ABSTRACT:
We investigated the effect of acute myocardial infarction (MI) on the hepatic clearance of theophylline in rats using the coronary artery ligation model. After 48 hr of ligation, there were significant changes in left ventricular performance in the MI rats, compared with controls, as indicated by elevated left ventricular end-diastolic pressure, reduced mean arterial pressure, and reduced left ventricular systolic pressure (20 ± 2 vs. 12 ± 3 mm Hg, p < 0.01; 90 ± 6 vs. 101 ± 6 mm Hg, p < 0.01; and 100 ± 8 vs. 114 ± 8 mm Hg, p < 0.01, respectively). Despite these changes, MI rats were able to maintain their cardiac output at rest (77.9 ± 6.8 vs. 77.2 ± 9.2 ml/min), and there was no change in mean central venous pressure (3 ± 1 vs. 2 ± 1 mm Hg). Although hepatic perfusion and oxygenation were preserved (17.3 ± 2.2 vs. 18.7 ± 3.3 ml/min and 127 ± 27 vs. 125 ± 19 μmol/min respectively), clearance of theophylline was reduced by 23% in the MI rats, compared with controls (0.86 ± 0.20 vs. 1.12 ± 0.17 ml/min, p = 0.01). There was no significant correlation between theophylline clearance and the infarct size (r = −0.038, p > 0.05). These findings demonstrate that the elimination of theophylline is impaired after acute MI, independent of any changes in hepatic perfusion or oxygenation.

Although cardiac disease is treated with a wide variety of drugs, surprisingly little is known about its effect on hepatic drug metabolism. A number of studies have provided evidence that drug clearance may be impaired in patients with chronic congestive heart failure (1–5). Reductions in the elimination of high clearance compounds, such as lignocaine, have been attributed to reduced hepatic perfusion (6). However, this cannot explain the observation that the elimination of low clearance compounds, such as theophylline and aminopyrine, may also be impaired (7, 8). These findings suggest that the intrinsic capacity of the liver to metabolize drugs is reduced in chronic heart failure. This is supported by studies in animal models of congestive cardiac failure in which reduced intrinsic hepatic metabolic capacity has been attributed to reduced enzyme content (9) or to hypoxia due to chronic hepatic congestion (10). Whether acute myocardial injury either alone or when complicated by heart failure also results in impaired hepatic drug metabolism is unclear.

There have been very few studies in patients that have examined the effects of MI on the clinical pharmacokinetics of drugs. Disappearance of aprindine from plasma was found to be slower in patients with acute MI (11), whereas disopyramide clearance was found to be significantly reduced in patients during their recovery from MI (12). Although a reduction in renal clearance may explain the impaired elimination of disopyramide, which is 60% excreted via the kidney unchanged (13), it cannot explain the reduced clearance of aprindine, because aprindine is predominantly eliminated via biotransformation in the liver (11). Neither can the reduced elimination of aprindine be attributed to reduced hepatic perfusion, because it is a low clearance drug whose elimination should not be influenced by changes in hepatic perfusion (14).

The aim of the present study was to evaluate in the rat the effect of acute left ventricular infarction on theophylline elimination. Theophylline was chosen as the model substrate for this study because it is a low clearance compound; at the dose level used in this study, only 10% of the dose is excreted unchanged in the urine, whereas the remainder undergoes oxidative biotransformation in the liver (15). It is 40% protein bound in the serum of the rat and does not penetrate erythrocytes (16). Therefore, changes in theophylline clearance in the rat in vivo are likely to be a true reflection of changes in the efficiency of hepatic drug oxidation.

Materials and Methods
Enflurane was purchased from Abbott Australasia Pty. Ltd. (Sydney, Australia). Theophylline was purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were of analytical grade. Radiolabeled microspheres were purchased from DuPont (Boston, MA). Monofilament polypropylene suture (6.0 Ethicon) was purchased from Johnson & Johnson Medical Pty. Ltd. (Sydney, Australia).

MI Model. The operative procedure used slight modifications to that previously described (17). In brief, it involved a left thoracotomy in the rat (male Sprague-Dawley, N = 15, age 7–8 weeks, weight 200–220 g) under anesthesia with a mixture of enflurane and oxygen. The heart was exteriorized briefly and the descending branch of the left coronary artery, near the origin of the pulmonary artery, was ligated with a monofilament polypropylene suture. The mortality of rats with left ventricular infarction as a result of the ligation (MI rats) was 40%, thus leaving N = 9 for study. The majority of deaths occurred while rats were under anesthesia or within the immediate postoperative period (i.e., within 24 hr of surgery). Sham-operated rats of the same age and weight (N = 8) underwent thoracotomy without ligation of the coronary artery.

Experimental Preparation. All rats were kept on standard chow and water ad libitum until the day of study, when food was withdrawn. On the day of the experiment (48-hr postcoronary occlusion or sham operation), under general anesthesia with enflurane, catheters were placed into the left jugular vein (for drug dosing and pressure monitoring), the left ventricle via the right carotid...
artery (for blood sampling, pressure monitoring, and microsphere injection), and the tail artery (for the collection of reference blood sample and pressure monitoring). Pressures were measured by an electronic pressure recording unit (Hewlett-Packard 78205A). All catheters were heparinised (5 IU/ml), and their exact positions were confirmed at autopsy. Catheters in the left ventricle and the left jugular vein were brought to the surface at the back of the neck, whereas the catheter in the tail artery was sealed and masked to prevent interference by the animal.

Theophylline Pharmacokinetic Studies. Theophylline pharmacokinetics were studied after allowing 2 hr for recovery from anesthesia. During this period of the experiment, the animals were alert, un restrained, and free to move about within their boxes (30 x 16 cm). At time 0, a drug-free blood sample was obtained and a bolus dose of theophylline (3 mg/kg) dissolved in 0.9% NaCl (3 mg/ml) was infused over 1 min via the left jugular vein. Blood samples (0.6 ml) were then withdrawn from the left ventricle at 5, 15, 30, 45, 60, 75, and 90 min after drug administration. Samples were immediately centrifuged, and the plasma separated and stored at −20°C. Plasma theophylline concentrations were subsequently measured by HPLC as previously described (10). The assay’s lower limit of quantitation was 0.625 μg/ml. Coefficient of variance and accuracy of the assay were 3% and 16%, respectively. After each sampling, erythrocytes were resuspended in saline and reinfused to avoid anemia and hemodynamic disturbance due to loss of intravascular volume.

Regional Blood Flow Studies. After completion of the pharmacokinetic study, the rat was restrained in a Perspex cylindrical tube (21 x 3 cm) and allowed to acclimatize to the new environment. Cardiac output and regional blood flows were then measured using radio-labeled microspheres as previously described (10), with minor modifications. Briefly, 100 μl of vigorously mixed 113-gadolinium-labeled microspheres (15.5 ± 0.1 μm in diameter and suspended in saline with 0.1% Tween 80) was injected into the left ventricle and flushed with 0.4 ml of heparinized saline over a period of 20–25 sec. For 5 sec before microsphere injection and for 60 sec thereafter, blood from the tail artery was collected in a preweighed tube (reference blood sample). Between 150,000 and 200,000 microspheres were injected into the rat, and >200 microspheres were collected in the reference sample in each experiment as previously described (18, 19). After completion of the microsphere injection and collection of the reference blood sample, the rat was anesthetized with pentobarbital (6 mg/100 g), and the left ventricle and flushed with 0.4 ml of heparinized saline over a period of 20–25 sec. For 5 sec before microsphere injection and for 60 sec thereafter, blood from the tail artery was collected in a preweighed tube (reference blood sample). Between 150,000 and 200,000 microspheres were injected into the rat, and >200 microspheres were collected in the reference sample in each experiment as previously described (18, 19).

Blood Gases and Hemoglobin Concentration Measurements. Two hundred microliters of whole blood was withdrawn from the left ventricle before commencement of pharmacokinetic studies and from the portal vein when the peritoneal cavity was opened after microsphere injection; for measurement of blood gases and pH using a ILs pH/blood gas analyzer (Instrument Laboratory, Lexington, MA). Hemoglobin concentration was also measured using a total hemoglobin measuring kit (Sigma Diagnostics, St. Louis, MO). Blood gases and hemoglobin concentration data were then used to calculate oxygen content in hepatic arterial and portal venous blood.

Measurement of Infarct Sizes. At the end of the experiment, the hearts were excised and washed in saline. The left ventricle, including the interventricular septum, was isolated from the right ventricle and the atria and fixed in 10% buffered formalin for histological processing. The ventricle was then cut into three transverse slices, representing the apex, middle, and base of the heart. Specimens were examined without knowledge of the pharmacodynamic and pharmacokinetic data using a dissecting microscope, and infarct size was assessed as the proportion of the total area of the myocardium that was infarcted, using an image analysis system equipped with “Video Pro-52 colour image analysis software” (© Leading Edge Pty. Ltd., Adelaide, Australia). Infarct size in each rat was designated as the mean of the infarct sizes from the three ventricular slices.

Calculations and Statistics. Cardiac output and regional blood flows were calculated using the following formula:

\[ Q = (Q_s \times N)/N_r, \]

where

\[ Q = \text{Blood flow (ml/min)}, \]

\[ Q_s = \text{Reference sample collection rate (ml/min)} \]

\[ N = \text{Radioactivity injected (for calculation of cardiac output) or radioactivity trapped in tissue (for calculation of regional blood flow)} \]

\[ N_r = \text{Radioactivity in reference sample}. \]

Total hepatic blood flow was taken as the sum of hepatic arterial and portal tributary flow. Hepatic arterial flow was calculated from the radioactivity trapped in the liver, whereas portal tributary flow was taken as the sum of the flows to the spleen, mesentery, stomach, and intestine. Hepatic oxygen delivery was calculated as the sum of hepatic arterial flow multiplied by arterial oxygen content and portal tributary flow multiplied by portal venous oxygen content. Pharmacokinetic parameters were calculated using standard noncompartmental formulas (20). Volume of distribution was calculated as \( V_d \) (20). Data are presented as means ± SD and compared using the unpaired Student’s \( t \) test. Probability of <0.05 was considered significant. Least squares linear regression analysis was used to examine the correlation between theophylline clearance and infarct size. All statistical analysis was performed using the statistics package, StatView SE (version 1.4; Abacus Concepts, Inc., Berkeley, CA).

**Results**

Effects of Coronary Artery Ligation. All animals that underwent coronary artery ligation (MI rats) developed myocardial infarcts. Infarct size ranged from 15% to 46% of the left ventricle (mean: 30 ± 10%). None of the sham-operated animals showed evidence of myocardial damage.

Hemodynamic effects of myocardial infarction are summarized in table 1. MI rats were able to maintain a normal cardiac output at rest; however, left ventricular systolic pressure and mean arterial pressure were decreased, whereas left ventricular end-diastolic pressure was found to be significantly elevated. In keeping with the increase in left ventricular end-diastolic pressure, there was a trend toward increased lung weight in MI rats (1.52 ± 0.49 g vs. 1.16 ± 0.15 g, \( p = 0.06 \)). As expected, right ventricular function was normal in the MI rats; mean central venous pressure was not different in the two groups, and there was no macroscopic or microscopic evidence of congestion in livers from MI animals. Mean liver weight in the MI group was significantly less than that in the sham group (9.04 ± 0.97 g vs. 10.12 ± 0.99 g, \( p = 0.038 \)).

Hepatic Perfusion and Oxygenation. MI did not alter total blood
flow in the gastrointestinal bed, with no difference between the two experimental groups in hepatic arterial, mesenteric, intestinal (including flow to stomach), portal tributary, and total hepatic blood flow (table 2). There was, however, a small but significant decrease in the splenic blood flow in MI rats. There was no difference in the hemoglobin content of the blood between the two groups (table 3). As a result of the unchanged hemoglobin content and oxygen saturation of the blood (results not shown), oxygen content in hepatic arterial and portal venous blood in the MI rats was unchanged (table 3). In the face of the maintained hepatic arterial and portal tributary flow and an unchanged oxygen content, total oxygen delivery to the liver was identical in the two groups.

Theophylline Pharmacokinetics. Representative plasma theophylline concentration-time profiles for sham and MI rats are shown in fig. 1. Within 30 min of drug dosing, plasma theophylline concentration declined in a monoexponential manner. Mean theophylline clearance in the MI rats was reduced to 77% of that in sham-operated rats (p = 0.01; table 4). Volume of distribution of theophylline at steady-state in the two groups was not significantly different. Terminal elimination half-life was significantly longer in the MI group (141 ± 49 vs. 96 ± 16 min, p < 0.05). No significant correlation was found between theophylline clearance and infarct size (fig. 2) (r = −0.038, p > 0.05).

Discussion
The major finding of this study is that theophylline elimination is significantly impaired in rats with acute left ventricular infarction. Theophylline clearance was reduced by 23% in MI rats, and terminal elimination half-life was prolonged by 47%. The reduction in theophylline clearance is likely to reflect decreased efficiency of hepatic metabolism. It is unlikely to be accounted for by increased drug binding to plasma proteins, because theophylline is predominantly bound to albumin (21), and albumin levels have been shown to fall after acute MI (22). In addition, plasma-free fatty acid concentrations rise after acute MI (23), and this results in reduced plasma protein binding of theophylline (21). Reduced clearance also cannot be explained by reduction in renal clearance, because theophylline is predominantly eliminated by biotransformation in the liver, with only 10% of the dose excreted unchanged in the urine at the dose used in this study (15). Theophylline is a low clearance compound that is predominantly eliminated via oxygen-dependent metabolism by hepatic cytochrome P450 isoenzymes, in particular cytochrome P4501A2 (24). Factors that might affect the rate of elimination of theophylline include the quantity and activity of cytochrome P450 enzymes and the availability of cofactors such as NADPH and oxygen. It has been suggested that, in conditions where hepatic oxygen delivery is likely to be reduced, such as cardiogenic shock, hepatic elimination of drugs such as theophylline may be impaired, because many cytochrome P450 isoenzymes seem to have a high requirement for oxygen (25). These enzymes are concentrated in the centrilobular region where oxygen tension is lowest (26, 27). However, our findings of reduced theophylline elimination in animals with MI in whom hepatic perfusion and oxygenation are preserved suggest that, in acute MI, oxidative drug elimination is reduced independently of changes in hepatic oxygenation. The lack of correlation between infarct size and theophylline clearance (fig. 1) supports the view that reduced theophylline clearance is not directly related to the effects of changes in systemic hemodynamics, hepatic perfusion, or oxygenation, because one would expect such effects to be most marked in animals with the largest infarcts. In a previous study, we found that theophylline pharmacokinetics was normal in rats with severe chronic left ventricular failure due to coronary artery ligation that had been performed 6 weeks earlier (28). This earlier finding suggests that the inhibition of the-

### TABLE 2

<table>
<thead>
<tr>
<th>Regional blood flows in sham and MI rats</th>
<th>Sham (N = 7)</th>
<th>MI (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic arterial flow (ml/min)</td>
<td>0.53 ± 0.19</td>
<td>0.57 ± 0.34</td>
</tr>
<tr>
<td>Splenic flow (ml/min)</td>
<td>1.48 ± 0.39</td>
<td>0.94 ± 0.45*</td>
</tr>
<tr>
<td>Mesenteric flow (ml/min)</td>
<td>1.24 ± 0.36</td>
<td>1.37 ± 0.31</td>
</tr>
<tr>
<td>Intestinal flow (ml/min)</td>
<td>15.4 ± 3.0</td>
<td>14.4 ± 1.8</td>
</tr>
<tr>
<td>Portal tributary flow (ml/min)</td>
<td>18.1 ± 3.2</td>
<td>16.7 ± 2.3</td>
</tr>
<tr>
<td>Total hepatic flow (ml/min)</td>
<td>18.7 ± 3.3</td>
<td>17.3 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD.

*p value is as follows: *significantly different from the values of the sham group, p < 0.05.

### TABLE 3

<table>
<thead>
<tr>
<th>Hemoglobin concentration, oxygen content in blood and oxygen delivery in sham and MI rats</th>
<th>Sham (N = 7)</th>
<th>MI (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin content (g/dl)</td>
<td>13.9 ± 1.0</td>
<td>14.1 ± 1.1</td>
</tr>
<tr>
<td>Arterial oxygen content (μmol/ml)</td>
<td>8.47 ± 0.67</td>
<td>8.58 ± 0.69</td>
</tr>
<tr>
<td>Portal oxygen content (μmol/ml)</td>
<td>6.68 ± 0.54</td>
<td>7.26 ± 0.81</td>
</tr>
<tr>
<td>Arterial oxygen delivery (μmol/min)</td>
<td>4.52 ± 1.67</td>
<td>4.94 ± 2.97</td>
</tr>
<tr>
<td>Portal oxygen delivery (μmol/min)</td>
<td>120 ± 19</td>
<td>122 ± 27</td>
</tr>
<tr>
<td>Total hepatic oxygen delivery (μmol/min)</td>
<td>125 ± 19</td>
<td>127 ± 27</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

### TABLE 4

<table>
<thead>
<tr>
<th>Theophylline pharmacokinetic parameters in sham and MI rats</th>
<th>Sham (N = 8)</th>
<th>MI (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (ml/min)</td>
<td>1.12 ± 0.17</td>
<td>0.86 ± 0.20*</td>
</tr>
<tr>
<td>t1/2b (min)</td>
<td>96 ± 16</td>
<td>141 ± 49*</td>
</tr>
<tr>
<td>Vdss (ml)</td>
<td>137 ± 15</td>
<td>151 ± 20</td>
</tr>
</tbody>
</table>

Values are means ± SD. t1/2b, terminal elimination half-life; Vdss, volume of distribution at steady-state.

*p value is as follows: *significantly different from the values of the sham group, p < 0.05.
ophylline elimination observed in the current study relate to the acute effects of MI and that these effects may resolve with time.

We previously found that, in the rat, chronic right heart failure substantially reduces total hepatic cytochrome P450 content and the content of several individual isoenzymes (29). Similar results were obtained by others who showed that chronic congestive heart failure reduces hepatic P450 content and activity in dogs (9). Studies by Tokola et al. (30) also showed reduced activities of several drug-metabolizing enzymes in liver biopsy specimens from patients with congestive heart failure. These findings indicate that, in chronic congestive heart failure, changes in cytochrome P450 content and activity may be an important determinant of the efficiency of metabolism of low clearance drugs such as theophylline.

Although the exact mechanism for the reduced theophylline clearance observed in the current study is not known, possible mechanisms include reduced cytochrome P450 content or inhibition of cytochrome P450 activity by vasoactive peptides or inflammatory cytokines that are released after MI. For example, there is evidence that plasma interleukin-1β levels are elevated in acute MI (31), and this cytokine downregulates expression of cytochrome P4501A2 (32), the principal cytochrome P450 responsible for theophylline metabolism (24). It should be noted, however, that in patients with severe heart failure or cardiogenic shock secondary to MI, hepatic oxygenation may be reduced sufficiently to result in hypoxic impairment of cytochrome P450 function, an effect that would add to the inhibition due to MI per se.

We conclude that elimination of theophylline in the rat is impaired in acute MI and that this is not due to reduced hepatic blood flow or deficiency of oxygen supply. Our findings support the view that acute MI may affect the activity of hepatic drug-metabolizing enzymes without inducing hemodynamic changes. Previous studies have emphasized that, in acute heart failure, elimination of high clearance drugs is reduced as a result of decreased hepatic perfusion. The findings of the present study indicate that, after acute MI, there may be a general impairment of cytochrome P450-dependent metabolism and that caution should be paid to the administration of drugs of low-hepatic clearance whose elimination is directly dependent on cytochrome P450 activity.

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References


