups for various organs according to unpaired t-testing (not shown), but the significance of these findings was unclear because variability was high. However, the results for blood might indicate that the increased flecainide concentrations in cardiac blood caused by cardiac massage increase because flecainide concentrations in the lung and liver are higher than levels in antemortem peripheral blood (Table I).

From the perspective of postmortem interval, significant differences in flecainide concentrations were observed between left/right cardiac blood and antemortem peripheral blood in Group II (samples collected 24 h after death), but no such differences were identified in Group I (samples collected immediately after death). Similarly, although no significant differences were observed by t-testing, mean flecainide concentrations in left/right cardiac blood were relatively higher in Group IV than in Group III. Postmortem redistribution of flecainide concentration in outflow of the lung with decreased pH of inflow in the perfusion experiment. Inflow contained 1000 (A) and 500 µg/L of flecainide (B).

Figure 3. Changes in flecainide concentration in outflow of the lung with decreased pH of inflow in the perfusion experiment. Inflow contained 1000 (A) and 500 µg/L of flecainide (B).

Figure 4. Changes in flecainide concentration in outflow of the lung with increased pH of inflow in the perfusion experiment. Inflow contained 1000 µg/L of flecainide.
Cainide might thus be affected by postmortem interval.

The present results show that cainide accumulates in the lungs before death, and concentrations in cardiac blood increase after death with acidification of blood/organs.

Conclusions

In the animal experiment, pH of postmortem cardiac blood was reduced and postmortem cainide concentration in cardiac blood increased after death. In the perfusion experiment, cainide concentration in outflow increased when inflow pH was lowered. These results indicate that acidification of blood represents an important contributor to postmortem increases in cainide concentration in cardiac blood.

In the perfusion experiment, cainide concentration in outflow immediately fell with increasing pH and remained low throughout the observation period, which indicated that cainide accumulates in the lung before death and must affect postmortem increase in cainide concentration.

Flecainide concentration in cardiac blood tended to increase with cardiac massage in the animal experiment, particularly in the left heart, and cardiac massage enhanced increases in flecainide concentration in cardiac blood after death. When concentrations of flecainide in postmortem cardiac blood are evaluated, levels can be expected to be at least 6.1 times higher than antemortem levels.

References