I.V. BOLUS ADMINISTRATION OF SUBCONVULSIVE DOSES OF LIGNOCAINE TO CONSCIOUS SHEEP: MYOCARDIAL PHARMACOKINETICS

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SUMMARY
Mass balance principles were used to study the myocardial pharmacokinetics of lignocaine in conscious sheep. After i.v. bolus doses of lignocaine 50, 75 or 100 mg, arterial lignocaine concentrations reached a peak in approximately 16 s and these increased linearly with dose. Coronary sinus concentrations reached a peak between 83 and 129 s and the values showed poor relationships with dose. Net myocardial lignocaine uptake lasted for approximately 60 s — this was much shorter than the reported initial distribution half-life of lignocaine. The maximum rate of uptake was proportional to both the dose and the peak arterial lignocaine concentrations. At 15 min, the myocardial lignocaine concentrations were 46 (SD 22) % of their peak values. Pseudo-equilibrium between blood and myocardial lignocaine concentrations was not observed. It is concluded that, despite the myocardium being very well perfused, lignocaine myocardial concentrations were not well represented by blood lignocaine concentrations for at least 15 min. A greater understanding of the determinants of myocardial drug concentrations is required. (Br. J. Anaesth. 1993; 70: 326-332)

KEY WORDS
Anaesthetics, local: lignocaine Heart drug uptake. Pharmacokinetics.

The concentration of a drug at its "receptor" sites in target tissues is one of the major determinants of its quantitative therapeutic or toxic effects [1, 2]. The predictability of pharmacological interventions depends on the extent to which a drug reaches its target tissues. Prediction of the concentration of a drug in target tissues depends on the characteristics of drug disposition in these tissues. When blood drug concentrations are at steady state after long-term administration, prediction of tissue concentrations is a simple matter because the target tissue concentrations may be assumed to be proportional to blood concentrations (i.e. they are in pseudo-equilibrium). However, studies in both experimental animals and patients have shown that, after short-term i.v. administration of drugs, the blood and tissue drug concentrations change rapidly. Depending on the rate of these concentration changes, there may not be pseudo-equilibrium between blood and tissue drug concentrations, even for the "well perfused" organs, for considerable periods [3]. In such situations, the commonly reported initial distribution volume and half-life of the drug in blood may be poor indicators of drug disposition in, and effect on, the well perfused tissues.

The myocardial pharmacokinetics of lignocaine were studied as an example of the disposition kinetics of a drug in a well perfused tissue after bolus drug administration. I.v. bolus doses of lignocaine are used to treat cardiac arrhythmias, and inadvertent i.v. injection is a risk when it is used as a local anaesthetic. Toxicity is characterized by both CNS effects and myocardial depression, and its narrow margin of safety has led to the investigation of its whole body and myocardial pharmacokinetics in both experimental animals and human patients [4–6]. Nevertheless, the limited number of methods available [7] and the difficulty of studying acute drug distribution in the myocardium have caused our understanding of the myocardial regional pharmacokinetics of drugs such as lignocaine to be far from complete [3]. In the previous paper in this series [8], we showed that lignocaine caused dose-dependent depression of myocardial contractility at doses small enough not to produce overt CNS effects. This paper describes an additional component of the studies reported previously. The aims of the studies were to develop an in vivo experimental preparation using conscious sheep in which the time-courses of myocardial drug concentrations could be determined using mass balance principles [9] whilst causing minimal interference to myocardial function, and to characterize the rate and extent of the uptake and elution of lignocaine by the myocardium and the relationships between its arterial, coronary sinus and myocardial concentrations after i.v. bolus administration of 50-, 75- and 100-mg doses.

MATERIALS AND METHODS
The study was approved by the institutional Ethics Review Committee.
The application of mass balance principles requires a knowledge of the blood flow to the relevant region of the body and the concentrations of drug in representative afferent and efferent blood vessels. In this study, myocardial blood flow was determined using a Doppler flow probe placed on the left main coronary artery as previously reported [8]. A modification of the method of Bond, Manning and Gonzalez [10] was used to determine the mass of the myocardium perfused by this artery. The hearts of 10 sheep (studied in other unrelated experiments) were studied at the end of their experimental lives. The sheep were systemically heparinized (heparin sodium 200iu kg⁻¹) and killed with a barbiturate overdose and the hearts collected. No obvious signs of previous myocardial infarction or other abnormalities were found. Cannulae (75-cm extension tubing, Lane Cove, NSW, Australia) were secured in the ostia of the left and right coronary arteries, with less than 3 mm of the cannulae placed inside the vessels. Each of the cannulae was connected to a sealed, 20-ml container (Terumo, Melbourne, Australia). One of the containers was filled with Indian ink (Penguin Indian ink, Melbourne, Australia) and the other with 0.9 % saline. The artery perfused by Indian ink was selected randomly and the containers were pressurized to 100 mm Hg to perfuse each artery with the appropriate fluid. The areas of the myocardium perfused by the left main coronary artery (six hearts) or right coronary artery (four hearts) were therefore stained with Indian ink. Immediately after perfusion of each heart, the stained tissue was dissected from the unstained tissue. The epicardial fat and the large blood vessels were removed, and both stained and unstained tissues were blotted dry and weighed.

**Animal preparation**

These studies were performed as an additional part of the haemodynamic studies of lignocaine in the five sheep reported in a previous paper [8]. The relevant measurement for the present study was left coronary artery blood flow which was measured and calibrated using a Doppler flowmeter method. Its values corresponding to the times of blood sample collection were determined from the calibrated blood flow velocities in the 5-s interval immediately before each blood sampling time.

The sheep were prepared also with chronic intravascular catheters, using the method of Rutten and colleagues [11]. Catheters were placed in the ascending aorta to sample afferent blood to the myocardium, in the coronary sinus to sample efferent blood from the myocardium, and in the inferior vena cava (IVC) for drug injection. The hemiazygous vein, which drains into the coronary sinus of sheep, was ligated outside the pericardium, to ensure the coronary sinus contained pure effluent blood from the myocardium. One week was allowed for the sheep to recover fully from surgery. At the end of each sheep’s experimental life, it was systemically heparinized and killed with a barbiturate overdose. The hearts were collected, the epicardial fat removed, and the mass of the myocardium measured.

**Study design**

Lignocaine hydrochloride (2 % Xylocard, Astra Pharmaceuticals Pty Ltd, NSW, Australia) in doses of 50, 75 or 100 mg was diluted to 10 ml with 0.9 % saline immediately before each experiment. One of these doses was selected randomly and given i.v. via the IVC catheter over 1 s using an Angiomat injector [8]. Blood samples were collected from the ascending aorta and the coronary sinus at the nominal frequencies of every 5 s until 1 min after injection, 15 s until 2 min, 30 s until 5 min and 60 s until 15 min. The “flush and withdrawal” method [12] was used to achieve the rapid rate of sampling during the first 120 s after injection, when samples were 3 ml. From 120 s onwards, 0.5-ml blood samples were collected with 1-ml syringes, after removal of twice the catheter deadspace volume. The 3-ml and 0.5-ml samples were transferred into 10-ml soda glass tubes (Johns Products, VIC, Australia) and 1.5-ml Eppendorf microtubes (Eppendorf, Hamburg, FRG), respectively. To determine the exact volume of each sample, these tubes were weighed both before and after the addition of samples. All samples were stored at −20 °C until required for assay.

**Drug assay**

Lignocaine was assayed using the single extraction gas chromatographic method described previously [13]. The lignocaine concentrations in blood samples collected by the “flush and withdrawal” method were corrected for the dilution that occurred during sampling and the actual volume sampled, as described previously [12]. The blood concentrations of lignocaine in the 0.5-ml samples were corrected for any discrepancies in sample volume detected by weighing.

**Mass balance calculations**

The equations to describe the rate and extent of myocardial drug uptake and elution have been described previously [9]. Their applications to the present experimental situation were as follows.

The myocardial net lignocaine flux (Jnetmyo) was calculated using the following equation:

\[
J_{\text{net,myo}} = Q_{\text{myo}} \times (C_a - C_{\text{myo}})
\]  

where \(Q_{\text{myo}}\) = left coronary artery blood flow; \(C_a\) and \(C_{\text{myo}}\) = lignocaine concentrations in arterial and coronary sinus blood, respectively. This is an index of the flux (or rate) of movement of lignocaine between the blood and the myocardium, and is a positive value for net uptake into the myocardium and a negative value for net elution. The integral of \(J_{\text{net,myo}}\) with respect to time is the total amount of lignocaine that has entered the myocardium via the left coronary artery, but has not left via the coronary sinus, at a given time. The mean myocardial lignocaine concentration (\(C_{\text{myo}}\)) in this region was determined by dividing this mass by the mass of this region of the myocardium (\(M_{\text{myo}}\)). Thus:

\[
C_{\text{myo}} = \frac{1}{M_{\text{myo}}} \int_0^t J_{\text{net,myo}} \, dt
\]
During the first 120 s after lignocaine injection, the blood samples were sometimes taken at times slightly different than the nominated times. In these cases, the blood drug concentration at the nominated sampling time was determined by linear interpolation between adjacent time points.

Statistical analysis

Paired t tests were used to compare peak drug concentrations in arterial or coronary sinus blood, the calculated myocardial drug concentrations, the times of the peak drug concentrations and the blood flow between the different doses. Linear regressions were used to analyse the relationships between the peak blood drug concentrations and the doses used. One Factor Repeated Measures Analysis of Variance was used to examine the trend of the peak arterial blood drug concentration, peak myocardial uptake flux and peak myocardial mass to dose ratios. The dose factor was partitioned into the linear and quadratic trend components by means of orthogonal polynomial contrasts. P < 0.05 was considered statistically significant.

RESULTS

In all 10 hearts of the in vitro study, blood flow to the right ventricular free wall, the right atrium and a small portion of the ventricular septum (near the base of the heart) was supplied by the right coronary artery while the left ventricular free wall, left atrium and most of the ventricular septum were supplied by the left coronary artery. This proportion was consistent—the left coronary artery supplied 77±2% (by weight; n = 10) of the total myocardium. The mean total lean tissue mass of the hearts of the five sheep used for in vivo studies was 233 ± 11 g. The myocardial mass perfused by the left coronary artery in each of these sheep was calculated as 77% of the individual total mass.

In one sheep, the blood sampling catheter had come out of the coronary sinus and was in the right atrium during one of the 75-mg lignocaine dose studies. This was deduced from the uncharacteristic time course of the coronary sinus blood lignocaine concentrations, which was similar to that in the pulmonary artery blood (measured as part of a study not included in this paper). The results from this experiment were excluded. Therefore, the numbers of studies of the 50-, 75- and 100-mg doses of lignocaine were 5, 4 and 5, respectively.

Left coronary artery blood flow

The time courses of several haemodynamic variables have been reported previously [8]. Of these, left coronary artery blood flow is of interest in this paper because of its use for the mass balance calculations. There were no significant changes in coronary artery blood flow from baseline values after any of the doses of lignocaine. The mean (SD) values of left coronary artery blood flow in the first 1 min after drug administration, during which rapid myo-

![Graph](image-url)
MYOCARDIAL PHARMACOKINETICS OF LIGNOCaine

Fig. 2. Linear correlation between lignocaine doses used and the peak arterial blood drug concentrations ($r = 0.713; P < 0.05$).

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Fig. 3. Time courses of the mean (SD) calculated net lignocaine fluxes for the myocardium after i.v. bolus injections of 50, 75 or 100 mg. The smaller graphs in the upper right corner of each panel present the data on a scale which better shows the negative net drug fluxes.

Fig. 3. Time courses of the mean (SD) calculated net lignocaine fluxes for the myocardium after i.v. bolus injections of 50, 75 or 100 mg. The smaller graphs in the upper right corner of each panel present the data on a scale which better shows the negative net drug fluxes.

Table II. Peak net lignocaine uptake flux ($J_{n_{\text{txt}}}^m$), times at which it occurred and times of the start of drug elution after the different doses of lignocaine (mean (SD)); *$P < 0.05$ compared with smaller dose.

<table>
<thead>
<tr>
<th>Lignocaine dose</th>
<th>Peak $J_{n_{\text{txt}}}^m$ (mg min$^{-1}$)</th>
<th>Time of peak (s)</th>
<th>Start of elution (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg ($n = 5$)</td>
<td>2.3 (0.4)</td>
<td>17 (5)</td>
<td>67 (23)</td>
</tr>
<tr>
<td>75 mg ($n = 4$)</td>
<td>4.3 (1.7)*</td>
<td>18 (5)</td>
<td>60 (13)</td>
</tr>
<tr>
<td>100 mg ($n = 5$)</td>
<td>5.8 (3.4)</td>
<td>16 (4)</td>
<td>63 (14)</td>
</tr>
</tbody>
</table>

cardial drug uptake was observed, were 139 (30) ml min$^{-1}$, 121 (23) ml min$^{-1}$ and 122 (21) ml min$^{-1}$ for the 50-, 75- and 100-mg doses, respectively.

Arterial and coronary sinus blood drug concentrations

Time courses of the mean (SD) lignocaine concentrations in arterial and coronary sinus blood after the 50-, 75- or 100-mg dose are shown in figure 1. It was apparent in all studies that the arterial blood lignocaine concentrations increased from 0 and reached their peak values within approximately 16 s after injection (table I). These peak values correlated linearly with the lignocaine doses used (fig. 2). In contrast, the peak coronary sinus lignocaine concentrations remained relatively small and did not increase significantly with the increasing dose. These peaks occurred between 83 and 129 s after the injections (table I).

Myocardial net lignocaine flux and net lignocaine mass

The period of myocardial net lignocaine uptake (positive flux) (fig. 3) lasted for approximately 60 s and was followed by longer periods of slower net lignocaine elution from the myocardium (negative flux) (fig. 3). The peak myocardial lignocaine uptake flux, the times of the peak net lignocaine uptake flux and the start of net lignocaine elution (when the flux first became negative) are listed in table II. The times of the peak myocardial uptake flux were approximately 17 s after lignocaine administration, which corresponds with the times of the peak arterial lignocaine concentration. The peak myocardial up-
The tissue staining study showed that the left main coronary artery of the sheep supplied a consistent portion of the tissue of the left side of the heart. The coronary blood flow to this region was measured with the Doppler flowmeter. The arterial blood samples collected from the ascending aorta provided representative samples of blood entering the left coronary artery. The coronary sinus of sheep differs from humans in that it receives some blood from the hemiazygous vein [14]. After ligation of the hemiazygous vein, blood from the coronary sinus of sheep was derived principally from the left ventricle and ventricular septum (85%) and the left atrium (8%), with a small proportion from the right ventricle (7%) [15]. Therefore, the region of myocardial tissue drained by the coronary sinus with the hemiazygous vein ligated consists predominantly of effluent venous blood from the region of the myocardial tissue perfused by the left main coronary artery. The staining study also enabled estimation of the mass of tissue for determining myocardial drug concentrations. Other criteria for the application of mass balance principles to the heart were also satisfied. For example, it has been reported that the mean intravascular transit times in the hearts of dogs and sheep (as determined using indocyanine green) were less than 5 s [9, 16], which would make a negligible contribution to the calculated myocardial net drug flux. The rates of diffusion of lignocaine from the surface of the heart and transport by lymph from the tissues were shown experimentally to make a negligible contribution to the myocardial net drug flux [17, 18]. Finally, lignocaine was not metabolized by, or eliminated from, the myocardium [19].

It has been recognized that the injection rate is a major determinant of the peak blood drug concentration and the resultant disposition of a drug in tissues [20-22]. The use of the Angiomat injector allowed the accurate control of injection volume and rate to 10 ml s^{-1}, which mimics the accidental i.v. injection of lignocaine during local anaesthesia. Previous work in our laboratory has also shown that the initial drug concentrations achieved after an i.v. bolus are inversely related to cardiac output [21]. The conscious sheep used in these studies had relatively large cardiac outputs—we would expect the results obtained in this study to be close, in this respect, to those obtained in critically ill patients with smaller cardiac outputs in whom slower, more clinically relevant injection rates were used. The rapid change in arterial concentrations of lignocaine were characterized using a new, rapid, blood sampling method [12].

The results of this study have several pharmacokinetic implications. The first of these concerns the relationships between blood and myocardial lignocaine concentrations. The initial peak lignocaine concentrations in the arterial blood were very large (fig. 1, table I). The nature of this peak is a function of the initial mixing of the drug in blood, of vascular transit and dispersion, and of the uptake and elution of lignocaine in the lungs. The linear relationship between the peak arterial lignocaine concentrations...
and doses used (fig. 2, table I) suggest that the uptake of lignocaine by the lungs was linear and not saturated as proposed [23]. The uptake and elution of lignocaine in the heart effectively "damped" the arterial concentration peak such that the peak coronary sinus blood lignocaine concentrations were only 7–13% of the arterial peak, and occurred about 67–114 s later (table I). It is apparent that the myocardial lignocaine concentrations did not achieve pseudo-equilibrium with either the arterial or coronary sinus blood concentrations within the 15-min period after the drug injection (fig. 5), despite the well-perfused nature of the myocardium. Thus, in this situation, the blood lignocaine concentrations did not give a reliable indication of the time course of the myocardial concentrations (fig. 5). It is important, therefore, to determine which of these concentrations (arterial, myocardial or coronary sinus) is in pseudo-equilibrium with the effects of lignocaine on the heart, as this is the concentration which must be manipulated to modify the cardiovascular effects of lignocaine.

The second implication is the apparent linearity of myocardial lignocaine uptake. The ratios of the peak myocardial uptake fluxes over dose were 0.045 (0.01), 0.057 (0.02) and 0.058 (0.03), and over the peak arterial lignocaine concentration were 0.127 (0.02), 0.129 (0.03) and 0.124 (0.03), respectively, for the 50-, 75- and 100-mg doses. These relatively constant ratios suggest that the rate of myocardial uptake was proportional to both the dose and the peak arterial blood lignocaine concentrations. Thus, within the constraints of the experimental system, it would be relatively easy to predict the myocardial concentrations and the rate of uptake resulting from a given dose, and on a more sophisticated level, the concentrations resulting from different dose regimens using linear systems analysis [24]. However, with the use of larger doses a maximum myocardial uptake of lignocaine may be reached [25]. It would be expected that factors influencing regional myocardial blood flow and drug binding in blood and myocardium, such as blood and tissue pH and myocardial mechanical activity, will also play important roles in the rate of myocardial drug uptake and elution [13, 26, 27]. Note that, in the present study, the concentrations of unbound lignocaine in sheep blood would be expected to be a constant fraction of the total blood concentrations [13].

The third implication is manifested by considering other methods for describing the initial distribution of drugs. The most common method when using blood concentration data is to determine the initial distribution volume (extrapolated to zero time) and the initial distribution half-life of the drug. Although the initial distribution volume (and thus the initial drug concentration) is of practical use in determining the loading dose of drugs, it is apparent from these data that there were large differences between the time courses of the arterial and coronary sinus lignocaine concentrations, and it is difficult to rationalize which of these concentrations represents the initial distribution volume. These data also reinforce the large influence of the site of blood sample collection (arterial or venous; central or peripheral blood) on the values of pharmacokinetic variables, such as initial distribution volume, determined using systemic blood [28–30]. By the same argument, it is apparent that the initial distribution half-life would differ greatly whether determined using arterial or coronary sinus blood. Furthermore, the initial distribution half-life of lignocaine in blood has been reported to be 1.76 (0.31) min [4], implying that distribution into the well perfused tissues would take four to five half-lives, or approximately 8 min. This is much greater than the 60-s period during which lignocaine distributed into the heart in our study, and suggests that this initial distribution half-life derived from blood concentrations provides little information about the distribution of lignocaine into vital, well perfused organs such as the heart. The fact that the time courses of the logarithmic myocardial lignocaine concentrations were not linear (fig. 4B) suggests that simple first-order compartmental models would not provide a satisfactory description of these data.

In conclusion, it would appear that systematic studies of the determinants of the drug concentrations in the myocardium in vivo are needed to provide a scientific basis for the design of dose regimens in different clinical situations. The application of mass balance principles allows the determination of the time course of the mean myocardial drug concentrations in awake animals with very little perturbation of their normal physiological status, and this approach may also be used in man [5]. This study is one step closer to measuring the concentrations of drugs at their sites of action after short-term i.v. use. Although the amount of drug in the blood vessels of the heart cannot be quantified, it would be expected to make a negligible contribution to the mean myocardial drug concentrations. The calculated myocardial concentration revealed little about drug uptake and elution at the cellular level or at receptor sites within the myocardium. This could be of importance if the effects of lignocaine on the myocardium were in equilibrium with its concentration at these sites, and these were different from the mean myocardial lignocaine concentration.

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