Rocuronium plasma concentrations during three phases of liver transplantation: relationship with early postoperative graft liver function

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Background. Previous studies have suggested that neuromuscular blocking agents might be used to assess liver function during liver transplantation. This study examines changes in rocuronium plasma concentration during liver transplantation, to assess graft function.

Methods. A constant-rate infusion of rocuronium was administered to 17 adult patients undergoing liver transplantation. Blood samples were taken at 30-min intervals throughout the procedure, which was divided into three phases: paleo-, an-, and neohepatic. Assay of plasma concentrations of rocuronium was by a gas chromatographic–mass spectrometry technique. Postoperative liver function was followed for up to five days by measuring plasma aminotransferases.

Results. In 14 of the 15 patients who survived the transplantation procedure, there was a 7–50% decrease in rocuronium concentration during the neohepatic phase compared with the anhepatic phase. In contrast, rocuronium concentrations increased in the two patients who died after surgery, one as a result of primary non-function and one from massive bleeding. In one patient who survived there was no change in rocuronium concentration. The increase in plasma rocuronium concentration during the neohepatic phase in the two patients who died was consistent with high levels of plasma aminotransferases.

Conclusions. Comparison of changes in plasma rocuronium concentration during the neohepatic phase with early postoperative liver function tests suggests the potential use of rocuronium as a pharmacokinetic probe for predicting liver function during liver transplantation. Further study of rocuronium’s potential as an intraoperative pharmacodynamic probe of liver function by measuring neuromuscular paralysis is suggested.

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Rocuronium is a quaternary aminosteroidal neuromuscular blocking agent with a rapid onset and intermediate duration of action.¹ In animals, rocuronium is mainly eliminated unchanged in the bile, while urinary elimination is a minor pathway.² Rocuronium is taken up rapidly by the isolated perfused rat liver, with a high extraction ratio, and was also excreted rapidly into bile.³ In humans, 12–22% of a 0.6 mg kg⁻¹ dose and 31% of a 1 mg kg⁻¹ dose appeared unchanged in the urine within 12 h of administration.¹⁴ In humans, 31% of rocuronium was recovered in the faeces and 27% appeared in the urine over 4–8 days; only negligible amounts of its metabolite, 17-desacyethylrocuronium, were found in urine and bile samples.⁵

The pharmacokinetics of rocuronium are not significantly altered during renal transplantation,⁶ which is consistent with...
the kidney being a minor organ of elimination, although in patients with chronic renal failure there is a decrease in clearance.7 In cirrhotic patients, rocuronium clearance is reduced, with a consequent prolongation of paralysis.8 For these reasons, rocuronium has been used as a probe of liver function during orthotopic liver transplantation in humans, and a strong correlation was observed between rocuronium recovery time and liver function following surgery.9 Another quaternary aminosteroidal neuromuscular blocking agent, vecuronium, has also been studied for the same purpose in animals and humans.10±12 The results were very similar to those with rocuronium. A pharmacokinetic study of rocuronium during the three phases of liver transplantation indicated that during the neohepatic period, clearance of rocuronium varied with the duration of warm ischaemia (the time from when the liver was removed from hypothermic storage and placed in the surgical field until its reperfusion). Increased duration of warm ischaemia was associated with decreased clearance of rocuronium.13

The aim of this study was to examine changes in plasma rocuronium concentration during various phases of liver transplantation and to assess if alterations in concentration were correlated with graft liver function tests. Specifically, patients were given a constant infusion of rocuronium, and the relationship between changes in plasma rocuronium concentration during different phases of the operation and graft liver function soon after surgery was examined.

Materials and methods

Patients

After obtaining institutional ethics approval and informed consent, we studied 17 adult patients (Table 1) with end-stage liver disease undergoing general anaesthesia and orthotopic liver transplantation at The National Liver Transplant Centre of Australia at Royal Prince Alfred Hospital (Sydney) from January to December 2000. Patients with neuromuscular diseases and/or whose body weight was either more than 20% below or 30% above ideal body weight (ideal weight in kg equals height in cm minus 100) were excluded.

Anaesthesia

Anaesthesia was induced with i.v. thiopental 3–5 mg kg⁻¹ and morphine 0.1 mg kg⁻¹ and the patient was intubated after succinylcholine 1–2 mg kg⁻¹. Anaesthesia was maintained with isoflurane (0.5–1.0% inspired concentration), morphine as required and an infusion of rocuronium. Patients were ventilated mechanically, with oxygen and air, adjusted to maintain end-tidal carbon dioxide in the range 4–5.3 kPa. ECG, heart rate, arterial pressure and oxygen saturation were monitored routinely. Both radial arteries were cannulated for continuous arterial pressure monitoring and blood sampling, and a Swan–Ganz® pulmonary artery flotation catheter was inserted via the right internal jugular vein for monitoring of central venous pressure, pulmonary artery pressure and cardiac output. Plasma electrolytes and acid–base balance were monitored by regular blood sampling using a blood gas machine. Acid–base balance was maintained by efficient airway ventilation and cell washing of all bank blood and salvaged blood before re-infusion. Body temperature was monitored by nasopharyngeal and pulmonary artery probes and was maintained above 35°C by thermal blankets and heating all fluids/blood infused into the patient. Potassium and/or calcium were given i.v. whenever plasma potassium decreased below 3.5 mmol litre⁻¹ or calcium decreased below 1.0 mmol litre⁻¹. Venovenous bypass was used for all patients during the anhepatic period to bypass blood from the lower limbs and portal vein to the jugular vein.

Table 1. Preoperative variables for patients who underwent liver transplantation. CTP, Child–Turcotte–Pugh;¹⁴ AST, aspartate aminotransferase; ALT, alanine aminotransferase

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>CTP score</th>
<th>AST (U litre⁻¹)</th>
<th>ALT (U litre⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>Male</td>
<td>Hepatitis C liver disease</td>
<td>7</td>
<td>37</td>
<td>31</td>
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<td>2</td>
<td>38</td>
<td>Male</td>
<td>Alcoholic cirrhosis</td>
<td>9</td>
<td>53</td>
<td>45</td>
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<tr>
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<td>63</td>
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<td>Hepatitis B liver disease</td>
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<td>58</td>
<td>39</td>
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<tr>
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<td>Alcoholic cirrhosis</td>
<td>9</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>Female</td>
<td>Chronic active hepatitis</td>
<td>7</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>Female</td>
<td>Hepatitis B cirrhosis</td>
<td>11</td>
<td>221</td>
<td>192</td>
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<tr>
<td>7</td>
<td>52</td>
<td>Male</td>
<td>Hepatitis B cirrhosis</td>
<td>9</td>
<td>64</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>Male</td>
<td>Hepatitis C cirrhosis</td>
<td>14</td>
<td>292</td>
<td>208</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>Male</td>
<td>Alcoholic liver disease</td>
<td>14</td>
<td>51</td>
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<tr>
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<td>Male</td>
<td>Alcoholic liver disease</td>
<td>11</td>
<td>70</td>
<td>34</td>
</tr>
<tr>
<td>11</td>
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<td>Male</td>
<td>Hepatitis B liver disease</td>
<td>6</td>
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<td>41</td>
</tr>
<tr>
<td>12</td>
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<td>Female</td>
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<td>8</td>
<td>82</td>
<td>35</td>
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<td>13</td>
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<td>Male</td>
<td>Cryptogenic cirrhosis</td>
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<td>138</td>
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<td>14</td>
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<td>Male</td>
<td>Primary sclerosing cholangitis</td>
<td>9</td>
<td>89</td>
<td>46</td>
</tr>
<tr>
<td>15</td>
<td>43</td>
<td>Male</td>
<td>Hepatitis B liver disease</td>
<td>11</td>
<td>91</td>
<td>73</td>
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<tr>
<td>16</td>
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<td>Female</td>
<td>Primary biliary cirrhosis</td>
<td>8</td>
<td>376</td>
<td>256</td>
</tr>
<tr>
<td>17</td>
<td>46</td>
<td>Male</td>
<td>Hepatitis C liver disease</td>
<td>10</td>
<td>47</td>
<td>71</td>
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</tbody>
</table>
salvage was used in all but two surviving patients. The salvaged blood was washed with a cell saver and checked for acid–base and electrolyte balance before re-infusion. Samples of the washed salvaged blood were assayed for rocuronium and its metabolite. Blood volumes infused during the operation were recorded precisely, and urine output was measured hourly.

**Rocuronium infusion strategy**

Infusion of rocuronium in each patient was commenced after full recovery of the twitch response in the adductor pollicis muscle to train-of-four stimulation following intubation with succinylcholine. A bolus of rocuronium 0.45 mg kg$^{-1}$ i.v. was given followed by an infusion started 30 min later. Rocuronium bromide 10 mg ml$^{-1}$ was diluted with 0.9% saline to a final concentration of 1 mg ml$^{-1}$ so that an accurate infusion rate was delivered. The initial infusion rate was 0.5 mg kg$^{-1}$ h$^{-1}$; this was maintained for 20 min. The initial infusion rate was then determined as the final one for that patient throughout the operation. All pharmacokinetic parameters were calculated by a computerized monitoring system (TOF-GUARD$^\text{®}$, Organon Teknika, Belgium) once the cannulation period was completed. A 0.1 Hz single stimulus was delivered to the ulnar nerve at the wrist and the response of the adductor pollicis muscle recorded. The response of the adductor pollicis muscle to this stimulus after full recovery from succinylcholine, which was used for intubation, was taken as the baseline (control) value. Intensity of paralysis was monitored throughout the operation.

**Neuromuscular function monitoring**

Neuromuscular paralysis was assessed by clinical methods during the period of cannulation. Paralysis induced by the constant infusion of rocuronium was measured by accelerometry (TOF-GUARD$^\text{®}$, Organon Teknika, Belgium) once the cannulation period was completed. A 0.1 Hz single stimulus was delivered to the ulnar nerve at the wrist and the response of the adductor pollicis muscle recorded. The response of the adductor pollicis muscle to this stimulus after full recovery from succinylcholine, which was used for intubation, was taken as the baseline (control) value. Intensity of paralysis was monitored throughout the operation.

**Blood sampling**

Liver transplantation can be divided into three phases. The first paleohepatic phase consists of the dissection procedure, which usually lasts 2–4 h. The second anhepatic phase begins when the hepatic artery, portal vein and inferior vena cava above and below the liver are cross-clamped. This phase lasts up to 2 h, during which the recipient’s liver is removed and the inferior vena cava and portal vein are anastomosed to the donor liver. The final neohepatic phase starts when the inferior vena cava and portal vein are unclamped and the hepatic artery and biliary duct anastomoses are performed.

Arterial blood samples (5 ml) were taken from the left radial artery cannula before administration of rocuronium and at 30-min intervals from the start of the procedure to the end of surgery ($n=3$–6 during each phase). Blood samples were collected in lithium heparinized tubes and kept at 4°C before harvesting plasma within 2 h of collection. Plasma was acidified to pH 5.5 with 1 M sodium dihydrogen phosphate solution (0.2 ml per ml plasma) to prevent rocuronium degradation and the samples were stored at −20°C until analysis. These plasma samples were assayed for rocuronium. Plasma enzymes were also measured in other blood samples collected on days 1–5 after surgery; these included plasma aspartate aminotransferase (AST), alanine aminotransferase, and γ-glutamyl transpeptidase assays.

**Rocuronium assay**

Plasma concentrations of rocuronium and its putative metabolite, 17-desacetylrrocuronium, were analysed using a gas chromatographic–mass spectrometry (GC–MS) technique with 3-desacetylvecuronium as the internal standard. This involved using a chemically bonded silica capillary column for separation of rocuronium, its metabolite, and the internal standard, and use of an electron impact ionization mass spectrometer as a detector. Selective ion monitoring using the most abundant (base) peak was employed for quantifying rocuronium (m/z: 413) and the internal standard (m/z: 425); 17-desacetylrocuronium was quantified using the fragment peaks at m/z: 236 and m/z: 447. The analytes were extracted from plasma by liquid–liquid extraction with dichloromethane using potassium iodide as the ion-pairing agent. Mean (SD) extraction efficiency was 75 (5)% for rocuronium and 50 (10)% for 17-desacetylrocuronium. The lower limit of quantification by this method was 26 ng ml$^{-1}$ for rocuronium and 870 ng ml$^{-1}$ for 17-desacetylrocuronium. Assay accuracy varied from −18% to +10% over the concentration range 500 ng ml$^{-1}$ to 50 μg ml$^{-1}$ for both analytes, and assay precision, as indicated by the intra- and inter-assay variability was <10% and <15% for rocuronium and 17-desacetylrocuronium, respectively.
Statistical analysis
All data are presented as mean (SD). Plasma rocuronium concentrations during the three phases of transplantation were compared by one-way analysis of variance for repeated measurements, followed by Tukey’s multiple comparisons test. Linear regression analysis was performed to verify the relationship between change in plasma rocuronium concentration after revascularization and blood infusion volume, and changes in concentration and postoperative liver function tests. For all statistical comparisons, differences were considered significant at \( P<0.05 \). All estimations were two tailed.

Results
Of the 17 patients studied, two died within one week of surgery, one as a result of primary non-function and one from massive bleeding. The average duration for the recipient operation in the survivors was 480 (114) min. Table 2 gives information about blood volumes infused during the operation and duration of ischaemia for the donor livers.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Duration of operation (min)</th>
<th>Blood infusion volume (ml h(^{-1}))</th>
<th>Duration of total ischaemia (min)</th>
<th>Warm ischaemia (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors (n=15)</td>
<td>480 (114)</td>
<td>1532 (619)</td>
<td>1872 (1207)</td>
<td>617 (171)</td>
</tr>
<tr>
<td>Non-survivors (n=2)</td>
<td>495 and 700</td>
<td>2343 and 3111</td>
<td>4650 and 17 580</td>
<td>656 and 1010</td>
</tr>
</tbody>
</table>

Rocuronium infusion requirement during various phases of transplantation
The infusion requirement reached a plateau at 0.2–0.4 mg kg\(^{-1}\) h\(^{-1}\) [0.30 (0.07), \( n=17 \); Table 3] 90–120 min after the start of the rocuronium infusion. This infusion rate was then kept constant in each patient from before the start of the surgery to the end of the operation. The durations of the various phases of transplantation in each patient are summarized in Table 3.

Rocuronium plasma concentration during the three phases of liver transplantation
Mean plasma rocuronium concentrations during the three phases of liver transplantation are summarized in Table 3. There were no statistically significant changes in plasma rocuronium concentrations during the anhepatic phase compared with the paleohepatic phase. Rocuronium concentrations decreased during the neohepatic phase in 14 of the 15 patients who survived the surgery (\( P<0.01 \)) compared with concentrations during the paleohepatic and anhepatic phases. In another patient (no. 13) who survived, concentrations remained almost unchanged throughout the procedure. In the two patients who died (nos. 4 and 14), rocuronium concentration increased during the neohepatic phase. These patients died on days 1 and 4 after surgery as a result of primary non-function and massive bleeding, respectively. Rocuronium metabolite was not detected in any of the blood samples.

Plasma rocuronium concentrations: anhepatic vs neohepatic phase
Mean plasma rocuronium concentrations during the neohepatic phase are presented as percentages of the mean concentrations during the anhepatic phase in Table 4 and Figure 1. Of the 15 surviving patients, plasma concentrations during the neohepatic phase were 20–50% lower than those during the anhepatic phase in six patients. In another eight patients, the concentrations decreased by 7–20%. In the remaining one patient, the concentration remained almost the same as during the anhepatic phase. In contrast, in the two patients who died within a week of surgery, the concentrations increased by 6 and 22%.

Intensity of neuromuscular paralysis
Quantitative data on neuromuscular block were not available until cannulae had been inserted in all four limbs of the
Thus, during the approximately 2 h period during which the cannulae were being inserted, the intensity of paralysis was assessed on the basis of clinical judgement. Usually, the rocuronium infusion rate was adjusted during this period to produce complete paralysis of the limbs and ablation of respiratory effort. Quantitative monitoring of paralysis in the right arm by accelerometry started once the cannulae were in place but before the start of transplantation. In most patients, no adductor pollicis muscle twitch response was detected using the TOF-GUARD® until the end of the surgery.

Discussion

The results of this study suggest that when rocuronium is infused continuously at a constant rate during liver trans-
Rocuronium in liver transplantation

plantation, there is a relationship between the changes in plasma rocuronium concentration during the different phases of transplantation and early postoperative graft liver function tests. A decrease in plasma rocuronium concentration during the neohepatic phase compared with that during the anhepatic phase was observed in 14 of 15 patients who survived the transplantation. In contrast, increases in rocuronium concentrations were noted in the two patients who died soon after the operation. This indicates that plasma rocuronium concentrations during liver transplantation may provide a useful pharmacokinetic tool for predicting graft liver function and survival after liver transplantation.

Plasma rocuronium concentrations decreased during the neohepatic phase in 14 patients who had normal liver function after surgery. Only three of these patients showed statistically significant changes, although several patients showed greater numerical deviation without significance, which was probably a reflection of the small number of samples taken during each phase in these patients. This decrease in plasma rocuronium concentration may be the result of several factors. First, as noted with vecuronium, rocuronium is probably taken up by the liver and excreted into bile by the newly grafted liver. Therefore, it is expected that reperfusion of the graft liver will lead to the initiation of rocuronium excretion via hepatic pathways and results in a reduction in plasma concentration, provided the liver is functioning properly. Second, rocuronium is also partly eliminated via the kidney. Therefore, the patient’s renal function may also influence plasma rocuronium concentration. However, of the 17 patients, all except one (no. 8) had normal renal function before the operation. Urine outputs were measured every hour and maintained above 1 ml kg\(^{-1}\) h\(^{-1}\) during the operation. No significant differences in urine output were observed during the different phases of liver transplantation. Third, volumes of blood infused during the three phases of the operation may also influence rocuronium plasma concentration. Transfusion of large volumes of blood may be expected to reduce rocuronium concentration by dilution. However, of all the patients in whom rocuronium concentrations decreased during the neohepatic phase, mean blood infusion volume during the anhepatic phase was significantly higher than during the paleohepatic and neohepatic phases (\(P<0.05\)).

During the operation could contribute to increases in rocuronium concentrations if any residual rocuronium is present in the blood that is re-infused into the patient. However, blood salvaged during the operation was routinely washed by the cell saver before re-infusion and no rocuronium was detected after washing.

Mean plasma rocuronium concentrations did not change significantly during the anhepatic phase when compared with the paleohepatic phase, suggesting that absence of liver function during this phase did not have a significant impact on the clearance of rocuronium. Fisher and colleagues also failed to observe significant differences in rocuronium clearance during the paleohepatic and the anhepatic periods in a pharmacokinetic study of the three phases of liver transplantation. In their study, 20 patients were given two doses of rocuronium 0.6 mg kg\(^{-1}\) after induction of anaesthesia and after re-perfusion of the transplanted liver. Their results suggested that rocuronium clearance is maintained, and is minimally affected by the anhepatic period. This would seem inconsistent with the concept that the liver has an important role in the elimination of rocuronium. It should be remembered, however, that all transplant patients have a severely diseased liver before transplantation. Thus, removal of a severely dysfunctional liver might be expected to cause minimal perturbation in rocuronium concentration.

Multiple physiological factors and events during the anhepatic phase, such as blood loss, alteration in cardiac output, hypothermia and loss of rocuronium to the venovenous bypass circuit, may have contributed to a lack of change in rocuronium clearance.

Our rocuronium assay using GC–MS can also detect the putative metabolite, 17-desacetylrocuronium, but the sensitivity for the metabolite is substantially less than that for the parent (unchanged) drug. That this metabolite was not detected in any plasma samples may reflect the low assay sensitivity for the metabolite or negligible plasma concentrations of it, caused by negligible conversion of rocuronium to the metabolite or its rapid plasma clearance. Previous studies also did not detect rocuronium metabolites using either HPLC or GC methods. Only minimal amounts of 17-desacetylrocuronium were detected in plasma even after a large dose of rocuronium (0.9 mg kg\(^{-1}\)). Similar results were also found in animals. These studies suggest that rocuronium is eliminated mainly unchanged, presumably in bile.

Previously a close relationship between recovery time from rocuronium-induced paralysis and early postoperative liver function after liver transplantation has been observed. This study produces further evidence that changes in rocuronium pharmacokinetics, as reflected by perturbations in plasma rocuronium concentration during different phases of liver transplantation, appear to be correlated with early postoperative graft liver function and patient prognosis. Thus, rocuronium may have potential as an on-line pharmacokinetic probe of liver function following reperfusion of the graft liver. Although assay methods for
Rocuronium are not available on-line in most transplantation centres, rocuronium may still prove a useful clinical indicator if its infusion rate is not kept constant, but allowed to vary according to clinical requirement during the three phases of transplantation.

Fisher and colleagues, observed a correlation between duration of warm ischaemia of the donor liver and rocuronium clearance during the neohepatic phase. Increased duration of warm ischaemia was associated with decreased clearance. In our study, the duration of warm ischaemia of the donor livers for the two patients who died (and who had increased rocuronium plasma concentrations during the neohepatic phase) were 52 and 110 min respectively, while mean duration of warm ischaemia was 73 (29) min (range 40–154 min). There was no correlation between the duration of warm ischaemia and individual changes in plasma rocuronium concentration during the neohepatic phase. Total duration of ischaemia in the donor livers for the two patients who died were 656 and 1010 min, respectively, compared with an average of 617 (171) min (range 325–1010 min) in the survivors (Table 2). Again, no association could be detected between the change in rocuronium concentration and the duration of ischaemia. However, the longest duration of ischaemia (1010 min) was observed in one of the two patients who died and who had an increased rocuronium concentration during the neohepatic phase.

Neuromuscular blocking agents have the advantage that their pharmacodynamic effects can be quantified clinically. We tried to quantify the intensity of paralysis during transplantation in this study. However, because the present study focused on the investigation of plasma rocuronium concentrations during three phases of liver transplantation, the patients were paralysed to an extent which guaranteed that no changes were made to the rocuronium infusion rate during the procedure. Thus, no extensive pharmacodynamic data were available from this study.

In conclusion, reperfusion of the graft liver during liver transplantation (neohepatic phase) resulted in a decrease in plasma rocuronium concentration compared with that during the anhepatic phase, indicating an important role of the liver in the elimination of rocuronium. The increase in rocuronium concentration during the neohepatic phase in the two patients who died after surgery correlated with their early postoperative liver function tests, which were abnormal. This is in agreement with a previous study which observed a close relationship between intensity of rocuronium-induced paralysis and postoperative graft liver function. Our study gives further encouraging information about the value of using rocuronium as a pharmacokinetic probe of liver function. Meanwhile, use of rocuronium as an on-line pharmacodynamic probe of graft liver function during transplantation by measuring neuromuscular block and infusion dose requirements is worthy of and is the subject of future studies by us.

References


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