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Theophylline Blood–Brain Barrier Transfer Kinetics in Dogs

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Abstract □ A simple diffusion-based pharmacokinetic model is proposed relating blood–brain barrier transfer kinetics of theophylline to the difference in the free concentrations of the drug in serum and cerebrospinal fluid (CSF). The model predicts that the CSF drug level is proportional to the serum drug level convoluted by $\exp(-kt)$, where k is the blood–brain barrier diffusion rate constant. An excellent agreement was found by nonlinear regression analysis between serum and CSF theophylline data in eight dogs and the proposed model for the blood–brain barrier transfer kinetics of theophylline. The ratio of the free fractions of theophylline in serum and CSF predicted from the model also agrees with the value determined experimentally.

Keyphrases □ Theophylline—blood–brain barrier transfer in dogs, kinetics □ Kinetics—of theophylline, blood–brain barrier transfer in dogs □ Blood–brain barrier—theophylline transfer in dogs, kinetics

The narrow therapeutic range of theophylline and the substantial intersubject variability in its disposition have resulted in extensive studies of the pharmacokinetics and clinical dosage management of the drug (1–8). Several investigations have related the bronchodilator effect of theophylline to its serum concentration level (5, 6, 9–13). However, it is the adverse effects rather than the therapeutic effect that dictates the dose administered and limits the therapeutic efficacy. The main adverse effects appear to be of CNS origin. It may be misleading, therefore, to use serum levels as a guide for the clinical management of the drug without any *a priori* knowledge of the kinetics of the blood–brain barrier transfer of theophylline. The theophylline concentration in the cerebrospinal fluid (CSF), should provide a better correlation to the CNS effects. The object of this study is to investigate the serum–CSF disposition of theophylline and the blood–brain barrier transfer kinetics. By establishing the kinetic relationship between the serum and CSF drug levels, a more rational approach to the usage of serum theophylline determinations can be established.

Although it was recognized early that theophylline enters the cerebrospinal fluid, neither the rate of equilibration with serum nor the serum–CSF concentration ratio have been defined adequately. In fact, little is known about the kinetics of transfer of drugs across the blood–brain barrier. The few CSF samples that have been correlated with serum samples in humans provide only a very limited insight into the kinetics (14–17). The use of dogs in the present study allowed comprehensive CSF sampling enabling a proper pharmacokinetic analysis of the serum–CSF theophylline disposition. Animals are often a poor predictor of human pharmacokinetics mainly due to substantial differences in the elimination processes. However, the present manner of analysis of the serum–CSF transfer kinetics is not influenced by absorption or elimination or other disposition processes. Furthermore, the tissues that constitute the blood–brain barrier apparently do not differ significantly between dogs and humans (18). The results from this study should therefore be of clinical interest.

EXPERIMENTAL

Study Design—After an 18-hr fast, eight dogs were anesthetized with 30 mg/kg iv of sodium pentobarbital; supplemental doses were given as needed during the remainder of the experiment. A polyethylene catheter in the left lateral saphenous vein was used for the infusion of aminophylline. Aminophylline[1] for intravenous use was utilized containing 25 mg of aminophylline (20.63 mg of anhydrous theophylline)/ml of solution. Aminophylline, 9 mg/kg (7.43 mg/kg theophylline) was diluted with saline to 19.4 ml and infused with a constant-infusion pump over a total of 20 min.

Blood samples for theophylline level determination were taken from a catheter in the left external jugular vein. An 18-gauge needle was percutaneously placed in the cisterna magna for obtaining the CSF samples. Cerebrospinal fluid and blood for theophylline levels were obtained at time zero (start of the infusion) and 20 (end of infusion), 50, 80, 140, 200, 260, 320, 350, and 380 min. The dogs were ventilated through a cuffed endotracheal tube using a constant-volume ventilator[2] with periodic hyperinflation to prevent atelectasis.

Theophylline Assay—The serum from 2–3 ml of blood and 0.5 ml of CSF were frozen and later assayed for theophylline, usually within 1–3 days. Theophylline concentrations were determined by the GC method of Least and coworkers (19) using 100-μl samples and substituting iodobutane for iodopentane in the derivation procedure. Theophylline concentrations were calculated using peak height ratios of theophylline to internal standards.

Protein Binding—The protein binding of theophylline was deter-

[1] Searle Laboratories.
[2] Harvard model 607.

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Table I—Pharmacokinetic Parameters Obtained by Fitting Eqs. 5 and 8 Simultaneously to Theophylline Serum and Cerebrospinal Fluid Concentration Data

Dog	k, min^{-1}	R	$t_{1/2}(\alpha)$, min	$t_{1/2}(\beta)$, min	r_T[a]	r_s[a]	r_c[a]
1	0.0135	0.690	0.00865	419	0.9906	0.9913	0.9716
2	0.0151	0.664	0.0489	634	0.9902	0.9943	0.9505
3	0.0668	0.674	0.0135	416	0.9963	0.9965	0.9771
4	0.0194	0.614	0.0144	617	0.9939	0.9963	0.9489
5	0.0323	0.670	0.0327	450	0.9918	0.9980	0.9391
6	0.0340	0.610	0.0172	587	0.9945	0.9923	0.9458
7	0.0158	0.642	0.00671	326	0.9971	0.9975	0.9777
8	0.0143	0.632	0.0249	560	0.9928	0.9980	0.9164
Mean	0.0264	0.600	0.0208	501	0.9934	0.9955	0.9533
SD	0.0182	0.029	0.0141	113	0.0025	0.0026	0.0212

[a] Correlation coefficients between observed and calculated theophylline levels: (r_T) total (serum and CSF), (r_s) serum, and (r_c) CSF.

mined by filtering serum and CSF samples through filter membrane cones[3] to yield an ultrafiltrate free of molecules with molecular weights >50,000. A 2-ml aliquot was centrifuged at 2000 rpm (not exceeding 1000×g) for 30 min yielding ~1 ml of filtrate. Both the filtrate and original sample were assayed for theophylline. The amount of theophylline bound to protein was determined by subtracting the amount of theophylline in the protein-free ultrafiltrate sample from the amount of theophylline in the original sample. The percent of protein-bound theophylline was calculated by dividing the amount of protein-bound theophylline by the amount of theophylline in the original sample. The free fractions of theophylline in serum were 0.86, 0.85, 0.89, 0.86, 0.91, 0.79, 0.80, and 0.83 and the cerebrospinal fluid 1.00, 1.00, 0.92, 1.00, 0.99, 1.00, 1.00, and 0.95 for dogs 1–8, respectively.

PHARMACOKINETIC ANALYSIS

The object of the pharmacokinetic analysis is to elucidate the blood–brain barrier transfer kinetics of theophylline on the basis of serum and CSF concentration data. Compounds may pass the blood–brain barrier by different mechanisms such as simple diffusion, facilitated passive diffusion with carrier substances, and active transport (20). The latter two are saturable processes. However, due to the narrow therapeutic range of theophylline, it may not be possible to establish kinetically if the drug crosses the blood–brain barrier by simple diffusion or by a saturable facilitated transport. For a diffusional process the rate of transfer of drug across the blood–brain barrier would be proportional to the difference between the free drug concentrations on the two sides of the barrier, *i.e.*:

$$\frac{d}{dt}\{V_cC_c(t)\} = k_1[F_sC_s(t) - F_cC_c(t)] \qquad \text{(Eq. 1)}$$

where subscripts c and s denote cerebrospinal fluid and serum, respectively; V, C, and F stand for volume, total drug concentration, and free (unbound) fraction, respectively; and k_1 is a positive diffusion constant. Equation 1 assumes that the drug is not metabolized in the CSF, which is consistent with our current knowledge about the metabolic systems present on the CNS side of the blood–brain barrier (20).

Equation 1 can be written simply as:

$$\frac{dC_c(t)}{dt} = k[RC_s(t) - C_c(t)] \qquad \text{(Eq. 2)}$$

where $k = F_ck_1/V_c$ is a diffusion rate constant with dimension time^{-1} and $R = F_s/F_c$ is the free fraction ratio. Laplace transformation of Eq. 2 yields, after rearrangement (bars denote transformed functions):

$$\bar{C}_c(s) = kR\bar{C}_s(s)\frac{1}{s+k} \qquad \text{(Eq. 3)}$$

which back-transformed gives:

$$C_c(t) = kRC_s(t) * e^{-kt} \qquad \text{(Eq. 4)}$$

where * denotes convolution. Equation 4 expresses how the concentration of theophylline in the CSF relates to the concentration of the drug in serum if the transfer of the drug across the blood–brain barrier is by simple diffusion. The diffusional transport hypothesis is tested kinetically in the following manner according to Eq. 4:

A suitable arbitrary function is chosen to approximate the $C_s(t)$ response by nonlinear least-squares curve fitting. The fitting of the arbitrary function to the serum data is done simultaneously with a fitting to the CSF data of a second function resulting from convoluting the first function with e^{-kt} and multiplying by a constant (kR) according to Eq. 4. The two constants k and R are treated as unknown parameters and determined in the simultaneous curve fitting.

The following empirical function was used to approximate the serum data resulting from a short ($t = 0$ to $t = T$) constant rate infusion:

$$C_s(t) = A\,[e^{-\alpha(t-T)_+} - e^{-\alpha t}] + B[e^{-\beta(t-T)_+} - e^{-\beta t}] \qquad \text{(Eq. 5)}$$

$$A, \alpha, B, \beta > 0$$

where:

$$(t-T)_+ = t - T \text{ for } t > T \qquad \text{(Eq. 6)}$$

$$(t-T)_+ = 0 \text{ for } t \le T \qquad \text{(Eq. 7)}$$

and A, α, B, β are positive constants that are adjusted in the curve fitting to provide a least-squares approximation of the serum level data by the $C_s(t)$ function.

Convoluting Eq. 5 according to Eq. 4 yields:

$$C_c(t) = kR\left\{\left[\frac{A\alpha}{k(\alpha-k)} + \frac{B\beta}{k(\beta-k)}\right][e^{-k(t-T)_+} - e^{-kt}] + \frac{A}{k-\alpha}[e^{-\alpha(t-T)_+} - e^{-\alpha t}] + \frac{B}{k-\beta}[e^{-\beta(t-T)_+} - e^{\beta t}]\right\} \qquad \text{(Eq. 8)}$$

Equations 5 and 8 were fitted simultaneously to serum and CSF theophylline data for each dog using the interactive nonlinear regression program FUNFIT (21).

RESULTS AND DISCUSSION

The present method of pharmacokinetic analysis differs in several aspects from classical pharmacokinetic approaches:

1. The study focuses on a single disposition process (the blood–brain barrier transfer kinetics) which is analyzed without interference from other pharmacokinetic processes.
2. The kinetic model for the process is physiologically meaningful.
3. The analysis does not rely on the many often unrealistic assumptions and concepts of classical pharmacokinetic approaches such as linear disposition, abstract multicompartmental drug transfer, rate processes that are proportional to the amount rather than the concentration of the drug, *etc.*
4. With the exception of the specific process under investigation, the method is completely model independent. No attempts are made to derive kinetic models to account for the drug's disposition and elimination kinetics.

The approach may perhaps best be characterized as a "response approximation approach" where the concentration response is estimated by fitting a suitable arbitrary function (Eq. 5) to the data. It is not necessary to attach any kinetic significance to the approximating function and its parameters. Its purpose is not to model the pharmacokinetics but to estimate (approximate) the concentration profile so it can be used to investigate changes in the theophylline concentrations in the serum and CSF in a way that is consistent with the proposed kinetic model for transfer of the drug across the blood–brain barrier (Eqs. 1 and 4).

The serum and cerebrospinal fluid data for each of the eight dogs showed excellent agreement with the proposed diffusion model for the transfer of theophylline across the blood–brain barrier (Fig. 1, Table I).

[3] Type CF50A Centriflo, Americon Corp.

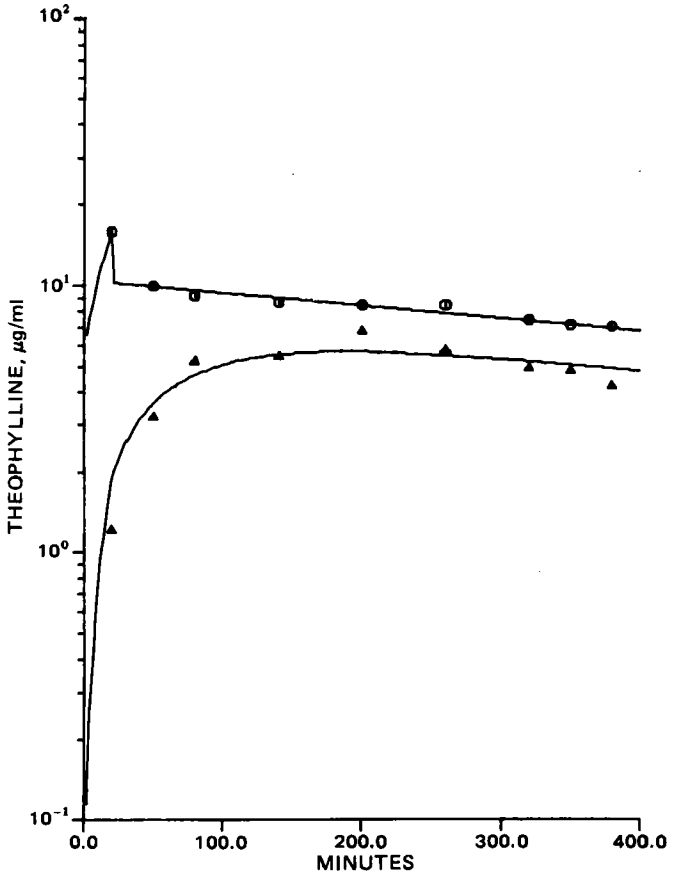

Figure 1—*Simultaneous least-squares fit of Eqs. 5 and 8 to serum (⊙) and CSF (▲) theophylline data resulting from a 20-min constant-rate intravenous infusion for dog 2. This fit has the smallest total correlation coefficient of the eight dogs studied (Table I).*

The k values are all of the same order of magnitude. Since $k = F_c k_1/V_c$ depends on both V_c, the volume of the CSF and F_c, the free fraction of theophylline in the CSF, this parameter is naturally expected to show greater intersubject variability than the more intrinsic diffusion parameter, k_1 (defined in Eq. 1). The determination of k_1 would have required V_c to be experimentally determined which was not done in the present study. The free fraction ratio $R = F_s/F_c$ differs remarkably little from subject to subject. The mean value of 0.60 ±0.03 (*SD*) for R comes fairly close to the ratio of 0.86 ± 0.06 (*SD*) calculated from experimentally determined values. The difference may well be due to the inherent inaccuracy of binding determination by ultrafiltration and to the fact that the free fraction is dependent on the total drug concentration. The microenvironment where the diffusion takes place may also have a different protein composition than that found in serum and CSF.

The binding of theophylline in serum is not likely to be affected by the presence of pentobarbital, because a significant change in the free fraction of a drug by competitive binding is usually only seen for drugs that are highly bound (>95%).

Theophylline serum data are used in clinical monitoring for dosage adjustments and in the management of overdose cases. However, since the side effects are of a CNS origin, the CSF drug level should provide a better basis for monitoring. In the postdistribution phase of a drug input where the ratio between the theophylline serum and CSF levels remains fairly constant (Fig. 1), the serum level should be a good indicator of the CNS level of the drug. However, in the distribution phase (lasting 1–1.5 hr after a rapid drug input), there appears to be a significant divergence in the serum and CSF drug levels[4]. In fact, during this phase the serum level may drop while the CSF level increases (Fig. 1). Evidently it is of great clinical significance to understand this kinetic phenomenon. During the distribution phase of a case of theophylline overdosing, it would be inappropriate to rely on serum levels for safety predictions. A decline in the serum level may not guarantee improvement; it would be wise to be prepared for delayed severe toxic effects. In a study of overdose cases resulting in seizure, it was reported that the most noteworthy phenomenon was the apparent absence of adverse effects in seven of the eight patients prior to the seizure (13). That study is consistent with what could be predicted from our pharmacokinetic investigation when the toxic effects are related to the CSF drug level rather than the serum level. Undoubtedly there is a need to further study the serum–CSF disposition of theophylline so a proper pharmacodynamic basis can be established for the rational clinical use of theophylline serum data.

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[4] The distribution phase in the present context is defined in reference to the CSF. Thus, although the distribution of theophylline to compartments other than the cerebrospinal fluid appears very rapid judged from the short α-phase in the serum, this is not the case for the CSF (Fig. 1).