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Prediction of the concentrations in rat tissues of the intravenous anaesthetic methohexital from a nonlinear pharmacokinetic model

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Abstract: A nonlinear pharmacokinetic model that describes the tissue distribution of intravenous anaesthetics was evaluated against experimental values for methohexital in the rat. There was excellent agreement between experimental and theoretical values for brain tissue, and good agreement for blood and adipose tissue. Agreement for lean tissue was good if it was assumed that some adipose tissue was present in skeletal muscle. Agreement was poor for all other visceral tissues. The experimental results justify further development of this mathematical model for use in accounting for differences in tissue distribution of anaesthetics, especially under various physiological conditions.

Keywords: *Nonlinear pharmacokinetic model; intravenous anaesthesia; methohexital; rats.*

Introduction

A variety of pharmacokinetic models have been described for the biological tissue distribution of intravenous anaesthetics. This is partly because pharmacokinetic models have been suggested as tools to predict dosage adjustments for some drugs in certain therapeutic situations [1–3]. Gibaldi *et al.* [4] have described the body distribution of thiamylal, ketamine and phencyclidine according to the compartmental analysis presented by Wagner [5]. A model has been devised to describe thiopental (thiopentone) pharmacokinetics [6]. Saidman and Eger [7] used a model to predict the effect of liver metabolism of thiopental on its rate of disappearance from plasma. A pharmacokinetic model has been developed to predict the distribution of thiopental in blood, viscera, lean and adipose tissues [8]. Gillis *et al.* [9] have reported a mathematical model that was used to predict the clinical concentrations of methohexital (methohexitone) in the blood and other tissues. Most of these models which described the pharmacokinetics of intravenous anaesthetics were never completely verified with experimental data, or were evaluated

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using literature data. The model by Gillis *et al.* [9] was evaluated for the distribution of methohexital in man by comparing calculated values with experimental drug concentrations taken from the literature. Since this model has the potential capability of enabling drug concentrations in blood, viscera, lean and adipose tissue to be calculated, it was thought that a better approach in its evaluation would be to compare calculated values of drug concentrations with experimental values measured directly in these tissues of the rat during anaesthesia.

The results indicate that experimental values for the tissue distribution of methohexital in rats correspond reasonably well with the theoretical interpretations predicted by the model, and support the further development of this model for use in accounting for differences in drug distribution in the presence of selected physiological changes.

The model comprises four compartments among which the injected drug is distributed; these are blood, visceral, lean and adipose tissues. The physiological parameters involved are the corresponding tissue volumes and blood flow rates, as well as rate of metabolism by the liver. Chemical equilibrium is assumed to exist in the blood in respect of drug ionization and reversible plasma protein binding of the drug. It is also assumed that chemical equilibrium is approached in the visceral and lean tissues, in respect of reversible protein binding, in a manner dependent upon a characteristic time. This characteristic time is related to how fast the blood is replaced in a specified tissue volume. In adipose tissue the drug concentration is assumed to be determined by its lipid solubility and by the characteristic time for this compartment.

Experimental

Details of the chemicals and analytical procedures have been reported previously [10].

Tissue extractions

Male Sprague–Dawley rats (approximately 250 g) were injected with methohexital at a dose of 10 mg/kg in the lateral tail vein and were killed by decapitation at various times after injection. Tissue samples of brain (whole), kidney, liver, fat, skeletal muscle and blood were taken. Blood was heparinized with heparin sodium (0.1 ml/ml of whole blood using a solution of 1000 units/ml); the plasma was separated by centrifugation and frozen at 4°C until assayed. Other tissue samples were dried with blotting paper, weighed, frozen in dry ice–acetone, and stored at 4°C until assayed.

Tissue homogenates were prepared by diluting the sample 1:10 (m/v) with distilled water and homogenizing by tissue-grinder with a Teflon pestle. Plasma was diluted 1:5 (v/v) with distilled water. The general extraction procedure reported previously [10] was found satisfactory for blood, brain, kidney and liver, but preliminary treatments were necessary for muscle and fat tissue. Muscle tissue was digested overnight in 1 M NaOH; the sample was diluted 1:5 (m/v) with distilled water and then treated by the general extraction procedure. Fat tissue was homogenized in 0.1 M NaOH, diluted 1:10 (m/v) with distilled water and centrifuged at 2000 ***g*** for 5 min; the aqueous layer was removed and treated by the general extraction procedure.

Recovery studies

Standard solutions of sodium methohexital in plasma, tissue homogenates and distilled water were prepared in the range of 0.2–5 mg per 100 ml (2–50 μg/ml) and extracted by the above procedure to determine the percentage recovery of the drug. Standards in fat

and muscle tissue were treated with NaOH before extraction. The mean recovery from plasma and tissue homogenates after extraction was 84% ± 6.6% (range 73–92%). Practically all the drug was recovered from distilled water. Extraction with two 10-ml portions of ether for 30 s each gave reproducible results and a greater percentage of drug was recovered than with one 20-ml portion. The recovery from a single extraction was 65% ± 7.1% (range 65–78%).

Results and Discussion

Among the input parameters for the theoretical model are tissue volumes, blood flow rates, characteristic equilibrium times and dosages. Values of these parameters were estimated for a 250-g rat. The tissue volumes shown in Table 1 were estimated using the approximation that the specific gravity of blood and tissue is unity. The blood volume was obtained from the literature [11]; the volume for viscera was obtained by summation of the organ weights reported for a 250-g rat [12]. The volumes for adipose and lean tissue were assumed to correspond to 10% and 70% of total body weight, respectively [13]. The blood flow rates in Table 1 were estimated on the basis that the total cardiac output of a rat is 60 ml/min [14] and that the distribution among visceral, lean and adipose tissue is in the same proportion previously used [9] for the case $Q_p = Q_1$. All other values were the same as those used previously [9], except that the characteristic equilibrium times listed in Table 1 were not calculated from the relation $t_i = V_i/Q_i$ but were adjustable parameters, selected to give a reasonable fit of the theoretical model to the experimental data.

Experimental against predicted concentrations

The experimentally determined drug concentrations in various tissues are plotted in Fig. 1; the experimental points are means ± S.D. for three different rats at a dosage of 10

Table 1
Values of parameters used to describe the four-phase model of the blood–tissue system for a 250-g rat

Tissue volumes	ml
Blood, V_b	15
Viscera, V_s	25
Lean, V_l	175
Adipose, V_p	25
Blood flow rates in tissues	**ml/min**
Viscera, Q_s	36
Lean, Q_l	12
Adipose, Q_p	12
Liver, Q_r	13
Characteristic equilibrium times	**min**
Viscera, t_s	2
Lean, t_l	30
Adipose, t_p	4

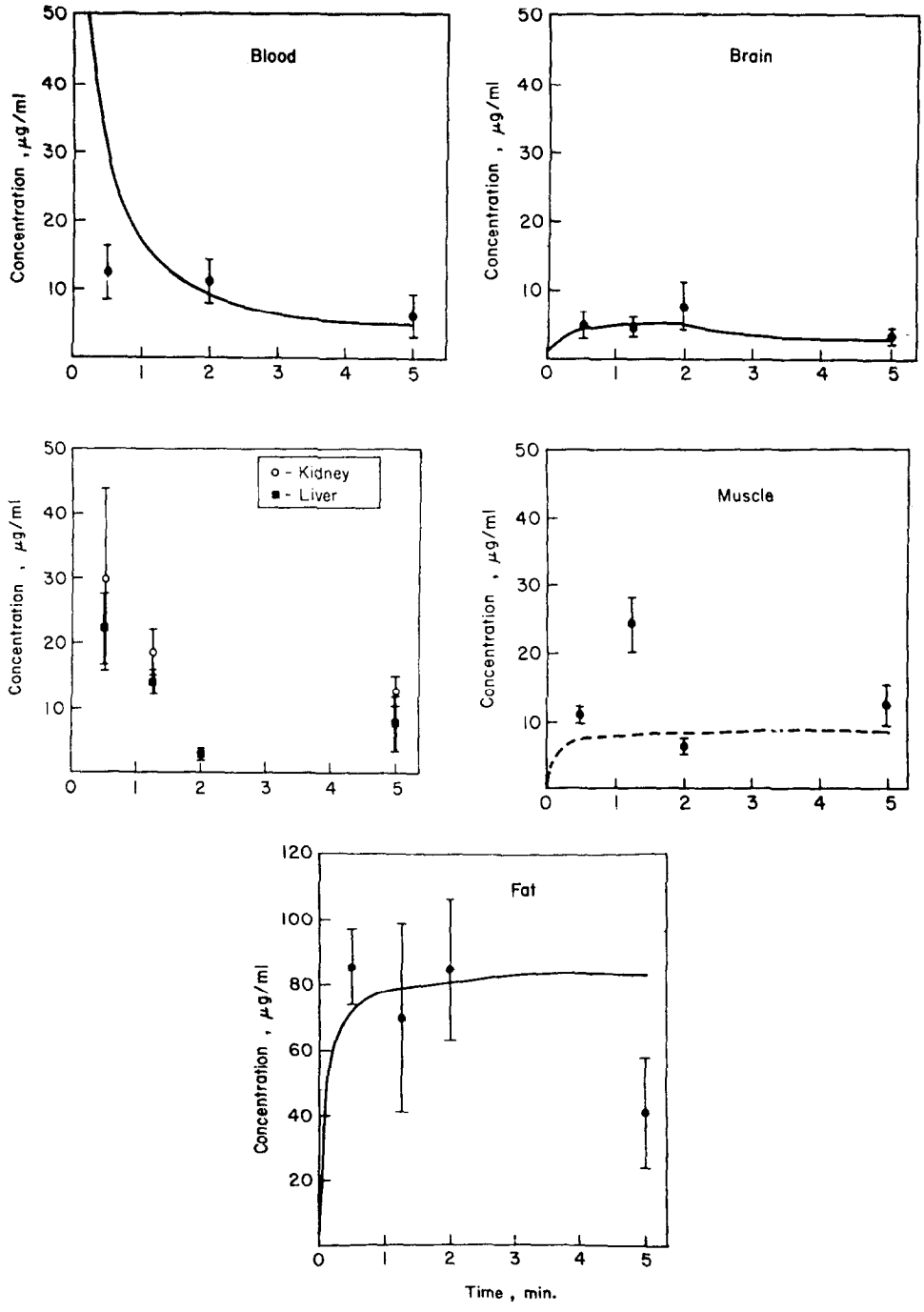

Figure 1
Methohexital concentration in blood plasma and other rat tissues as a function of time. Experimental points are means ± S.D. of results for three rats at an i.v. dose of 10 mg/kg. The solid curves are the theoretical predicted concentration–time graphs using the empirical parameters in Table 1. The theoretical predictions for kidney and liver tissues would be exactly the same as that in brain since the model makes no distinction between specific visceral tissues. The dashed line for concentration in muscle represents the theoretical prediction for a tissue assumed to be 90% lean but 10% adipose.

mg/kg. The curve drawn on a particular graph is the theoretically predicted concentration–time graph obtained from the model using the empirical parameters in Table 1.

The concentration of methohexital in plasma as a function of time is presented in Fig. 1; the theoretical curve is reasonably close to experimental values. For times less than 1 min the degree of agreement is not so good, but this was originally recognized as a limitation of the model. The maximal blood concentration of drug for the present model (A/V_b) occurs at zero time because the model assumes homogeneity of the blood compartment, i.e. the drug concentration in blood is uniform throughout all blood vessels and tissues. Sunshine *et al.* [15], however, have shown that the concentration of methohexital increases from zero to values larger than A/V_b during the first minute after injection. Thus the present model is restricted to describing redistributions at times greater than that at which the drug has become uniformly distributed in blood. For methohexital injected intravenously into rats, this time appears to be 1 min.

The drug concentration in brain tissue as a function of time is also shown in Fig. 1. The corresponding theoretical curve is in excellent agreement with the experimental observations. This is particularly significant in that the brain is the location of anaesthetic effect. Both experimental values and theoretical predictions show a rather low, almost constant, concentration of drug over this time period; there is very little change in drug concentration in the brain whether or not the rat is in an anaesthetized state. For example, at the dosage used in the present work, total anaesthesia is induced almost immediately and the rat recovers after about 5–7 min; over this period of time, predicted brain drug concentrations change by about 60%.

In contrast, Fig. 1 also shows drug concentration in kidney and liver tissue as a function of time; there is a distinct difference between brain and these other visceral tissues. Although experimental values for methohexital concentration in three different visceral tissues were obtained, the model includes all visceral tissues in one 'compartment'. Experimentally there is a 3- to 5-fold difference in the amount of drug in kidney or liver compared with that in brain at times up to 75 s. However, the theoretical prediction for kidney and liver would be exactly the same as that for brain because the model does not distinguish between the various visceral tissues. This seems to be a significant defect of the analysis that warrants further investigation. Experimental determinations of binding site concentrations and association constants for various tissue types seems to be highly significant projects in relation to further refinement of analytical models of drug distribution. The present model takes into account protein–drug binding site concentrations and association constants as adjustable parameters. The values for binding site concentrations used were those for the binding of thiopental by bovine serum albumin determined by Goldbaum and Smith [16]; association constants were then calculated from these results. Since no corresponding data are available for visceral tissues, the same values were used for these tissues. In view of the experimental results, however, these values may not be similar in all tissues.

There is good agreement between theoretical predictions and experimental values for drug concentration in adipose (fat) tissue. The uptake of methohexital in fat is both very rapid and very high. The degree of agreement could be termed excellent except that the experimentally observed fall in concentration at 5 min is significantly larger than the theoretical fall. This may be explained partly by postulating that methohexital is metabolized via a cytochrome-P_{450} system at sites other than the liver [17]. Increased amounts of drug metabolized would result in a decrease in the amount of parent drug taken up in adipose tissue or a greater release of drug from these tissues at later times. If

extra-hepatic metabolism actually occurs, the effect after a long time (e.g. 5 min) would be observed mainly in adipose tissue because that is where most of the drug resides by then. The present model only accounts for metabolism of the drug in the liver.

Figure 1 also shows the drug concentration in lean (muscle) tissue as a function of time. The corresponding theoretical curve from the model would show no agreement with the experimental observations because it predicts concentrations of less than 1 μg/ml. One possible reason for this discrepancy is that there may be a dramatic difference between lean tissue and plasma protein, whose experimental binding site concentrations and association constants were assumed to describe all tissues. Another reason may be the experimental difficulty in obtaining a lean tissue specimen entirely free from lipid tissue. For the muscle curve, the dashed line represents the theoretical results for a tissue that is assumed to be 90% lean but 10% adipose. In a general sense, the experimental observations correspond to this calculation.

The present model is of the physiological type in that it incorporates as many as possible of the variables known to affect drug distribution. This type of approach was selected in order to predict dosage adjustment in selected therapeutic situations. It has been evaluated [9] against clinical data taken from the literature and animal data obtained in this study. These studies indicate that overall prediction of trends by the model is correct but that some discrepancies exist between theoretical and experimental values.

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