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Lignocaine kinetics in the rat

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After intravenous injection of lignocaine 2·5, 5·0 or 10·0 mg kg^{-1} in the rat blood concentration declined in a bi-exponential manner. The data were analysed according to a two-compartment open model with elimination from the central compartment. Analysis of variance revealed no significant differences in k_{12}, k_{21}, k_{13}, $t\frac{1}{2}_{\alpha}$, $t\frac{1}{2}_{\beta}$, AUC or in the volume constants for the different doses used. After lignocaine 50·0, 70·0 or 90·0 mg kg^{-1} by mouth there was no significant change in $t\frac{1}{2}_{\beta}$ for the different doses or in peak plasma concentration normalized for dose. For both routes of administration blood concentration-time curves were superimposible and AUC was linearly related to dose. Renal excretion was negligible. Systemic availability of lignocaine was the lowest of species so far studied (mean 0·019 ± 0·001) did not alter with dose, and was very similar to the values in the literature quoted for the isolated perfused liver. Clearance of lignocaine related to anticipated liver mass was almost identical to the values for liver blood flow quoted using other techniques. The data indicated high hepatic extraction of lignocaine by the intact rat and the absence of dose dependency within the range studied.

In the study of drug extraction by the liver, lignocaine is frequently selected to represent substances whose clearance is highly dependent on hepatic blood flow. In man (Boyes et al 1971; Bennett et al 1982) and in dog (Branch et al 1973) clearance values of lignocaine approach those for liver blood flow. The rat is an especially suitable species for such work since extraction of lignocaine is almost complete in a single pass through the perfused liver (Shand et al 1975; Pang & Rowland 1977) and hepatic clearance should virtually equate to blood flow.

Data in the intact rat including a study of dose dependency have not previously been published and we now report such a study.

MATERIALS AND METHODS

Lignocaine hydrochloride [2-diethylamino-*N*-)2,6-dimethylphenyl) acetamide hydrochloride] was supplied by Astra Chemicals Ltd. Lignocaine was assayed in whole blood by gas-liquid chromatography (Mather & Tucker 1974). The standard curve was linear over the range 0·05–8·0 mg litre^{-1} and the coefficient of variation of 5 assays at 0·05 mg litre^{-1} was 8·29%. Doses and concentrations of lignocaine quoted refer to the base form.

Male albino rats of CRF strain (Anglia Laboratory Animals, Huntington, United Kingdom), 350–500 g, were housed in controlled photoperiod conditions (14 h light, 10 h dark) at a temperature of 21 °C with food (Oxoid diet 41B) and water freely available until experimental conditions were imposed.

Samples of whole blood, 0·3–0·5 ml were taken from the tip of the tail at specified times and were stored in heparinized polyethylene tubes at −20 °C. Following dosing, certain animals were housed singly in metabolism cages with a mesh floor placed over a polyethylene funnel so that urine could be collected separately from faeces. Urine was collected for 8 h after dosing.

Intravenous dosing. Rats received lignocaine dissolved in isotonic saline in doses of 2·5 (n = 6), 5·0 (n = 8) or 10·0 (n = 6) mg kg^{-1} as a bolus injection through a tail vein, the duration of the injection being one minute. Free access to food and water was allowed throughout the experiment.

Oral dosing. Rats received lignocaine in distilled water in doses of 50·0 (n = 8), 70·0 (n = 8) or 90·0 (n = 8) mg kg^{-1} by direct oesophageal intubation of the manually restrained animal. These rats were starved the night before and for the duration of the experiment but were allowed free access to water at all times.

Pharmacokinetics

The drug concentration-time data following an intravenous injection of lignocaine were fitted to precribed functions using the non-linear least squares regression analysis programme NONLIN (Metzler et al 1974). Preliminary investigation indicated that the data could be described by the expression

$$C_t = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

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The coefficients and exponents of this equation were used to calculate the following parameters assuming a two compartment open model with drug elimination from the central compartment (Gibaldi & Perrier 1975a)—k_{12}, k_{21}, k_{13}, $t\frac{1}{2}\alpha$, $t\frac{1}{2}\beta$, V_1, V, V_{ss}.AUC was calculated by the trapezoidal rule, estimating the area beyond the last observation by dividing its value by the value of the respective terminal exponent (Gibaldi & Perrier 1975b). On average the area beyond the last observation contributed 15·1% to the total area.

Preliminary analysis of data for orally administered lignocaine indicated that in the terminal phase blood concentration declined log-linearly with time. The rate constant for elimination of the drug from blood in the terminal phase was calculated from the slope $(-\beta/(2{\cdot}303))$ of the regression of the semi-logarithmic plot of blood concentration against time. The $t\frac{1}{2}\beta$ was calculated from the estimated value for β. AUC was calculated as for the intravenous data and on average the area beyond the last observation contributed 13·1% to the total area. The $t\frac{1}{2}_{abs}$ was calculated by the method of residuals (Gibaldi & Perrier 1975c). F was calculated as the ratio of the AUC for different oral doses to the mean AUC for all intravenous doses, all values for AUC having been normalized for dose.

RESULTS

Intravenous administration. Mean blood concentration-time data appear in Table 1a. Plots of blood concentration against time, normalized for dose were superimposible. Analysis of variance revealed no significant differences in k_{12}, k_{21}, k_{13}, $t\frac{1}{2}_{\alpha}$, $t\frac{1}{2}_{\beta}$, AUC, V_1, V or V_{ss} between the doses used (Table 2).

A plot of AUC against dose was linear ($r = 0{\cdot}883$, $P < 0{\cdot}001$) and the 95% confidence interval of the regression line included the origin of the plot.

Less than 3% of the 2·5 mg kg^{-1} dose of lignocaine was recovered in urine collected for 8 h after dosing.

Oral administration. Mean blood concentration-time data appear in Table 1b. Lignocaine was detected in blood within 10 min of dosing and reached maximum concentrations between 30 and 45 min. Plots of blood concentration against time, normalized for dose were superimposible. Analysis of variance revealed no significant differences in $t\frac{1}{2}_{abs}$, $t\frac{1}{2}\beta$, peak blood concentration, AUC or F between the doses used (Table 3). A plot of AUC against dose was linear ($r = 0{\cdot}873$, $P < 0{\cdot}001$) and the 95% confidence interval of the regression line included the origin of the plot.

Less than 1% of the 50 mg kg^{-1} dose of lignocaine was recovered in urine collected for 8 h after dosing.

DISCUSSION

After intravenous injection, blood concentration of lignocaine declined in a bi-exponential manner. This is also a characteristic of the drug in other species; the mean $t\frac{1}{2}$ of the terminal phase in rat (57 min) exceeded that found in monkey (15 min) (Benowitz et al 1974) and dog (40 min) (Boyes et al 1970) but was less than the usual values quoted for man (100 min) (Boyes et al 1971; Thomson et al 1973; Perucca & Richens 1979). The volume constants in

Table 1. Blood concentrations of lignocaine in rats after intravenous and oral administration (means ± s.e.m.).

Dose mg kg^{-1}	n	Body wt (g)	Blood concentration mg $litre^{-1}$							
(a) Intravenous			5	10	20	40	60	90	120	180 min
2·5	6	395 ± 10	3·16 ±0·56	2·45 ±0·37	1·72 ±0·16	1·23 ±0·13	0·84 ±0·09	0·67 ±0·13	0·46 ±0·09	0·19 ±0·04
5·0	8	399 ± 5	7·32 ±0·47	6·04 ±0·48	4·99 ±0·50	3·14 ±0·43	2·52 ±0·32	1·56 ±0·17	0·94 ±0·15	0·55 ±0·09
10·0	6	431 ± 15	13·66 ±0·89	10·34 ±0·53	8·15 ±0·27	5·49 ±0·54	3·94 ±0·44	2·52 ±0·44	1·90 ±0·34	0·76 ±0·16
(b) Oral			10	20	30	45	60	90	120	180 min
50·0	8	427 ± 11	0·41 ±0·11	0·60 ±0·10	0·57 ±0·09	0·56 ±0·08	0·50 ±0·08	0·36 ±0·05	0·24 ±0·04	0·13 ±0·03
70·0	8	414 ± 13	0·69 ±0·16	0·88 ±0·09	0·85 ±0·08	0·73 ±0·09	0·61 ±0·06	0·47 ±0·04	0·32 ±0·04	0·13 ±0·02
90·0	8	405 ± 9	0·83 ±0·18	0·96 ±0·15	1·11 ±0·12	1·29 ±0·09	1·18 ±0·12	0·73 ±0·07	0·57 ±0·04	0·29 ±0·02

Table 2. Kinetic parameters* of lignocaine following intravenous administration to rats (mean ± s.e.m.).

Dose mg kg^{-1}	k_{12} min^{-1}	k_{21} min^{-1}	k_{13} min^{-1}	$t\frac{1}{2}_{\alpha}$ min	$t\frac{1}{2}_{\beta}$ min	AUC† mg litre^{-1} min	V_1 litre kg^{-1}	V litre kg^{-1}	V_{ss} litre kg^{-1}
2·5	0·078 ±0·032	0·078 ±0·022	0·028 ±0·006	5·3 ±1·2	61·8 ± 6·0	74·6 ±11·2	0·65 ±0·12	1·23 ±0·15	1·09 ±0·13
5·0	0·065 ±0·032	0·082 ±0·017	0·024 ±0·002	8·5 ±2·8	51·4 ±11·0	89·1 ± 9·2	0·52 ±0·06	0·62 ±0·27	1·05 ±0·12
10·0	0·087 ±0·042	0·076 ±0·022	0·029 ±0·005	8·0 ±2·7	57·9 ± 6·0	76·6 ± 7·0	0·59 ±0·09	1·16 ±0·19	0·92 ±0·07

* Standard terminology.
† Normalized to 1·0 mg kg^{-1} dose.

rat were similar to those quoted for dog, monkey and man and indicate extensive distribution of the drug into tissues, a conclusion which is supported by tissue analysis following intravenous injection of radioactively labelled lignocaine to the rat (Katz 1968; Keenaghan & Boyes 1972).

Blood concentrations following lignocaine by mouth indicated rapid absorption of the drug from the rat alimentary tract. Disappearance of radioactively-labelled lignocaine from the gut and recovery of lignocaine and metabolites in urine after oral administration further shows that absorption from the rat alimentary tract is complete (Keenaghan & Boyes 1972). Systemic availability of lignocaine in the rat was the lowest of species so far studied, the mean value of 0·019 contrasting with 0·22 in the dog (Branch et al 1973), 0·3 in man (Boyes et al 1970; Perucca & Richens 1979; Bennett et al 1982), 0·34 in the monkey (Benowitz et al 1974) and 0·66 in the cat (Lautt & Skelton 1976). Low availability in the presence of negligible renal excretion and complete absorption from the gut suggests high hepatic clearance. Direct measurement of heptatic extraction of lignocaine by the isolated rat liver perfused through the portal vein gives figures in excess of 0·99 (Pang & Rowland 1977; Ahmad et al 1981). These values accord with the calculated figure for hepatic extraction (E = 1 − F) of 0·981 in the present study. The findings attest to the validity of the perfused rat liver in-situ as a system for studying hepatic drug extraction. It would also appear that gut wall metabolism plays a negligible role in the elimination of lignocaine.

Table 3. Kinetic parameters of lignocaine following oral administration to rats (means ± s.e.m.).

Dose mg kg^{-1}	$t\frac{1}{2}_{abs}$ min	$t\frac{1}{2}_{\beta}$ min	Peak conc* mg litre^{-1}	AUC* mg litre^{-1} min	F
50	12·0 ± 2·2	60·1 ± 7·0	0·014 ±0·002	1·54 ±0·21	0·019 ±0·003
70	12·3 ± 2·4	55·8 ± 3·6	0·013 ±0·002	1·36 ±0·15	0·017 ±0·002
90	11·0 ± 1·1	63·0 ± 3·9	0·015 ±0·001	1·78 ±0·07	0·022 ±0·001

* Normalized by 1·0 mg kg^{-1} dose.

Assuming an hepatic extraction ratio approaching unity, clearance of lignocaine in the rat can be taken to be virtually equal to liver blood flow. In the animals used liver accounts for 3·5% of total body weight which equates to 14·28 g for those rats that received lignocaine intravenously. The mean total body clearance of lignocaine (intravenous dose/AUC) in these animals of 13·51 ± 0·82 ml min^{-1} is thus equivalent to a liver blood flow of 0·93 ± 0·06 ml min^{-1} g^{-1} of liver. This value is similar to that obtained with radioactively-labelled microsphere technique in the rat in this laboratory (1·17 ± 0·11 ml min^{-1} g^{-1} of liver) (Supradist 1980) and by others (1·06 ± 0·05 ml min^{-1} g^{-1} of liver) (Yates et al 1979). Similar values of liver blood flow relative to liver weight has been obtained in several other species (Neutze et al 1968; Sasaki & Wagner 1971; Benowitz et al 1974).

The absence of dose dependency within the range tested is indicated by the constancy of the kinetic parameters after different intravenous and oral doses, by the superimposibility of the lignocaine blood concentration-time curves when normalized for dose and by the direct proportionality between dose and AUC. Evidence for dose dependency of lignocaine has been reported in dog (Vicuna et al 1979). In the present study the highest oral dose relative to liver weight greatly exceeds the corresponding value in the human studies and indicates a high Km of the enzyme system responsible for the metabolism of lignocaine in the rat.

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