COMPARISON OF THE EFFECTS OF ISOFLURANE OR FENTANYL–NITROUS OXIDE ANAESTHESIA ON PROPRANOLOL DISPOSITION IN DOGS

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Drug disposition has been shown to be altered in the period around surgery [1,2]; however, the exact nature of these changes remains unclear. Anaesthetic agents themselves might alter drug disposition by a number of mechanisms: for example by changes in the volume of distribution of a drug or in the extent of protein binding in plasma, by alterations in regional blood flow or by changes in drug metabolizing enzyme activity.

Halothane inhibits drug metabolism markedly in animals [3] and man [4]. Halothane anaesthesia in dogs has been shown to slow the elimination of propranolol [5] and verapamil [6]. However, little is known of the effect of the i.v. anaesthetic agents or opioids on drugs disposition.

For drugs metabolized by the liver, changes in drug disposition result from effects on hepatic blood flow or hepatic drug-metabolizing enzyme activity, or both. The exact nature of this effect will depend on the extent of the hepatic extraction of the drug. The clearance of a drug of high hepatic extraction given i.v. will be determined principally by its rate of delivery to the liver (that is, by the hepatic blood flow), whereas the clearance of a drug of low hepatic extraction given i.v. will be determined primarily by hepatic enzyme activity. When administered by mouth or intraorally, the clearance of drugs of both high and low hepatic extraction reflects hepatic enzyme activity and is unaffected by changes in hepatic blood flow [7].

SUMMARY

The disposition of propranolol was studied, using dual-route administration, in two groups of six dogs. Each dog was studied on three consecutive days: day 1 awake, day 2 during anaesthesia, and day 3, 24 h after anaesthesia. Anaesthesia was with isoflurane 2.0 MAC (in oxygen) in one group and with a fentanyl–nitrous oxide–atracurium regimen in the other group. In the group receiving fentanyl, anaesthesia caused a significant decrease (63%) in intrinsic clearance from the day 1 value (P < 0.05) and a 45% decrease in systemic clearance (P < 0.05). Hepatic plasma flow decreased by 27% (ns). A similar pattern was found with isoflurane: intrinsic clearance decreased by 53% (P < 0.05) and systemic clearance by 40% (P < 0.05). Hepatic plasma flow decreased by 40% (ns). In both groups, the values 24 h after anaesthesia were not significantly different from those obtained on day 1. Anaesthesia with either fentanyl–nitrous oxide–atracurium or isoflurane has a marked, but short-lasting effect on the disposition of propranolol, in part as a result of a decrease in intrinsic clearance.

disposition of a high extraction drug such as propranolol given simultaneously by mouth and i.v., it is possible to measure hepatic blood flow and intrinsic clearance [8–10].

In a previous study we used dual-route administration (simultaneous radio-labelled i.v. and unlabelled portal administration of propranolol) to measure the effect of halothane anaesthesia on drug disposition in the dog [5]. In the present study, using similar methods, we have compared the effects of isoflurane and
fentanyl-nitrous oxide anaesthesia on hepatic blood flow and hepatic enzyme activity.

METHODS

Twelve male mongrel dogs were studied. Femoral artery, femoral vein and portal vein cannulae were implanted in each dog, under pentobarbitone anaesthesia (30 mg kg$^{-1}$) 5 days before the first study day. One group ($n = 6$, mean ± SD weight $20.7 ± 7.6$ kg) received a fentanyl-based and the other ($n = 6$, mean weight $21.3 ± 7.1$ kg) received an isoflurane-based anaesthetic. The disposition of propranolol was studied in each dog on three consecutive days: day 1 = 24 h before anaesthesia while awake; day 2 = during anaesthesia; day 3 = awake, 24 h after anaesthesia.

Anaesthetic regimens

In both groups, anaesthesia was induced with thiopentone 5–8 mg kg$^{-1}$ i.v. and the trachea was intubated. The lungs were ventilated mechanically to maintain normocapnoea ($P_{aCO_2}$ 4.6–5.5 kPa). Intra-arterial pressure was monitored continuously throughout the study.

Fentanyl anaesthesia. Following the induction of anaesthesia, fentanyl was infused i.v., initially at a rate of 0.75 ng kg$^{-1}$ min$^{-1}$ for 20 min (15 μg kg$^{-1}$ given over 20 min), and then at a rate of 0.22 ng kg$^{-1}$ min$^{-1}$ for the remainder of the study period. This was based on a regimen described by Murphy and Hug [11], which resulted in plasma fentanyl concentrations of approximately 8–10 ng ml$^{-1}$. Ventilation was with 67% nitrous oxide in oxygen. Muscle paralysis was achieved with atracurium at an initial dose of 0.3 mg kg$^{-1}$ with subsequent doses of 0.1 mg kg$^{-1}$ every 20 min. One hour of anaesthesia was completed before the start of the 4-h study period. At the end of the study period the infusion of fentanyl and the administration of nitrous oxide were discontinued and the dog was allowed to recover spontaneous respiration before the trachea was extubated.

Isoflurane anaesthesia. After tracheal intubation anaesthesia was maintained with isoflurane 2.0 MAC (2.3 %) in oxygen. End-tidal isoflurane concentrations were measured by gas chromatography every 15 min until 2.0 MAC was obtained and then hourly until completion of the study. To allow stable haemodynamic conditions and constant end-tidal concentrations, 2 h of anaesthesia was completed before the start of the 4-h study period. The total MAC hours of volatile agent was calculated. At the end of the study the dog was allowed to recover consciousness before extubation of the trachea.

Drug disposition study

The same procedure was carried out on each of the three consecutive study days in both groups. Each dog received a bolus injection into the femoral vein of a trace dose of $^{3}$H-propranolol 200 μCi (specific activity 67 mCi mg$^{-1}$, Amer-sham Searle Corp., Arlington Heights, IL) and, simultaneously, unlabelled propranolol 40 mg was given directly into the portal vein over 10 min by a constant rate infusion pump. The intraportal route was used to bypass the variable absorption which may occur following oral administration and which might be affected by anaesthesia.

Arterial blood samples, for measurement of both unlabelled and $^{3}$H-propranolol plasma concentrations, were obtained every 5 min for the first 1 h and then every 15 min for a further 3 h. Blood removed for sampling was replaced with twice the volume of Hartmann’s solution. Plasma concentrations of unlabelled propranolol were measured by high pressure liquid chromatography and the plasma concentrations of $^{3}$H-propranolol by liquid scintillation counting of the HPLC eluent corresponding to the propranolol peak [9]. Plasma samples were obtained on each day before the propranolol was administered and the propranolol binding in plasma measured by equilibrium dialysis [12].

Systemic clearance ($C_{ls}$), portal clearance ($C_{lp}$), hepatic extraction ratio ($E$), bioavailability ($F$), i.v. half-life ($T_{1/2,i.v.}$), volume of distribution ($V_d$) and hepatic plasma flow ($Q$) were calculated as described below. As propranolol is metabolized only by the liver and was injected into the portal vein (the equivalent of 100% absorption of an oral dose) the apparent clearance of the portally administered propranolol ($C_{lp}$) is numerically equal to the total intrinsic clearance ($C_{lin}$) [7].

Calculations

The clearance of propranolol following i.v. or systemic administration ($C_{ls}$) was calculated as:

$$C_{ls} = \frac{X_{d,i.v.}}{AUC_{i.v.}}$$

where $X_{d,i.v.}$ is the dose of labelled propranolol administered i.v. and $AUC_{i.v.}$ is the area under
the time-concentration curve calculated by the trapezoidal rule for the i.v. labelled propranolol.

The clearance of the unlabelled propranolol administered into the portal vein \( (Cl_p) \) was calculated as:

\[
Cl_p = \frac{Xd_p}{AUC_p}
\]

where \( Xd_p \) is the dose of unlabelled drug administered into the portal vein and \( AUC_p \) is the area under the time-concentration curve for the unlabelled drug. Thus:

intrinsic clearance \( (Cl_{im}) = Cl_p = Xd_p/AUC_p \)

The elimination rate constant \( (k_{10}) \) was calculated by linear regression analysis and \( T_{1/2,v} \) as:

\[
T_{1/2,v} = \frac{0.693}{k_{10}}
\]

Volume of distribution \( (Vd) \) was calculated as:

\[
Vd = \frac{Cl_p}{k_{10}}
\]

Hepatic plasma flow \( (Q) \) was calculated as follows:

By definition, following intraportal administration of a drug of dose \( (Xd_p) \), the amount of the drug entering the systemic circulation is equal to \( Xd_pF \) where \( F \) is the fractional systemic availability. Thus:

\[
Cl_s = \frac{Xd_pF}{AUC_p}
\]

but, as \( Xd_p/AUC_p = Cl_p \) (equation (2)), and by definition \( F = 1 - E \) where \( E \) is the hepatic extraction ratio:

\[
Cl_s = (1 - E) Cl_p
\]

Also by Fick’s principle:

\[
Cl_s = QE
\]

where \( Q = \) apparent hepatic plasma flow.

Thus substituting for \( E \) in equation (4):

\[
Q = \frac{Cl_s-Cl_p}{Cl_p-Cl_s}
\]

Therefore, by substituting for \( Cl_p \) and \( Cl_s \):

Hepatic plasma flow = \( \frac{Xd_{i,v} \times Xd_p}{AUC_{i,v} \times Xd_p - AUC_p \times Xd_{i,v}} \)

Statistical analysis

Statistical comparisons were performed using analysis of variance followed by Student’s \( t \) test for paired data, where appropriate. The minimal accepted level of significance was taken as \( P < 0.05 \). Data are given as mean ± SEM.

RESULTS

In the fentanyl study, the mean duration of anaesthesia was 5.38 ± 0.08 h. Mean total doses of fentanyl 1565 ± 150 \( \mu \)g and atracurium 73.0 ± 6.5 mg were administered. In the isoflurane study a mean of 11.2 ± 0.8 MAC hours of isoflurane was given.

Fentanyl study

The effects of fentanyl–nitrous oxide anaesthesia (plus atracurium-induced myoneural blockade) on the disposition of propranolol are shown in table I. The clearance of the intraportally administered unlabelled propranolol decreased significantly \( (P < 0.05) \) from a mean value on day 1 of 1775.2 ± 384.6 ml min\(^{-1}\) to 663.6 ± 94.7 ml min\(^{-1}\) on day 2. On day 3 (24 h after anaesthesia) the mean portal clearance of 1648.9 ± 445.0 ml min\(^{-1}\) was not significantly different from the pre-anaesthetic value. The decrease of 63% in the portal or intrinsic clearance was the result of a reduction in hepatic drug metabolizing ability which was reflected in the decrease in hepatic extraction \( (E) \) from 0.61 on day 1 to 0.46 on day 2.

There was a significant \( (P < 0.05) \), but smaller, decrease of 35% in the i.v. or systemic clearance of the labelled propranolol from 535.5 ± 29.2 ml min\(^{-1}\) on day 1 to 346.9 ± 41.0 ml min\(^{-1}\) on day 2. The systemic clearance measured on day 3 was not significantly different from that on day 1. As the volume of distribution was unchanged during the three study days, the change in systemic clearance is reflected by the significant increase in the i.v. half-life from 64.5 ± 6.4 min on day 1 to

<table>
<thead>
<tr>
<th>Day</th>
<th>I.v. clearance ( (ml\ min^{-1}) )</th>
<th>Hepatic plasma flow ( (ml\ min^{-1}) )</th>
<th>Volume of distribution ( (litre) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>1775 ± 385</td>
<td>536 ± 29</td>
<td>65 ± 6</td>
</tr>
<tr>
<td>Day 2</td>
<td>664* ± 95</td>
<td>347* ± 41</td>
<td>98* ± 9</td>
</tr>
<tr>
<td>Day 3</td>
<td>1649 ± 445</td>
<td>615 ± 84</td>
<td>65 ± 4</td>
</tr>
</tbody>
</table>
TABLE II. Effect of isoflurane anaesthesia on propranolol disposition in six dogs (mean values ± SEM). Significantly different compared with day 1: *P < 0.05; **P = 0.053

<table>
<thead>
<tr>
<th>Volume of distribution</th>
<th>Intrinsics Systemic half-life flow</th>
<th>L.v. Hepatic plasma flow (ml min⁻¹) (ml min⁻¹) (min) (ml min⁻¹) (litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>1552 ± 210</td>
<td>479 ± 50</td>
</tr>
<tr>
<td>Day 2</td>
<td>723 * ± 50</td>
<td>286 * ± 41</td>
</tr>
<tr>
<td>Day 3</td>
<td>1853 ± 556</td>
<td>454 ± 51</td>
</tr>
</tbody>
</table>

97.9 ± 9.2 min on day 2. This returned to the pre-anaesthetic value on day 3.

The hepatic plasma flow showed a non-significant decrease of 27% from 1044.1 ± 233.4 ml min⁻¹ on day 1 to 762.2 ± 98.5 ml min⁻¹ on day 2.

During anaesthesia the free fraction of propranolol in plasma increased from 7.0 ± 0.8% on day 1 to 8.4 ± 0.6% on day 2 (P < 0.05) returning toward control value on day 3 (7.8 ± 0.7%).

**Isoflurane study**

The effects of isoflurane anaesthesia on propranolol disposition are shown in table II. The intrinsic clearance of propranolol decreased significantly (P < 0.05) from 1552 ± 210 ml min⁻¹ on day 1 to 723 ± 50 ml min⁻¹ on day 2 (a decrease of more than 50%). This returned to 1852 ± 556 ml min⁻¹ on day 3. The hepatic extraction ratio decreased from 0.66 on day 1 to 0.61 on day 2.

The systemic clearance of the labelled propranolol decreased significantly from 479 ± 60 ml min⁻¹ on day 1 to 286 ± 41 ml min⁻¹ on day 2 (a decrease of 40%) and returned towards control value (454 ± 51 ml min⁻¹) on day 3. As there was no change in the volume of distribution, the change in clearance resulted in a significant increase in the i.v. half-life from the day 1 value of 68.5 ± 6.7 min to 171.6 ± 42.5 min on day 2 and 84.6 ± 8.9 min on day 3.

Hepatic plasma flow was decreased (but not significantly) from 884 ± 304 ml min⁻¹ on day 1 to 506 ± 96 ml min⁻¹ on day 2 and 720 ± 79 ml min⁻¹ on day 3. There was no significant change in propranolol plasma binding during the isoflurane study.

**DISCUSSION**

The disposition of propranolol was altered markedly during both isoflurane, and enflurane–nitrous oxide–atracurium, anaesthesia. The portal or intrinsic clearance of propranolol, which is a reflection of hepatic enzyme metabolizing capacity, decreased by over 50% during anaesthesia in both studies. The extent of inhibition was similar to that seen previously with halothane [5]. In addition, using a similar experimental design, we have shown that enfurane anaesthesia also resulted in a marked inhibition of drug metabolism in the dog [13]. However, enzyme inhibition after halothane anaesthesia lasted for more than 24 h, while the effects of the anaesthetic regimens studied in the present investigation were shorter, and drug disposition had returned to pre-anaesthetic values by 24 h.

In this study, plasma propranolol concentrations were measured which, with a blood-to-plasma ratio for propranolol in the dog of 0.9, are slightly higher than blood concentrations. Thus the hepatic flow measured by this technique approximates to hepatic blood flow. The decrease in measured hepatic blood flow is of a magnitude similar to that found in other studies during anaesthesia (in the absence of surgery) with inhalation agents [14-16], and with subarachnoid anaesthesia [17]. Beta-adrenoceptor blockade is known to influence hepatic blood flow and has been shown to cause a decrease of up to 20% [18]. However, in this study, plasma propranolol concentrations were sufficient to cause near maximal beta-blockade on all three days. The increase in propranolol concentrations during anaesthesia would not explain the decrease in hepatic plasma flow that occurred during anaesthesia.

The cause of the marked decrease in hepatic drug metabolism during anaesthesia is not immediately clear, but several possible mechanisms may be considered. The inhalation anaesthetic agents may inhibit directly the cytochrome P-450 enzyme system, as has been demonstrated in vitro for halothane [19, 20]. Enflurane has been shown to have an inhibitory effect on oxidative metabolism, but possibly to a lesser extent than halothane [3, 21]. In dogs the only significant non-oxidation pathway of propranolol metabolism is glucuronidation, which accounts for 16% of the dose [22]. Even if glucuronidation were completely blocked, it could not account for the large reduction in clearance found in this study. Anaesthesia-induced changes in hepatic blood flow may alter drug disposition [23]. Anaesthesia
and controlled ventilation of the lungs per se may alter drug metabolizing ability. In support of this suggestion is the finding that the changes in drug disposition produced during isoflurane, fentanyl–nitrous oxide–atracurium, halothane or enflurane anaesthesia were similar. Our study did use nitrous oxide (in the fentanyl group), which does cause a small decrease in hepatic blood flow [24], and further studies are required to determine whether an i.v. anaesthetic technique without nitrous oxide has a similar effect. However, halothane anaesthesia produced an effect which was still present 24 h after anaesthesia, when all others had returned to pre-anaesthetic values.

Hypoxia may have important effects on drug metabolism [25] and antipyrine clearance (a measure of drug metabolizing ability) is related linearly to oxygen delivery and consumption [26]. In the isolated perfused rat liver the elimination of propranolol is reduced during hypoxia, a result consistent with reduced activity of the mixed function oxidase system [25]. It is possible that anaesthesia results in a relative intrahepatic hypoxia, which then produces inhibition of the mixed function oxidase system which is responsible for the biotransformation of a wide variety of drugs.

In conclusion, anaesthesia with fentanyl–nitrous oxide–atracurium or isoflurane produced a marked, but short-lasting alteration in drug disposition, in part as a result of the inhibition of drug metabolizing enzyme activity. This alteration in drug metabolism may result in considerable increases in plasma drug concentrations.

REFERENCES

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