Testing the porphyrinogenicity of propofol in a primed rat model

H. BÖHRER, H. SCHMIDT, E. MARTIN, R. LUX, K. BOLSEN AND G. GOERZ

Summary
We evaluated the porphyrinogenicity of propofol in a rat model. After a pilot study had been conducted to determine an optimal dose, 48 fasting male Sprague–Dawley rats were allocated randomly to six groups. The animals in groups 1–3 received saline i.p. In groups 4–6, the animals were given allylisopropylacetamide (AIA). Twelve hours later, animals in groups 1 and 4 received saline, groups 2 and 5 were given propofol 150 mg kg\(^{-1}\) i.p., followed by 75 mg kg\(^{-1}\) 3 h later, and groups 3 and 6 received phenobarbitone 50 mg kg\(^{-1}\) i.p. and 25 mg kg\(^{-1}\) i.p. The animals were anaesthetized and killed 3 h after the second drug bolus and we measured the concentration of cytochrome P450, total porphyrin content and the activity of δ-aminolaevulinic acid synthase (ALAS) in the liver. Urinary δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG) concentrations were measured. Analysis of variance and the \(t\) test with Bonferroni’s correction were used to compare data. The hepatic cytochrome P450 concentration in the non-primed groups varied from 28.1 to 31.1 nmol g\(^{-1}\); administration of AIA decreased this to 20.1–20.9 nmol g\(^{-1}\). Total hepatic porphyrins were between 0.78 and 1.22 nmol g\(^{-1}\) in the non-primed groups and between 2.71 and 3.54 nmol g\(^{-1}\) in the AIA-primed groups. Hepatic ALAS activity was 29.2 and 35.5 nmol h\(^{-1}\) g\(^{-1}\) in groups 1 and 2. In the primed saline group, ALAS activity was measured at 134.5 nmol h\(^{-1}\) g\(^{-1}\). There was a tendency towards an increase in activity in the primed propofol group (169.7 nmol h\(^{-1}\) g\(^{-1}\)) when this was compared with the primed saline group. The highest values were obtained in the primed phenobarbitone group (262.5 nmol h\(^{-1}\) g\(^{-1}\)). Corresponding results were obtained for measurements of urinary ALA and PBG. We conclude that propofol is a safe agent in the porphyric patient when given as a single bolus dose. Caution may be necessary, however, when large cumulative doses are administered. (\textit{Br. J. Anaesth.} 1995; 75: 334–338)

Key words

Acute hepatic porphyrias, which include acute intermittent porphyria, variegate porphyria and hereditary coproporphyria, are rare disorders of the haem biosynthetic pathway. They are characterized by excessive accumulation of porphyrins and porphyrin precursors because of partial block of the pathway caused by single or dual enzyme deficiencies. It is the latent phase of acute porphyrias that is usually present, but this can be turned rapidly into an acute attack when porphyrinogenic agents are administered. Anaesthetic agents such as barbiturates have long been known to precipitate such acute porphyrac attacks [1, 2].

In recent years propofol has been recommended for anaesthesia in patients suffering from acute hepatic porphyria, and the majority of reports consider propofol to be safe in this condition. However, there are three case reports that warn against the use of propofol in acute porphyria [3–5]. In addition, propofol was found to exert a potent porphyrinogenic effect in the primed chick embryo model [6].

We have evaluated the porphyrinogenic potency of propofol in an in vivo rat model. Propofol was administered to normal rats and to rats made porphyrptic with the chemical substance allylisopropylacetamide (AIA). When AIA is given to rats in appropriate doses, it induces a condition which resembles the latent phase of an acute hepatic porphyria in humans. The rats become sensitive to drugs which may then precipitate a metabolic phenomenon that exhibits the biochemical characteristics of the acute attack in humans.

Materials and methods

ANIMALS

All aspects of this study were reviewed and approved by the Governmental Animal Protection Committee. Healthy, male Sprague-Dawley rats, weighing 300–400 g, were obtained from Save (Kisslegg, Germany). They were housed in groups of four on a 12:12-h light–dark cycle with food and water ad libitum. A temperature of 22 °C and humidity 55–60 % were maintained in the room. Before the experiment, the animals were fasted for approximately 13 h with free access to water.

H. BÖHRER, MD, DEAA, H. SCHMIDT, MD, DEAA, E. MARTIN, MD, Department of Anaesthesia, University of Heidelberg, Im Neuenheimer Feld 110, D-69120 Heidelberg; R. LUX, MD, K. BOLSEN, BS, G. GOERZ, MD, PhD, Department of Dermatology, University of Düsseldorf, Postfach 101007, D-40001 Düsseldorf, Germany. Accepted for publication: March 24, 1995. Correspondence to H.B.
Testing the porphyrinogenicity of propofol

PILOT STUDY

Appropriate doses of propofol and phenobarbitone were administered to ensure comparability of results. A total of 12 animals were used to determine these doses, with each animal being treated repeatedly. Doses of propofol 150 mg kg\(^{-1}\) administered i.p., followed by 75 mg kg\(^{-1}\) 3 h later and phenobarbitone 50 mg kg\(^{-1}\) i.p., followed by 25 mg kg\(^{-1}\) 3 h later were capable of inducing sleep, with the animals remaining easily arousable. Higher doses of the drugs resulted in a comatose state with irregular respiratory movements and rapid development of hypothermia.

EXPERIMENTAL PROTOCOL

A total of 48 animals were allocated randomly to one of six different groups (n = 8). The animals in groups 1–3 received saline i.p. In groups 4–6 the animals were primed with AIA, which was applied i.p. at a dose of 300 mg kg\(^{-1}\). Twelve hours later, propofol 150 mg kg\(^{-1}\) i.p. was administered to the animals in groups 2 and 5, while groups 3 and 6 received phenobarbitone 50 mg kg\(^{-1}\) i.p. Saline was given to groups 1 and 4. Three hours later, a second dose of propofol 75 mg kg\(^{-1}\), phenobarbitone 25 mg kg\(^{-1}\) or saline, respectively, was administered. Propofol was used in its commercially available 10 % Intralipid emulsion (propofol 10 mg ml\(^{-1}\), 10 % soybean oil, 2.25 % glycerol and 1.2 % purified egg phosphatidate). Phenobarbitone was used as a commercially available solution dissolved in propylene glycol, ethanol and water.

All animals breathed spontaneously throughout the study period. External heating was applied to all animals in the propofol and phenobarbitone groups. Three hours after administration of the second drug bolus, all animals were briefly anaesthetized with diethyl ether and rectal temperature was obtained to exclude hypothermia. A median laparotomy was then performed and aortic blood was obtained, thereby killing the animals by exsanguination.

Arterial blood-gas values were determined to exclude hypoxia and acidosis. In addition, samples for measurement of propofol concentrations were placed into tubes that contained potassium oxalate and then stored at 4 °C before analysis. Blood concentrations of propofol were measured after extraction into cyclohexane using high-pressure liquid chromatography with electrochemical detection [7]. Plasma concentrations of phenobarbitone were measured using a commercially available fluorescence polarization immunoassay (TDx System, Abbott).

BIOCHEMICAL MEASUREMENTS (SEE FIG. 1)

After exsanguination, the liver was perfused with Tris-HCl buffer, excised and homogenized in 0.9 % (w/v) NaCl–EDTA 0.5 mmol litre\(^{-1}\)-Tris-HCl buffer 10 mmol litre\(^{-1}\), pH 7.4 (1 : 3 w/v). The concentration of cytochrome P450 in the homogenate was measured using the method of Matsubara and colleagues [8], while δ-aminolaevulinic acid synthase (ALAS) was also assayed in whole homogenates according to the method of Marver and colleagues [9]. Total hepatic porphyrins were measured by the method of Seubert and Seubert [10], and urinary δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG) concentrations were measured according to Doss and Schmidt [11] using ion exchange chromatography columns.

STATISTICAL ANALYSIS

Analysis of variance and the t test with Bonferroni’s correction were used to compare data. Drug concentrations were compared using the t test. Changes were considered statistically significant if P < 0.05. All data are reported as mean (SEM).

Results

Data from one animal in each of groups 2, 3 and 6 were excluded from the study because of hypothermia. Acidosis and hypoxia, assessed by blood-gas analysis, did not occur in any of the remaining animals. A total of 45 rats were thus considered for statistical evaluation and in the final data set there were 7–8 animals in each of the six groups. At the end of the study, mean blood concentration of propofol was 2.22 (0.32) µg ml\(^{-1}\) in group 2 and 2.06 (0.19) µg ml\(^{-1}\) in group 5. The plasma concentration of phenobarbitone was 90.4 (7.3) µg ml\(^{-1}\) in group 3.
Table 1  Concentration of cytochrome P450 in liver homogenates, total hepatic porphyrin concentration, urinary δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG) concentrations in the six groups (mean (SEM)). All concentrations in the allylisopropylacetamide (AIA)-primed groups differed significantly (*P < 0.05) from those in the non-primed groups. †P < 0.05 compared with the two other AIA-primed groups

<table>
<thead>
<tr>
<th>Group</th>
<th>P450 concn (nmol/g liver)</th>
<th>Total hepatic porphyrins (nmol/g liver)</th>
<th>Urinary ALA concn (nmol litre⁻¹)</th>
<th>Urinary PBG concn (nmol litre⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>30.1 (1.8)</td>
<td>0.78 (0.2)</td>
<td>13.5 (3.0)</td>
<td>5.1 (0.9)</td>
</tr>
<tr>
<td>Propofol</td>
<td>28.1 (2.2)</td>
<td>1.22 (0.3)</td>
<td>10.1 (3.4)</td>
<td>9.3 (1.9)</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>31.1 (2.2)</td>
<td>0.82 (0.3)</td>
<td>14.0 (2.6)</td>
<td>7.7 (1.6)</td>
</tr>
<tr>
<td>AIA + NaCl</td>
<td>20.9 (1.2)*</td>
<td>2.71 (0.4)*</td>
<td>33.2 (5.5)*</td>
<td>51.5 (7.2)*</td>
</tr>
<tr>
<td>AIA + propofol</td>
<td>20.1 (1.6)*</td>
<td>3.27 (0.7)*</td>
<td>39.7 (5.7)*</td>
<td>65.5 (7.1)*</td>
</tr>
<tr>
<td>AIA + phenobarbitone</td>
<td>20.3 (1.9)*</td>
<td>3.54 (0.8)*</td>
<td>58.2 (9.1)*†</td>
<td>89.1 (10.7)*†</td>
</tr>
</tbody>
</table>

There was a non-significant increase in activity in the primed propofol group (169.7 nmol h⁻¹ g⁻¹). The highest values were achieved in the primed phenobarbitone group (262.5 nmol h⁻¹ g⁻¹).

Urinary concentrations of ALA and PBG were normal in groups 1–3, whereas increases in ALA and PBG concentrations were found in groups 4–6. In the AIA-primed propofol group, there was a non-significant increase in ALA and PBC concentrations compared with group 4. The highest concentrations of ALA and PBG were found in the primed phenobarbitone group.

Discussion

Porphyrias are characterized by a deficiency in one of the eight enzymes in haem biosynthesis. This leads to a decrease in the pool of free haem and it may, via a negative feedback mechanism, markedly stimulate the enzyme ALAS so that the supply of haem precursors exceeds the activities of the subsequent enzymes of the pathway. The haem precursors ALA and PBG accumulate and are excreted in excess. ALAS is the initial and rate-controlling enzyme of haem biosynthesis. Hepatic ALAS has an extremely short half-life of 30 min in the rat [12, 13], which implies that in order to obtain significant differences in our model of acute testing, it was necessary to have a study period of only a few hours. However, it would have taken several more hours to obtain evidence of marked inducing effects of phenobarbitone on the cytochrome system; this would have become evident in group 3.

There were no significant differences in ALA and PBG concentrations and in ALAS activities within the non-primed groups. Only ALAS showed a tendency towards an increase in activity in the non-primed phenobarbitone group. However, priming with AIA increased the sensitivity of the animals markedly. Administration of appropriate doses of AIA causes rapid loss of hepatic cytochrome P450 and haem, thus sensitizing the animals to porphyrinogenic agents [14, 15]. Porphyrinogenicity testing of drugs is, therefore, only significant when primed animals or systems are used.

We chose the in vivo system of the intact rat to screen for the porphyrinogenicity of propofol. Alternative systems are the exquisitely sensitive chick embryo liver cell culture and the chick embryo in vivo. However, the primed chick embryo might be too sensitive, which would give rise to false positive
results [16]. Furthermore, the porphyrinogenic effect of drugs is a dose-related phenomenon, making it difficult to find a dose which is equivalent to that administered to humans [17]. In our model, we were able to administer a clinically relevant dose according to the findings of the pilot study.

Priming the animals with AIA induced a state of latent porphyria. This was evident by the increased amount of total hepatic porphyrins in groups 4–6. Porphyrin accumulation may take longer than 6 h in the rat and thus administration of propofol or phenobarbitone was not expected to yield higher porphyrin values in groups 5 and 6 than in group 4. In contrast, the short half-life of the rate-controlling enzyme ALAS allows early recognition of a porphyrinogenic agent when this agent is added to a sensitized system. Therefore, drugs that cause a further increase in ALAS activity and subsequently in the concentrations of ALA and PBG are considered to have a definite porphyrinogenic effect. On the other hand, the addition of a non-porphyrinogenic agent to primed control animals should not further increase these values and thus exert no influence on this sensitive system.

On this basis, phenobarbitone must definitely be regarded as porphyrinogenic, as evidenced by ALAS activity and ALA and PBG concentrations in study group 6. Propofol did not exert significant porphyrinogenic effects in our AIA-primed in vivo rat model. However, there was a tendency in the primed propofol group towards increased values in all measurements relating to the haem biosynthetic pathway.

Propofol (2,6-diisopropylphenol) is a highly lipophilic compound and is rapidly metabolized by the liver; less than 0.3 % of the administered dose is excreted unchanged in the urine [18]. Total body clearance of propofol even exceeds estimates of total hepatic blood supply, so that additional, extrahepatic sites of propofol metabolism have been postulated. The principal metabolites in the urine are glucuronide conjugates of propofol, the corresponding quinol and the 4-sulphate conjugate of the quinol [19]. In order to be porphyrinogenic, chemicals or drugs must interact with the cytochrome P450 system [20]. Because propofol is metabolized extensively by the hepatic microsomal system, theoretically it has the potential to exert porphyrinogenic effects.

In the primed chick embryo model, propofol was found to exert a potent porphyrinogenic effect [6]. However, this model may be too sensitive when testing for porphyrinogenicity. In contrast, non-primed rat models [21] and those primed with dicarbethoxydihydrocollidine [22] have not shown any evidence of porphyrinogenicity associated with administration of propofol.

The literature contains several reports on the use of propofol in patients with acute hepatic porphyrias. With the exception of one small series of 13 patients from South Africa [23], these publications have usually appeared as single case reports [24, 25] or as letters to the editor [26–32]. In general, propofol is considered to be a safe agent in acute porphyria. Biochemical alterations in the haem biosynthetic pathway after administration of propofol have only been seen in three patients. Two of these patients had received known porphyrinogenic agents in addition to propofol [3, 4], while the third patient had been given a total dose of 1300 mg of propofol over a 40-min period [5].

Propofol is commercially available in an oil-in-water emulsion. There is no evidence from the literature that fat or fat emulsions exert porphyrinogenic effects in animals or humans. We therefore believe that vehicle control studies requiring the killing of additional animals are not warranted.

In summary, we found that propofol did not exert any significant porphyrinogenic effects in our AIA-primed in vivo rat model of acute hepatic porphyria. However, there was a trend in the primed propofol group towards increased values in all measurements relating to the haem biosynthetic pathway. Because the induction of an acute attack in a patient with acute hepatic porphyria is a largely dose-dependent phenomenon, we would extrapolate from our animal data that the induction of anaesthesia with a single bolus of propofol in the porphyric patient should be considered a safe technique. Caution may be necessary, however, when large cumulative doses of propofol are administered, such as in total i.v. anaesthesia.

References


