Influence of incubated atracurium on rat liver function


SUMMARY
Degradation of atracurium by Hofmann elimination and ester hydrolysis depends mainly on pH and temperature and is said to be independent of liver and kidney function. Consequently atracurium is used widely in patients with liver failure. However, there is evidence that incubation of atracurium at 37 °C and pH 8 leads to leakage of LDH from hepatocyte cell cultures. We have tested the hepatotoxic effects of incubated atracurium in an isolated perfused rat liver model. After equilibration, atracurium 2010 μmol ml⁻¹ (preincubated at pH 8 and 37 °C for 120 min) was administered over a period of 10 min followed by perfusion of Krebs–Henseleit bicarbonate buffer for 60 min. We found that incubation resulted in considerable degradation of atracurium and formation of laudanosine. Administration of incubated atracurium did not produce either biochemical or morphological damage to liver cells, but caused considerable increase in bile flow. We conclude that administration of preincubated atracurium did not produce impairment of liver cell function. The increase in bile flow could be beneficial if it occurs clinically. (Br. J. Anaesth. 1994; 72: 324–327)

KEY WORDS
Neuromuscular block: atracurium. Toxicity: hepatic.

The use of neuromuscular blocking agents in patients with multiple organ failure is a major problem during anaesthesia and intensive care medicine. Atracurium, a short-acting non-depolarizing neuromuscular blocking agent, may be useful in such patients because of its unique breakdown by Hofmann elimination and ester hydrolysis [1–3], as both are said to occur independently of kidney and liver function [4–8]. The main breakdown products of atracurium are laudanosine, quaternary alcohols, quaternary acids and monoacylates [9]. The chosen elimination pathway of atracurium depends mainly on pH and temperature. In vitro studies showed that at 37 °C the plasma half-life of atracurium is approximately 18 min, whereas at 5 °C the plasma half-life is 15.5 h [9].

In previous studies we demonstrated, in the isolated perfused rat liver model, that administration of atracurium did not cause impairment of hepatic function [10, 11]. However, Nigrovic and colleagues reported that administration of atracurium, which had been incubated at 37 °C and pH 8, resulted in increased leakage of LDH in hepatic cell culture [12, 13]. Therefore, it appears that pretreatment of atracurium leads to degradation of the drug and formation of hepatotoxic breakdown products. Thus, in this study, we investigated if degradation products of incubated atracurium (incubation at 37 °C and pH 8 for 120 min), formed during pretreatment, results in liver cell damage in the whole organ similar to that found in liver cell culture.

MATERIALS AND METHODS
We diluted atracurium 50 μg (Tracrium, Wellcome, Wellcome Foundation Ltd, London, U.K.) with distilled water to a volume of 20 ml giving a final concentration of 2010 μmol ml⁻¹. In one group this mixture was incubated at 37 °C for 120 min and in the second group the solution was adjusted to pH 8 with KOH and incubated at 37 °C for 120 min. Samples were obtained at 15-min intervals during incubation for measurement of atracurium and laudanosine by high pressure liquid chromatography (values expressed as μmol ml⁻¹) and pH, as described by Nagl, Weindlmayr-Goettel and Fitzal [14]. In addition, concentrations of laudanosine were measured in collected bile after the perfusion period.

Livers were harvested from non-fasted male Louvain rats obtained from Forschungsinstitut für Versuchstierzucht, Himberg, Austria (n = 5 animals in each group). After anaesthesia with thiopentone 5–10 mg/100 g body weight i.p. and heparinization (300 iu/100 g body weight into the spleen), the portal vein, inferior vena cava and common bile duct were cannulated. The livers were excised and perfused via the portal vein with oxygenated Krebs–Henseleit bicarbonate buffer (KHB) containing glucose 5.5 mmol litre⁻¹ at 37 °C, as described previously [10]. After 20 min of normothermic perfusion for equilibration, the incubated atracurium was administered over 10 min using a Braun Perfusor (B. Braun, Austria GmbH), followed by...
additional normothermic perfusion with KHB for 60 min. Livers perfused with KHB only served as controls. Aliquots of perfusate samples were collected at defined times before, during and after drug administration. LDH in perfusate was measured using commercially available kits (Boehringer Mannheim GmbH, Mannheim, Germany). Results are expressed as IU litre\(^{-1}\).

Bile flow (mg/g liver min\(^{-1}\)) was measured continuously during the whole perfusion period using an automatic drop counter. Bile was collected, weighed and concentrations of laudanosine measured. At the end of perfusion, tissue specimens were fixed in neutral buffered formalin and prepared for light microscopy. Tissues were stained with haematoxylin–eosin, van Gieson and a connective tissue stain with chromotroph aniline blue (CAB), according to Mallory.

All data are presented as mean (SD) of five different rat livers in each group. Data were analysed by Student's t-test. Probability values of \(P < 0.05\) were considered statistically significant.

RESULTS

As described previously [10], we found that almost 20% of atracurium had been degraded in the commercially available ampoules. Incubation of atracurium at 37 °C for 120 min did not produce additional degradation of the substance, the pH remained constant at 3 (0.1). In contrast, at pH 8 and 37 °C, marked degradation of atracurium and formation of laudanosine occurred. Atracurium concentrations decreased from 2010 (93) to 1226 (81) μmol ml\(^{-1}\) after 120 min, whereas concentrations of laudanosine increased from 241 (23) μmol ml\(^{-1}\) at the beginning to 456 (55) μmol ml\(^{-1}\) after the incubation period (fig. 1). pH values decreased from 8 (0.1) at the beginning to 6.89 (0.09) at the end of the incubation period.

As before [10], we found rapid appearance and subsequent increase in the concentrations of atracurium and laudanosine in the perfusate samples after starting the infusion, but also rapid disappearance after stopping the infusion (data not shown). We found no pathological alterations in release of LDH into the perfusate (fig. 2) in comparison with control livers. LDH concentrations remained within the normal range both during administration of the drug and in the following perfusion period. A marked choleretic response occurred in the incubated atracurium group. Bile flow increased immediately by about 80% after starting drug administration and returned rapidly to baseline values after stopping the drug (fig. 3). For laudanosine, 15 (2)% of the administered dose was found in the bile collected.

Under light microscopy, all liver biopsies had normal hepatic architecture with typical normal lobules, regular portal tracts and only minimal dilatation of sinusoids and central veins caused by perfusion. The thin-walled central veins were surrounded by radiating columns of hepatocytes and bordered peripherally by two to four portal tracts. Sinusoidal lining cells, Kupffer cells and hepatocytes in the specimens could not be distinguished from those in control livers. Occasionally, some of the hepatocytes contained a number of scattered small fat droplets. Neither cell necrosis nor an increase of single acidophilic bodies was detected. The bile canaliculi and bile ducts were well preserved.

DISCUSSION

Because of its unique breakdown by Hofmann elimination or ester hydrolysis [1–3], atracurium is used widely in patients with hepatic or renal failure [4–8]. In contrast with many reports on its remarkable safety in clinical use [15, 16], Nigrovic and colleagues demonstrated concentration-dependent...
LDH leakage of incubated atracurium in liver cell cultures [12, 13], which was most pronounced when hepatocytes were pretreated with triorthotolylphosphate to inhibit enzyme-catalysed hydrolysis of atracurium. They attributed the hepatotoxicity to the action of acrylates, which are formed during Hofmann decomposition, the preferred elimination pathway at pH 8 [17–20]. Laudanosine and acrylates are generated concomitantly and in equimolar amounts. Detoxification of acrylates in vivo proceeds either by hydrolysis to acrylic acid or by conjugation to glutathione.

In our previous study [10], we found that administration of an extremely large dose of atracurium did not cause impairment of liver function. In this study we aimed to investigate both the rate and extent of atracurium degradation occurring in 120 min of incubation at 37 °C and pH 8, conditions which may occur in vivo, and the consequent possible hepatotoxic effects of the breakdown products, especially acrylates, which are formed in considerable amounts during incubation.

As reported elsewhere [17–20], we found that incubation of atracurium at 37 °C and pH 8 caused extensive degradation of atracurium and formation of the main breakdown product, laudanosine. Formation of laudanosine is one mechanism for the increase in bile flow by about 80% in comparison with control livers. The choleretic effect of laudanosine is caused by several mechanisms. It is a tetrahydropapaverine and therefore a chemical relative of papaverine, a substance known to cause vaso-
diatation [21]. However, this may explain only partly the large cholestatic effect of this substance. We found that approximately 15% of the laudanosine administered was excreted in bile, but it is not known if this occurs by an active or passive transport mechanism. However, each molecule of laudanosine may bind water and therefore contribute to the choleretic effect of this substance. Laudanosine causes an increase in bile flow and may be of benefit in patients undergoing liver transplantation to induce bile flow [22].

In summary, we observed that incubation at 37 °C and pH 8, in contrast with pH 3, caused extensive degradation of atracurium and formation of laudanosine, and reduced the neuromuscular blocking effect of this substance. Laudanosine causes an acute hepatocellular injury, which may be of benefit in patients undergoing liver transplantation to induce bile flow [22].

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References