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Steady-State Determination of the Contribution of Lung Metabolism to the Total Body Clearance of Drugs: Application to Carbamazepine

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Abstract □ A steady-state approach is proposed to examine the contribution that the lung makes to the total body elimination of medium- to high-clearance drugs. Carbamazepine, a potential candidate of pulmonary metabolism, was investigated by infusion into the femoral vein in seven unrestrained Sprague–Dawley rats (250–300 g). Blood samples (0.45 ml), taken simultaneously from the jugular vein and carotid artery in each rat during the infusion (2–5 days), were assayed in duplicate for carbamazepine by GLC/CI/MS. Venous blood concentrations were used to calculate the total body clearance of carbamazepine, 440 ± 38 ml/hr (mean ± *SEM*), and the difference between simultaneous venous and arterial blood concentrations were used to calculate the extraction ratio of carbamazepine by the lung. The mean extraction ratio of 0.0058 (n = 28) suggests that the lung only contributes ~5% to the total body clearance of carbamazepine. It is proposed that this technique could be useful in examining the importance of the lung in the total body clearance of other drugs, and that it has several advantages over some currently used techniques.

Keyphrases □ Carbamazepine—elimination *via* pulmonary metabolism in the rat, steady-state determination □ Metabolism, pulmonary—of carbamazepine in the rat, steady-state determination of drug elimination *via* the lungs □ Drug clearance—contribution to lung metabolism, steady-state determination using carbamazepine in the rat

Numerous articles and reviews have appeared over the last 10 years establishing the xenobiotic-metabolizing capability of *in vitro* lung preparations (1–5). However, the extrapolation of *in vitro* data on pulmonary metabolism to drug elimination by the lungs *in vivo* is fraught with difficulties and limitations (6–8). Several approaches are available for quantitation of lung metabolism *in vivo*, including isolated lung perfusion (6–9), ratios of area under the curve following venous and arterial bolus doses (10), and measurement of the extraction of drug across the lung at steady state (11). While each approach has advantages and disadvantages, measurement of drug extraction across the lung following achievement of steady-state drug levels constitutes a reliable and convenient method of delineating the contribution of the lung to the total body clearance of drugs. In the present study, the steady-state approach was used in rats to investigate the possible contribution of the lung to the total body clearance of the antiepileptic drug,

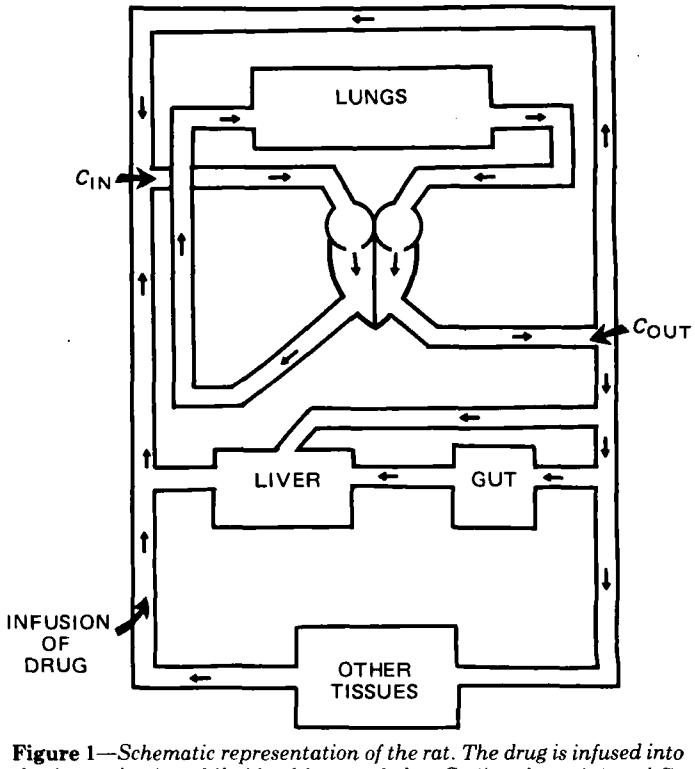

Figure 1—*Schematic representation of the rat. The drug is infused into the femoral vein, while blood is sampled at* C_i *(jugular vein) and* C_o *(carotid artery).* $CL_L = Q_B \times ER$ *[lung clearance = lung blood flow ×* $(C_i - C_o)/C_i$*].*

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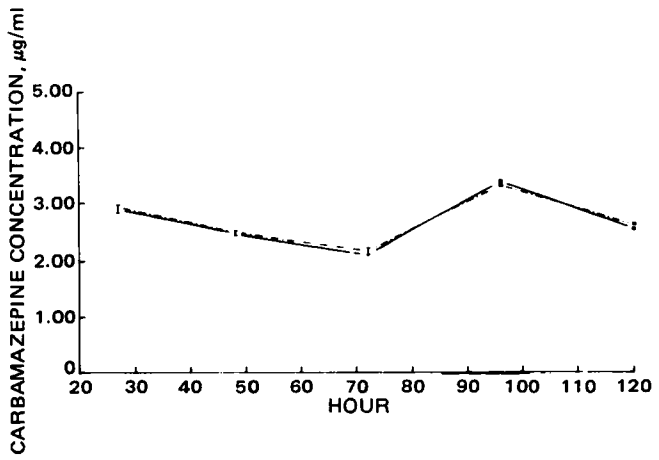

Figure 2—*Arterial (—) and venous (- - -) carbamazepine blood levels in rat 13 during a long-term carbamazepine infusion.*

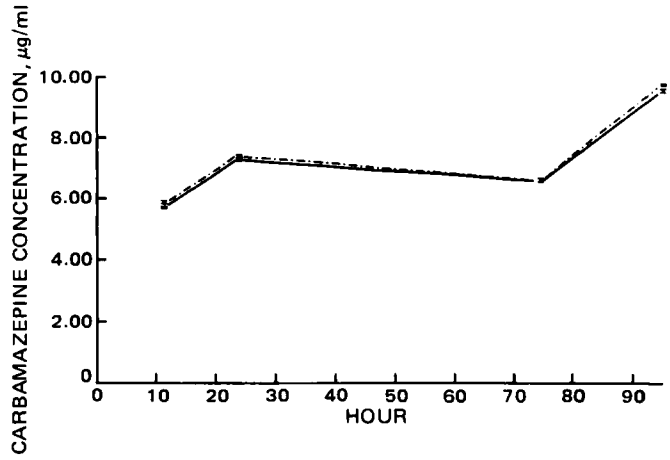

Figure 3—*Arterial (—) and venous (- - -) carbamazepine blood levels in rat 27 during a long-term carbamazepine infusion.*

carbamazepine. Carbamazepine has a large total body clearance in rats with a very small fraction (2%) eliminated unchanged in urine (12–14). Also, the reported clearance of carbamazepine in rat liver perfusions is too low to account for the large total body clearance observed (15, 16). Therefore, the potential contribution of the lungs to carbamazepine elimination was investigated.

THEORETICAL

Based on mass balance principles, the clearance of a drug by an organ can be defined by the relationship:

$$CL_o = Q_B \times ER = Q_B \times \frac{C_i - C_o}{C_i} \quad \text{(Eq. 1)}$$

where CL_o is the organ clearance, Q_B is the blood flow through the organ, and ER, estimated from the blood concentration before (C_i) and after (C_o) the organ circulation, represents the extraction ratio of the drug across the organ (17, 18). By placing cannulas in the jugular vein and carotid artery, one can measure the change in drug levels across the heart and lungs (Fig. 1). With the assumption that the heart does not metabolize the drug, one can calculate the pulmonary clearance of the drug at steady state using Eq. 1.

EXPERIMENTAL

Materials—Infusion solutions of carbamazepine (5 mg/ml) were made by dissolving the drug in 60% polyethylene glycol 400. The solutions were filtered and autoclaved before use. Harnesses and swivel joints for long-term infusions in unrestrained rats were purchased[1]. Constant-rate infusion pumps[2] were used for drug delivery. Cannulas were made of a short piece of silicone tubing[3] (for insertion into the veins and artery) connected to a longer section of polyethylene-50 tubing. Ether (USP grade) was used as the anesthetic.

Animals—Seven male Sprague–Dawley rats (250–300 g) were cannulated under ether anesthesia. Cannulations were performed on the right carotid artery and jugular vein for blood sampling and on the femoral vein for drug infusion. The polyethylene-50 section of the cannula was run subcutaneously beneath the skin and out through a small incision in the back of the neck. All operations were performed under aseptic conditions using sterilized instruments and cannulas. Rats were then attached for unrestrained long-term infusions using previously described methods (19–21).

Following the operation, animals were infused with sterile saline for 3 days. During this time, the carotid artery and jugular vein cannulas were flushed once a day with sterile saline solution (without heparin) to prevent blood clotting in the line.

[1] Instech Laboratories, Pittsburg, Pa.
[2] Sage pump models 352 and 355. Harvard pump model 2620.
[3] Silastic Medical-Grade tubing, i.d. 0.020 in., o.d. 0.037 in., Dow-Corning, Midland, Mich.

Drug Treatment and Blood Sampling—Three days after the cannula implantation, the rats were started on a carbamazepine infusion (0.25–0.30 ml/hr). Blood samples (0.45 ml) were drawn simultaneously from jugular vein and carotid artery once every 12–24 hr after the beginning of the infusion. The blood was frozen immediately and maintained at −20° until assayed.

Analytical Procedure—Whole blood (200 µl) was assayed for carbamazepine using a GLC/CI/MS assay developed in this laboratory (22). Each sample was assayed in duplicate, and the average values were used for all calculations.

RESULTS AND DISCUSSION

The concentration–time profiles of carbamazepine from jugular vein and carotid artery blood samples in rats 13 and 27 are shown in Figs. 2 and 3, respectively. Little, if any, difference in carbamazepine blood levels were found between these sampling sites.

A plot of the arterial *versus* venous carbamazepine blood levels for 28 simultaneous samples in all seven rats is given in Fig. 4. Theoretically, any slope from zero (reflecting complete extraction) to 1.0 (no pulmonary extraction) could result. The slope of 0.992 (not significantly different than 1.0) suggests very little or no carbamazepine elimination during passage through the pulmonary circulation.

The mean (± *SEM*) extraction ratio of carbamazepine by the lung was 0.0058 (±0.004) based on the 28 pairs of arterial–venous blood levels. Assuming a mean cardiac output of 62.5 ml/min in rats (23–25), the average lung clearance of carbamazepine would be approximately 22 ml/hr. The total body clearance of the drug (CL_T) was calculated using the infusion rate (Ko) and steady-state concentration (C_v) in the venous blood:

$$CL_T = \frac{Ko}{C_v} \quad \text{(Eq. 2)}$$

Based on a mean (± *SEM*) total body clearance of carbamazepine of 440 (± 38) ml/hr, the lung appears to contribute only ~5% to the total elimination of carbamazepine *in vivo*. The lack of significant lung metabolism of carbamazepine and the failure of liver perfusion experiments to account for its large total body clearance suggest either the presence of some other extrahepatic sites of drug metabolism or an error in the total liver clearance of carbamazepine determined by liver perfusion.

While the steady-state approach may be a useful technique to quantitate the role that lung metabolism plays in the total body clearance of drugs, several aspects of the present study design need further consideration before an appreciation can be gained for the limits of this approach. The blood flow through the pulmonary circulation is 3–4 times larger than the blood flow through other organs that are capable of drug elimination. Hence, even a small drug extraction ratio by the lungs can contribute significantly to the total elimination of a drug. To measure a potentially small pulmonary extraction ratio of a drug, a very sensitive and accurate assay is required in addition to a large number of samples. These problems were overcome in the present experiment by: (*a*) the use of a GLC/CI/MS assay for carbamazepine with a median percent difference between duplicate determinations of only 1.4% and (*b*) the inclusion of 28 pairs of observations from a group of seven rats.

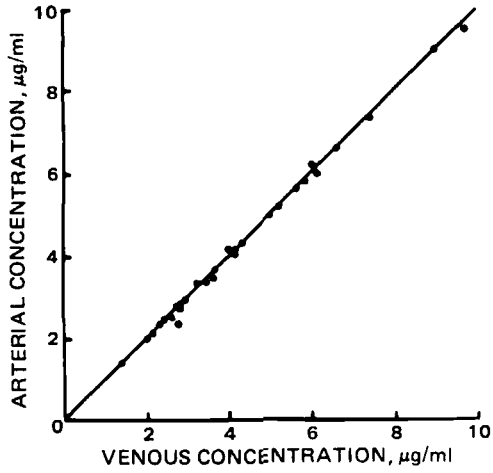

Figure 4—*Relationship between venous and arterial carbamazepine blood levels during long-term infusions in seven rats. The slope is 0.992 (not significantly different than 1.000);* $R^2 = 0.997$.

Despite the need for an accurate and reproducible assay, the steady-state approach has several advantages over other available techniques for quantitating the importance of lung metabolism. First, it does not require measuring the amounts of metabolites formed by the lung. This may be advantageous, from an analytical point of view, when there are a large number of potential metabolites which could be formed or novel metabolites specific to lung metabolism. Second, it allows a quantitation of the contribution of the lung to the total body clearance of drugs in the intact animal rather than under low pulmonary blood flow rates or other conditions optimal to *in vitro* systems. Third, it eliminates problems of intraanimal variability in drug metabolism (a potential problem with single bolus doses given by different routes) by using instantaneous differences in blood levels. Fourth, the contribution of lung metabolism to the total body clearance of drugs can be determined at drug concentrations of therapeutic importance.

The results of this study suggest that the steady-state approach can be a reliable and simple means of quantitating the contribution of lung metabolism to the total body clearance of drugs. This may be particularly important for drugs that are metabolized extensively, and where significant extrahepatic elimination is suspected. In addition, the steady-state approach can be a useful and direct means for examining the role of pulmonary metabolism for medium- to high-clearance drugs or model compounds which, based on other approaches, are postulated or appear to undergo significant lung metabolism.

Finally, it should be noted that although a number of substances are metabolized by isolated perfused lungs or lung homogenates (26, 27), little work has been directed toward quantitating the importance of lung metabolism to the total body clearance of these substances (11, 28). Despite the role played by lung metabolism in lung cancer and its induction by cigarette smoke (29–32), the importance of pulmonary metabolism in the overall elimination of drugs and its potential for producing toxic metabolites has only just begun to be appreciated (28, 33).

ADDENDUM

Following submission of this manuscript for publication, a theoretical treatment of the importance of lung metabolism to total body clearance by Collins and Dedrick was published (34).

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