Administration of atracurium during reperfusion of rat livers after 21 h of cold ischaemic storage in different solutions

H. RECKENDORFER, M. SPERLICH, H. BURGMANN, M. WEINDLMAVR-GÖTTEL, P. G. SPIECKERMANN AND S. SCHWARZ

SUMMARY

The pharmacokinetics of atracurium are not altered by impaired hepatic function. The drug is therefore used widely in liver transplant patients. In previous work on the hepatotoxic effects of atracurium in an isolated, perfused rat liver model, we could not detect biochemical (release of lactate dehydrogenase or aspartate aminotransferase) or histological evidence of liver cell damage, except a reduction in hepatic tissue ATP content. In the present study, rat livers were reperfused with Krebs–Henseleit bicarbonate buffer with or without atracurium after 21 h of cold ischaemic storage in University of Wisconsin (UW), Bretschneider’s HTK or Eurocollins solution. UW-protected livers showed a complete restoration of ATP, total adenine nucleotides and energy charge during reperfusion, but the addition of atracurium diminished the regeneration capacity to about 50%. The energy charge (an index for determination of liver viability) was also reduced markedly. (Br. J. Anaesth. 1994; 72: 89–92)

KEY WORDS

Neuromuscular relaxants atracurium. Liver metabolism.

The pharmacokinetics of atracurium, a bis-quaternary competitive neuromuscular blocking agent, are not altered by impaired hepatic or renal function because of its unique degradation pathway by Hofmann decomposition and ester hydrolysis [1–3]. It should therefore be ideal for patients with hepatic failure and is used widely in liver transplant patients [14] and intensive care medicine [5]. In contrast, some studies have indicated considerable hepatotoxicity, notably lactate dehydrogenase (LDH) leakage from isolated rat hepatocytes, produced by atracurium and its breakdown products [6]. In a previous study using an isolated, perfused rat liver model [7], we could not detect biochemical or morphological changes, except for a reduction in ATP, during or after administration of atracurium. However, as proposed by Nigrovic and colleagues [8], it is possible that the intact organ may retain sufficient detoxification systems to repair any damage caused by atracurium. This study was designed to test this hypothesis under conditions of depleted glutathione such as occur in cold-conserved liver grafts before transplantation. Rat livers were stored on ice for 21 h in University of Wisconsin, Eurocollins or Bretschneider’s HTK solution and then reperfused with Krebs–Henseleit bicarbonate buffer (KHB) with or without atracurium.

MATERIALS AND METHODS

Livers were harvested from non-fasted male Louvain rats (body weight 280–300 g) (obtained from Forschungsinstitut für Versuchstierzucht, Himberg, Austria) as described previously [9]. Briefly, after the animal was anaesthetized with thiopentone 5–10 mg/100 g body weight i.p and heparinized (300 IU/100 g body weight into the spleen), the portal vein, common bile duct and inferior vena cava were cannulated. The livers were flushed in situ via the portal vein with 50 ml of cooled (4 °C) conserving solution, then excised, immersed in 100 ml of the same solution and stored on ice for 21 h. The following solutions were compared (for detailed specifications see [9]): University of Wisconsin (UW) solution (DuPont Critical Care, Waukegan, IL, U.S.A.), Eurocollins (EC) solution (Fresenius AG, Oberursel, Germany) and Bretschneider’s (HTK) solution (Dr F. Köhler Chemie GmbH, Alsbach-Haehnlein, Germany). Krebs–Henseleit bicarbonate buffer (KHB) was used to create inadequate storage conditions (n = 10 in each group).

After 21 h of cold ischaemic storage, the livers were reperfused in a non-recirculating model [9] via the portal vein for 1 h at 37 °C, using oxygenated (95% oxygen–5% carbon dioxide) Krebs–Henseleit bicarbonate buffer (KHB) containing glucose 5.5 mmol litre⁻¹ as perfusate. The flow rate was 3 ml/g liver min⁻¹ and the perfusion pressure 10–15 cm of perfusate. In each group, five livers were reperfused, with the addition of atracurium (Tracrium, Wellcome, The Wellcome Foundation Ltd, London, U.K.) 12.5 mg/5 ml for the first 10 min of reperfusion, using a Braun Perfusor (B. Braun Austria Gmbh) followed by 50 min of KHB perfusion. The pH, measured continuously during reperfusion, but the addition of atracurium diminished the regeneration capacity to about 50%. The energy charge (an index for determination of liver viability) was also reduced markedly. (Br. J. Anaesth. 1994; 72: 89–92)
the whole reperfusion period, remained at 7.45–7.55 and was kept constant also during administration of atracurium.

Aliquots of perfusate were collected 1, 5, 10, 15, 30 and 60 min after reperfusion was started. Perfuse lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were measured using commercially available kits (Boehringer Mannheim GmbH, Mannheim, Germany). Enzyme release into the 21-h storage solution was also measured. Atracurium and laudanosine concentrations were measured by high pressure liquid chromatography (HPLC) as described previously [10]. Concentrations were measured in the infusion preparations at the start and end of infusion, and in the perfusate during and after infusion.

In addition, liver samples were taken for measurement of hepatic tissue high energy phosphates immediately after the flushing period, after 21 h of storage and at the end of reperfusion. Analyses were performed using an LKB HPLC System (LKB-Producenter AB, Bromma, Sweden) [9]. Total adenine nucleotide concentrations were the sum of AMP, ADP and ATP. The energy charge ((ATP + 0.5 ADP)/(AMP + ADP + ATP)) was calculated according to the formula of Atkinson [11]. All data are presented as mean (sd). Data were analysed statistically using Students’ t test, analysis of variance and Scheffe’s test. P < 0.05 was considered statistically significant.

RESULTS

After flushing with the different solutions, all rat livers had comparable mean concentrations of high energy phosphates (table I) and energy charge values. As shown previously [9], livers conserved in UW solution maintained significantly greater concentrations of high energy phosphates after 21 h of cold ischaemic storage compared with those in the other groups. Mean ATP values of the UW preserved livers decreased from 2.42 to 1.01 μmol/g wet weight after 21 h. Using HTK or EC as conserving solution, a decrease in ATP concentration to 0.38 and 0.37 μmol g⁻¹, respectively, was observed. KHB was a poor protecting medium, as shown by the largest decrease in ATP concentration. Mean energy charge of the UW solution decreased from 0.59 to 0.24 after storage. After flushing with atracurium-containing solutions, ATP concentrations of the UW group decreased to 0.38 μmol g⁻¹ after storage. An increase in AMP to 2.31 μmol g⁻¹ occurred in the UW group. A significantly smaller increase in AMP concentration occurred in organs stored in HTK (1.65 μmol g⁻¹), EC (1.54 μmol g⁻¹) and KHB (1.02 μmol g⁻¹). Concentrations of ADP were also maintained significantly better as a result of storage in UW. As a consequence, mean total adenine nucleotide concentration was 4.28 μmol g⁻¹ in the UW group. Similar, but significantly smaller total concentrations occurred in EC- and HTK-treated livers; the smallest content occurred in the KHB group (table I).

Analysis of the infusion preparations of atracurium showed considerable amounts of the main metabolite, laudanosine, in the commercial ampoules, as already reported [7]. The preparation contained atracurium 2116 (61.8) μmol ml⁻¹ and laudanosine 197.8 (31.7) μmol ml⁻¹ at the start of infusion and atracurium 2105.9 (101.8) μmol ml⁻¹ and laudano-

<table>
<thead>
<tr>
<th>Adenine nucleotide concn (μmol/g wet wt)</th>
<th>Total</th>
<th>Energy charge</th>
</tr>
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<tbody>
<tr>
<td>AMP</td>
<td>ADP</td>
<td>ATP</td>
</tr>
<tr>
<td>UW</td>
<td>After flush</td>
<td>1.28 (0.20)</td>
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<td></td>
<td>21 h</td>
<td>2.31 (0.33)</td>
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<tr>
<td>HTK</td>
<td>After flush</td>
<td>1.30 (0.25)</td>
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<td></td>
<td>21 h</td>
<td>1.65 (0.20)**</td>
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<tr>
<td>EC</td>
<td>After flush</td>
<td>1.25 (0.10)</td>
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<td></td>
<td>21 h</td>
<td>1.54 (0.17)**</td>
</tr>
<tr>
<td>KHB</td>
<td>After flush</td>
<td>1.35 (0.15)</td>
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<td></td>
<td>21 h</td>
<td>1.02 (0.16)**</td>
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</tbody>
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ATRACURIUM AND HEPATIC ENERGY METABOLISM

Table II. Hepatic tissue adenine nucleotides and energy charge after 60 min of normothermic reperfusion with Krebs-Henseleit buffer without or with atracurium (for the first 10 min of reperfusion); n = 5 in each group. Solutions as in table I. Significant differences compared with KHB reperfused organs without atracurium: *P < 0.05; **P < 0.01; ***P < 0.001.

<table>
<thead>
<tr>
<th>Adenine nucleotide concn (μmol/g wet wt)</th>
<th>AMP</th>
<th>ADP</th>
<th>ATP</th>
<th>Total</th>
<th>Energy charge</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UW</strong></td>
<td>Without</td>
<td>0.45 (0.16)</td>
<td>0.97 (0.30)</td>
<td>2.97 (0.24)</td>
<td>4.39 (0.39)</td>
</tr>
<tr>
<td>With</td>
<td>1.18 (0.33)**</td>
<td>1.50 (0.36)</td>
<td>1.30 (0.42)***</td>
<td>3.52 (1.1)</td>
<td>0.51 (0.01)***</td>
</tr>
<tr>
<td><strong>HTK</strong></td>
<td>Without</td>
<td>0.55 (0.20)</td>
<td>0.60 (0.06)</td>
<td>1.25 (0.03)</td>
<td>2.40 (0.29)</td>
</tr>
<tr>
<td>With</td>
<td>0.80 (0.12)</td>
<td>0.48 (0.22)</td>
<td>0.53 (0.24)***</td>
<td>1.81 (0.53)</td>
<td>0.42 (0.06)**</td>
</tr>
<tr>
<td><strong>EC</strong></td>
<td>Without</td>
<td>0.41 (0.04)</td>
<td>0.77 (0.16)</td>
<td>1.68 (0.40)</td>
<td>2.86 (0.50)</td>
</tr>
<tr>
<td>With</td>
<td>0.60 (0.16)</td>
<td>0.63 (0.09)</td>
<td>0.94 (0.15)**</td>
<td>2.17 (0.33)</td>
<td>0.61 (0.04)**</td>
</tr>
<tr>
<td><strong>KHB</strong></td>
<td>Without</td>
<td>0.38 (0.06)</td>
<td>0.85 (0.07)</td>
<td>1.39 (0.20)</td>
<td>2.62 (0.20)</td>
</tr>
<tr>
<td>With</td>
<td>0.35 (0.07)</td>
<td>0.64 (0.31)</td>
<td>0.78 (0.28)*</td>
<td>1.74 (0.65)</td>
<td>0.63 (0.02)*</td>
</tr>
</tbody>
</table>

Atracurium and hepatic energy metabolism.

Since 196.3 (30.2) μmol ml⁻¹ at the end of infusion. During the period of study, no additional degradation of atracturium occurred. We observed a rapid appearance and increase in the concentrations of atracturium and laudanosine in the perfusate, to plateau values of 15.8 (4.5) nmol ml⁻¹ and 6.8 (0.9) nmol ml⁻¹, respectively. These are comparable to the drug concentrations measured in freshly prepared and perfused rat livers [7].

After 1 h of normothermic reperfusion without addition of neuromuscular blocking drugs, complete restoration of ATP (2.97 (0.24) μmol g⁻¹) and energy charge (0.79 (0.05)) occurred only in UW-conserved livers; the capacity to recover from ischemic insult during ex vivo conservation was reduced markedly in all other groups. There were no significant differences in hepatic high energy phosphate concentrations between KHB-, EC- and HTK-conserved livers after 1 h of reperfusion (table II).

When atracurium was added for the first 10 min of reperfusion only, considerable changes in hepatic high energy phosphate content were observed after 1 h of reperfusion. In UW-conserved organs, ATP regeneration capacity was reduced markedly (1.30 (0.42) μmol g⁻¹), whereas the AMP content remained high (1.18 (0.33) μmol g⁻¹ compared with 0.45 (0.16) μmol g⁻¹ without atracurium). Mean energy charge, reflecting the cellular energy balance between energy utilization and generation, was reduced significantly (0.51 vs 0.79). Total adenine nucleotide content was also reduced, but the difference was not statistically significant.

Similar results were obtained in HTK-, EC- and KHB-stored organs. We found a significant reduction in ATP regeneration capacity and a decrease in energy charge in all three groups compared with organs reperfused with KHB only. Mean hepatic tissue ATP concentrations were 0.53 (vs 1.25) μmol g⁻¹ in the HTK group, 0.94 (vs 1.68) μmol g⁻¹ in the EC group and 0.78 (vs 1.39) μmol g⁻¹ in the KHB group. Changes in concentrations of all hepatic high energy phosphates are given in table II.

We found no additional increase in enzyme release into the perfusate during administration of atracurium compared with livers conserved in the same protecting solution and reperfused with KHB only. After the start of reperfusion, poorly preserved livers (represented by the KHB group) released significantly more LDH than livers stored in any of the other test solutions. The peak value, obtained 1 min after starting reperfusion, was 840 (80) iu litre⁻¹. Enzyme liberation from livers conserved in HTK or EC was similar (600 (40) iu litre⁻¹ and 580 (50) iu litre⁻¹, respectively), but greater than that from livers in the UW group (180 (20) iu litre⁻¹). The same pattern was obtained for AST. The smallest release (peak value 1 min after starting reperfusion) occurred in the UW group (25 (7) iu litre⁻¹) compared with 68 (10) iu litre⁻¹ in the EC group, 70 (10) iu litre⁻¹ in the HTK group and 135 (30) iu litre⁻¹ for KHB-stored organs.

**DISCUSSION**

Because of its unique degradation pathway, the pharmacokinetics of atracturium are not altered by impaired hepatic and renal function [1]. An infusion of atracturium is therefore recommended and used widely as a safe and convenient way of providing neuromuscular block in patients undergoing liver transplantation [4]. In contrast with its remarkable safety in clinical use [12], studies by Nigrovic and co-workers [6,8] indicated considerable hepatotoxicity (massive LDH leakage from isolated rat hepatocytes) of atracturium and its breakdown products such as reactive electrophilic metabolites. Detoxification of these acrylates in vivo proceeds by hydrolysis to acrylic acid and by conjugation to glutathione in the liver [13]. As suggested by Nigrovic and colleagues [6], the stores of glutathione in an intact organ might be sufficient to act as a scavenger of acrylates and may repair minor cellular damage.

In previous studies using the isolated, perfused rat liver model [7,14], we did not detect either morphological or biochemical (LDH, AST release) changes during or after administration of atracturium or its main metabolite, laudanosine. However, we found a significant reduction in hepatic tissue ATP and total adenine nucleotide content only in the
atracurium-treated livers, compared with laudanosine and control groups. It was the aim of this study to investigate the effects of atracurium on hepatic high energy phosphate metabolism and enzyme release under conditions of depleted glutathione such as occur during cold ischaemic storage of liver grafts before transplantation. As shown by Vreugdenhil and colleagues [15], glutathione was reduced to about 30% of control values during 24 h of cold storage of rabbit livers. Only the University of Wisconsin (UW) preservation solution, developed in 1987 by Wahlberg and colleagues [16] and now the “gold standard” in liver transplant surgery, contains glutathione, but efforts have been made to promote the use of Bretschneider’s and Eurocollins solutions as liver protecting solutions [17].

In a previous study [9], using the same isolated, perfused rat liver model as in the present work, we compared the protecting capacity of UW, HTK and EC solutions. KHB was used to show the effects of simple hypothermia. We found that only UW-conserved livers were able to restore completely ATP, total adenine nucleotides and energy charge under reperfusion conditions after 21 h of cold ischaemic storage. Release of enzymes (LDH, AST) into either the 21-h storage solution or the perfusate during reperfusion was significantly less with storage of organs in UW solution.

In the atracurium groups, the pharmakokinetic properties were not changed compared with those of immediately perfused rat livers. If atracurium was added only for the first 10 min of reperfusion, significant depression of ATP regeneration capacity (to about 50%) occurred in all four groups compared with organs reprefused with only KHB. In addition, the energy charge, which reflects cellular energy balance between utilization and generation and is known as a reliable index for determination of liver viability [18], was reduced drastically as a result of administration of atracurium. As reported previously [19, 20], recovery from ischaemic damage after transplantation depends on the cellular capacity for ATP resynthesis and therefore the rate and extent of liver ATP regeneration after recirculation reflects the viability of the graft and can serve as a prognostic marker in orthotopic liver transplantation [19].

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REFERENCES


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