Time-Dependent Disposition of β-Naphthoflavone in the Rat

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The pharmacokinetics of β-naphthoflavone (BNF) have been investigated in rats following various modes of intravenous administration. From intravenous bolus studies it was established that BNF showed a high blood clearance (130 ml/min/kg) and no detectable excretion of unchanged compound in the urine. The volume of distribution for BNF was large (6 L/kg), and binding to plasma proteins extensive (96%). Intravenous infusion studies where the length of infusion was increased from 1 to 8 hr showed marked signs of time-dependent pharmacokinetics. During continuous infusions the plasma concentrations accrued for approximately 1 hr, after which plasma concentrations declined in an apparent exponential fashion to a plateau value. In the short infusion studies the postinfusion half-life (27 min) was significantly shorter than the terminal half-life after bolus administration (40 min). Time-dependent clearance of BNF resulting from enhancement/induction of P450IA enzymes is proposed as the mechanism for these unusual pharmacokinetic features. The use of antipyrine as an independent probe for P450 activity gave similar trends in antipyrine clearance for various modes of BNF administration. Computer simulations based on an autoinduction model for time-dependent clearance were consistent with the observations on BNF in the rat.

KEY WORDS: β-naphthoflavone; time-dependent clearance; enzyme induction; β-naphthoflavone–antipyrine interaction.

INTRODUCTION

β-Naphthoflavone (BNF) is a synthetic flavone. In common with other flavones and several polycyclic aromatic hydrocarbons [e.g., 3-methylcholanthrene, benzo(*a*)pyrene], BNF is a potent inducer of mammalian hepatic microsomal monooxygenases (1,2). A major component of this enzyme system which metabolizes a wide variety of chemical structures is the cytochrome P450 (P450) family. Induction of P450 by flavones and polycyclic aromatic hydrocarbons is selective for the P450IA subfamily (3). To date two members of this subfamily have been identified, isolated, and purified (3). Their primary protein structure and much of the molecular biology involved in their induction are known (4,5).

The P450IA members are possibly the most studied of all the P450 enzymes, due to the early association of polycyclic aromatic hydrocarbon induction with cell toxicity via the generation of active metabolites (6,7). Induction of P450IA members in the rat may be as high as 70-fold [3-methylcholanthrene, 25 mg/kg (8)] and their substrate specificity is relatively narrow. Numerous similarities between induction by BNF and induction by 3-methylcholanthrene, the most extensively investigated polycyclic aromatic hydrocarbon inducer, have been documented using various systems (9,11). However, unlike most of these hydrocarbon inducers, whose inductive effect may be correlated with their carcinogenicity, BNF is believed to be noncarcinogenic (12). This has led to extensive use of this compound in enzyme induction investigations and, especially, in studies focusing on the multiplicity of the cytochrome P450 family.

Despite the wide use of BNF as an inducer of hepatic microsomal monooxygenases, very few investigations have been carried out on the disposition of BNF itself. Boobis *et al.* (9) used radiolabeled BNF to demonstrate that BNF was absorbed and eliminated faster than 3-methylcholanthrene. More recently the metabolic fate of BNF was investigated in liver microsomes and reconstituted cytochrome P-450 systems (13). However, the pharmacokinetics of BNF have not been characterized and hence the relationship between its disposition and its inductive effect remains unexplored. We have applied a recently developed HPLC method of analysis of BNF in plasma (14) to investigate the pharmacokinetics of BNF in rats. The intravenous route of administration has been used in preference to the traditionally used intraperitoneal route of administration for BNF. The data demonstrate a marked time dependence in BNF pharmacokinetics. Further studies presented using computer simulation and antipyrine pharmacokinetics, as an independent marker of P450 activity, are consistent with P450 enhancement/induction as the mechanisms for the unusual pharmacokinetic behavior of BNF.

MATERIALS AND METHODS

Animal Experiments

Male Sprague–Dawley rats (200–250 g) obtained from the University of Manchester Medical School Animal Unit were used for all experiments. The rats were cannulated in the jugular vein and carotid artery. BNF was administered via the jugular vein as a bolus injection or as an infusion of a solution in polyethylene glycol 400 and propylene glycol mixture (PEG/PPG; 9:1, by volume). Serial blood samples (250 μl) were collected via the carotid artery over 4–8 hr into heparin-containing tubes, and plasma was obtained by centrifugation. The cannula was flushed (0.1 ml) after each sample with heparinized saline (100 U/ml) to prevent blood clotting.

Single-Dose Studies

One group of rats (n = 5) was anesthetized with urethane (1200 mg/kg), cannulated, and given a single bolus dose of BNF (10 mg/kg). The experiment was conducted entirely under anesthesia. A second group of animals (n = 7) was cannulated under light ether anesthesia and allowed to recover from the operation for about 24 hr before being used for the experiment. They were housed individually in an approved apparatus to restrict movement while sampling (15) and given a single bolus dose of BNF (10 mg/kg). After

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the blood sampling, the rats were transferred to approved metabolism cages that allowed separate collections of urine and feces. Voided urine was collected for a total period of 24 hr from the start of the experiment.

Infusion Studies

Rats (n = 9) that had been allowed to recover from the cannulation operation for about 24 hr were given BNF as a continuous infusion (via a syringe pump, Model Sage 351, A. R. Howell Ltd., London) at the rate of 1.5, 3, and 6 mg/hr/kg for 6 hr, and BNF was monitored during this period. Another group of rats (n = 12) was given BNF at the rate of 6 mg/hr/kg for 1 or 2 hr, and BNF monitored during and after infusion.

Antipyrine–BNF Interaction Studies

These studies were carried out in two series of experiments: (i) simultaneous administration of BNF and antipyrine and (ii) administration of antipyrine 4 hr after BNF administration. The experiments were performed in a similar manner to those described above. In the simultaneous-administration study, the rats were given BNF (as a single bolus dose, 10 mg/kg, or as an infusion at a rate of 6 mg/hr/kg for 2 hr) or an appropriate volume of vehicle (as both a bolus and an infusion) immediately after a single intravenous bolus dose of antipyrine (50 mg/kg). In a separate series of studies, antipyrine was given 4 hr after a single bolus dose (1 or 10 mg/kg) of BNF or an appropriate volume of vehicle (2 ml/kg).

Analysis

BNF was measured by an HPLC method. Samples of plasma (100 μl) or urine (500 μl) were extracted with ethylene chloride as detailed in a previous publication (14). A reverse-phase Zorbax TMS 25-cm column was employed, with UV detection at 275 nm (14). Antipyrine concentrations were measured by the same extraction and chromatographic procedures as described for BNF.

The area under the curve (AUC) was calculated using the log trapezoidal rule. Clearance (BNF and antipyrine) and volume of distribution at steady state (BNF) were calculated by noncompartmental methods (16). Antipyrine volume of distribution was calculated by dividing the dose by the back-extrapolated zero-time concentration.

Chemicals

β-Naphthoflavone and antipyrine were obtained from the Sigma Chemical Company Ltd. (Poole, England). Polyethylene glycol 400 and propylene glycol were obtained from BDH Chemicals Ltd. (Poole, England).

Determination of Plasma Binding and Blood-to-Plasma Distribution of BNF

The binding of BNF to rat plasma was investigated by an ultracentrifugation technique (17). Two concentrations, 10 and 20 mg/L of BNF, were prepared in rat plasma. An aliquot of this solution (1.8 ml) was put in a polycarbonate tube (MSE type, Fisons Scientific Equipment, Loughborough, England) and subjected to centrifugation at 135,500g and 37°C under vacuum for 16 hr. The supernatant was then analyzed for BNF.

The distribution of BNF between whole blood and plasma was investigated by preparing various concentrations of BNF (1, 5, and 10 mg/L) in rat whole blood. This was then divided into two, with one-half centrifuged (2000g for 10 min) to separate the plasma. The plasma and the blood solution were then analyzed for BNF and the blood-to-plasma BNF concentration ratio was calculated.

Simulation Studies

The pharmacokinetic behavior of BNF was simulated using an enzyme induction model proposed by Levy and co-workers (18) where clearance is time dependent [Eq. (1)].

$$\mathrm{CL}(t) = \mathrm{CL}' - (\mathrm{CL}' - \mathrm{CL})e^{-k(t-\theta)} \quad (1)$$

where CL(t) is the time-dependent clearance, CL and CL′ are the initial and final clearances, k is the rate constant for the change in enzyme activity (induction), and θ is the time lag prior to induction.

The degree of induction observed can be related to the dose administered [Eq. (2)] by a sigmoid relationship where (CL'_{max} − CL) is the maximal effect and (Dose/Dose + S) defines the steepness of the curve.

$$\mathrm{CL}' = \mathrm{CL} + \frac{\mathrm{Dose}}{\mathrm{Dose} + S}(\mathrm{CL}'_{max} - \mathrm{CL}) \quad (2)$$

Dose is the BNF dose administered, S a dose sensitivity parameter, and CL'_{max} the maximally induced clearance.

For the iv bolus and infusions simulations Eqs. (3) and (4) were used. For simplicity a monoexponential decline in BNF eliminations from the plasma was assumed.

$$V \cdot \frac{dC}{dt} = -\mathrm{CL}(t) \cdot C \quad (3)$$

$$V \cdot \frac{dC}{dt} = R - \mathrm{CL}(t) \cdot C \quad (4)$$

where V, C, and R are the volume of distribution, plasma concentration, and zero-order infusion rate, respectively, for BNF.

Simulations were carried out with the continuous simulation program DARE-P (19). Basal values for BNF were selected from observed data: V, 6 L/kg; CL, 100 ml/min/kg; and half-life, 40 min. Unless stated otherwise, the other model parameters values used were k (0.02 min^{-1}), θ (60 min, infusion; 45 min, bolus), S (2 mg/kg), and CL'_{max} (200 ml/min/kg).

RESULTS

BNF Disposition Following Intravenous Bolus Administration

Figure 1 illustrates a typical plasma concentration–time profile for BNF following an intravenous bolus injection of 10 mg/kg of BNF. The decline is biexponential, with an initial half-life between 40 and 60 min. The low plasma concentrations achieved following BNF administration are a con-

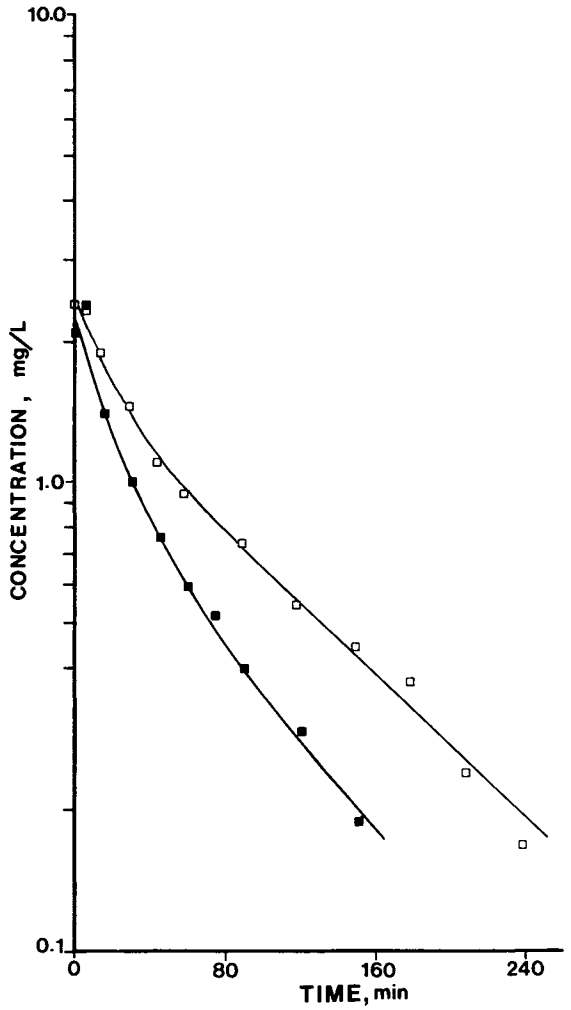

Fig. 1. Typical BNF plasma concentration–time profiles from anesthetized (□) and unanesthetized (■) rats following intravenous administration of a bolus 10 mg/kg dose of BNF.

sequence of the large volume of distribution and the high clearance (see Table I).

Binding within the plasma is extensive; fractions unbound at 10 and 20 mg/L (n = 24) are 0.041 ± 0.006 and 0.039 ± 0.002, respectively. Hence binding within the tissues must be considerable to produce a volume of distribution of 6 L/kg. When an allowance is made for plasma binding, the volume of distribution for unbound BNF reaches 150 L/kg.

The plasma clearance of BNF is high, over 90 ml/min/kg (Table I). The blood-to-plasma concentration ratio is 0.7 ± 0.08 over the concentration range 1–10 mg/L; therefore a blood clearance of 130 ml/min/kg may be calculated. This high value approximates hepatic blood flow in the rat (20), indicating that the clearance of BNF may be blood flow limited. No BNF could be detected in the urine of these rats, suggesting that clearance is predominantly hepatic (either metabolic or biliary or both).

Studies in anesthetized rats also show a biexponential decline in plasma concentrations with time (Fig. 1). A similar volume of distribution is obtained, but a lower clearance and longer terminal half-life (Table I). The latter findings are consistent with a reduction in hepatic blood flow, which has been reported during urethane anesthesia (21).

BNF Disposition Following Intravenous Slow Infusion

Using the half-lives and clearance data obtained from the intravenous bolus study, the time course for BNF plasma concentrations during an intravenous slow infusion can be calculated (22). Figure 2 shows both the predicted and the observed BNF concentrations at various times during 8-hr infusions of BNF at rates of 1.5, 3, and 6 mg/hr/kg. The observed data are at variance with that anticipated for a continuous infusion. The maximum concentrations achieved are similar to the predicted steady-state concentrations but occur after 45–90 min rather than after 200–300 min. Further, these maxima are not maintained, although the infusion is continued, but decrease in an approximately exponential fashion to a plateau concentration. Both the maximum concentration and the area under the curve appear to be linearly related to the infusion rate (Table II).

Figure 3 illustrates BNF plasma concentration–time profiles during and following short-term infusions of BNF over 1 and 2 hr at a rate of 6 mg/hr/kg; both observed and predicted profiles are shown. Most of the data points are postinfusion, to allow calculation of a half-life. For the 1- and 2-hr infusions half-lives of 27 ± 10 min (n = 6) and 25 ± 3 min (n = 6), respectively, are obtained. These half-lives are not statistically different from each other but both differ from the bolus-dose half-life ($P < 0.01$ by t test).

Antipyrine Disposition Following Simultaneous Intravenous Administration of Antipyrine and BNF

Simultaneous administration of antipyrine (50 mg/kg by intravenous bolus) with BNF (10 mg/kg intravenous bolus or

Table I. Pharmacokinetic Parameters Describing the Disposition of β-Naphthaflavone Following Intravenous Bolus Administration to Rats

Parameter	Animals[a] Unanesthetized (n = 7)	Anesthetized (n = 5)	Statistical significance between groups[b]
Initial half-life (min)	8 ± 3	8 ± 3	NS
Terminal half-life (min)	4.3 ± 9	64 ± 15	$P < 0.05$
Volume of distribution (L/kg)	5.9 ± 1.7	6.1 ± 1.5	NS
Clearance (ml/min/kg)	94 ± 1.0	69 ± 24	$P < 0.05$

[a] Mean ± SD.
[b] By t test; NS, not significant.

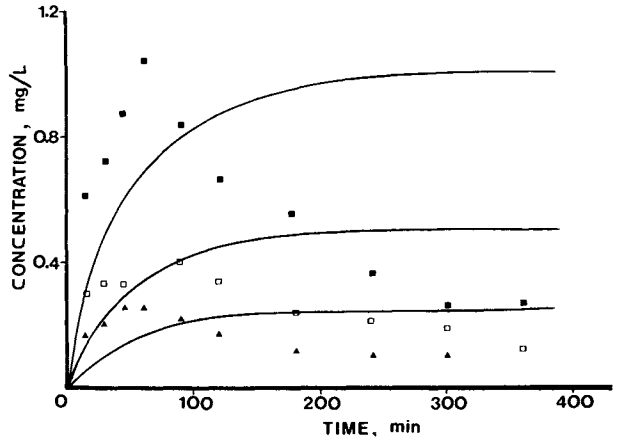

Fig. 2. Typical BNF plasma concentration–time profiles in rats receiving a continuous intravenous infusion of BNF at a rate of either 1.5 (▲), 3 (□), or 6 (■) mg/hr/kg. Solid lines denote predicted profiles based on the parameter values reported in Table I.

6 mg/hr/kg intravenous infusion over 2 hr) results in marked changes in the antipyrine plasma concentration–time profile compared to the profile following antipyrine administration alone. This is manifested as an increase in the rate of elimination over the time of the experiment to produce a convex log concentration–time profile (Fig. 4). These profiles have been analyzed as two distinct phases in order to describe the changes in kinetics. The 240-min time point was taken to divide the curve into two phases. Estimating the half-lives for the two phases produces an average half-life of about 110 min for the first phase and about 70 min for the second phase (Table III). Thus, a change in elimination kinetics of antipyrine is evident 240 min after BNF administration. This change in antipyrine kinetics can also be seen in the reduction in the area under the curve from these experiments (Table III). The volume of distribution of antipyrine did not differ in any of the experiments. This parameter was calculated from the initial phase data for the BNF animals.

Antipyrine Disposition in Rats Pretreated with BNF

Table IV shows the effect of BNF on the disposition of antipyrine administered 4 hr after either a 1 or a 10 mg/kg BNF intravenous dose. In both cases marked changes in antipyrine kinetics result in a larger clearance and shorter half-life. No change in volume of distribution is observed. Figure 5 illustrates typical concentration–time profiles. In control and low-dose BNF-treated rats, the profiles are adequately described by a monoexponential decline; however, in the higher-dose BNF-treated rates, the profiles show signs of a convex behavior. Thus, the terminal half-life and clearance may have been overestimated by imposing a monoexponential decline on these data.

DISCUSSION

Time-Dependent BNF Pharmacokinetics

BNF displays a large volume of distribution (6 L/kg) and a high blood clearance (130 ml/min/kg). The latter is close to hepatic blood flow in the rat and suggests that the clearance of BNF is blood flow limited. This is supported by the smaller clearance observed in anesthetized rats. The high clearance and large volume of distribution explain our inability to detect BNF in plasma following either oral or intraperitoneal administration of the commonly employed 100 mg/kg BNF dose. Similar pharmacokinetic properties have been reported for polycyclic aromatic hydrocarbons (23).

The pharmacokinetic behavior of BNF following continuous infusion differs dramatically from that observed after bolus administration. The total dose administered in the 2-hr infusion of the highest BNF concentration is comparable to the bolus dose (12 and 10 mg/kg, respectively) and provides unequivocal evidence for time-dependent pharmacokinetics of BNF. The decrease in the plasma concentration despite continuing infusion (Fig. 2) and the faster than expected decline in postinfusion concentration (Fig. 3) are both indicative of an increasing rate of elimination of BNF with time. This explanation is also consistent with the eventual plateau concentration being much lower than expected.

There are two main differences between the observed and the predicted time profiles in the infusion studies: first, in the early stages of the experiments, when BNF concentrations always exceed those predicted, and second, in the latter stage of the experiments, when BNF concentrations are considerably less than predicted. The single-dose data used to calculate the pharmacokinetic parameters for infusion prediction were obtained over a period of time during which the kinetics were charging. The parameters are therefore average values over the experiment and do not represent the value at a particular time. The calculated clearance will be an overestimation in the initial stages and an underestimate of the final stage. This could account for why the accrual phase of the infusion studies has higher than pre-

Table II. Observed and Predicted Disposition Parameters for β-Naphthoflavone Administered as a Continuous Intravenous Infusion to Rats

Infusion rate (mg/hr/kg)	Concentration (mg/L)			Area under curve (mg/L · hr)[a,b]
	Predicted steady-state	Observed maximum[a]	Plateau[a]	
1.5	0.25	0.25 ± 0.10	0.12 ± 0.03	49.8 ± 8.7
3	0.50	0.45 ± 0.16	0.11 ± 0.01	96 ± 12.6
6	1.00	1.04 ± 0.22	0.13 ± 0.06	186 ± 15.6

[a] Mean ± SD (n = 3).
[b] Calculated over the infusion time of 8 hr.

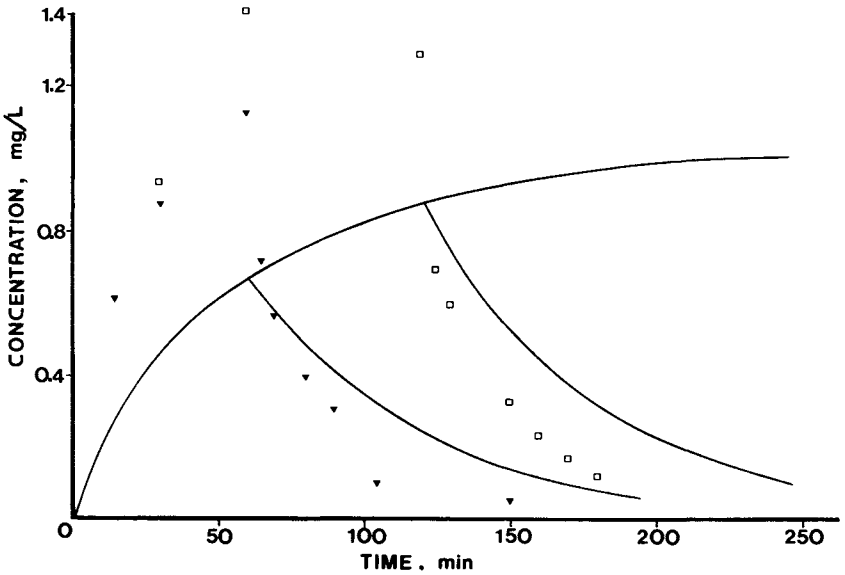

Fig. 3. Typical BNF plasma concentration–time profiles in rats during and after an intravenous infusion of 6 mg/hr/kg for either 1 (▼) or 2 (□) hr. Solid lines denote predicted profiles based on the parameter values reported in Table I.

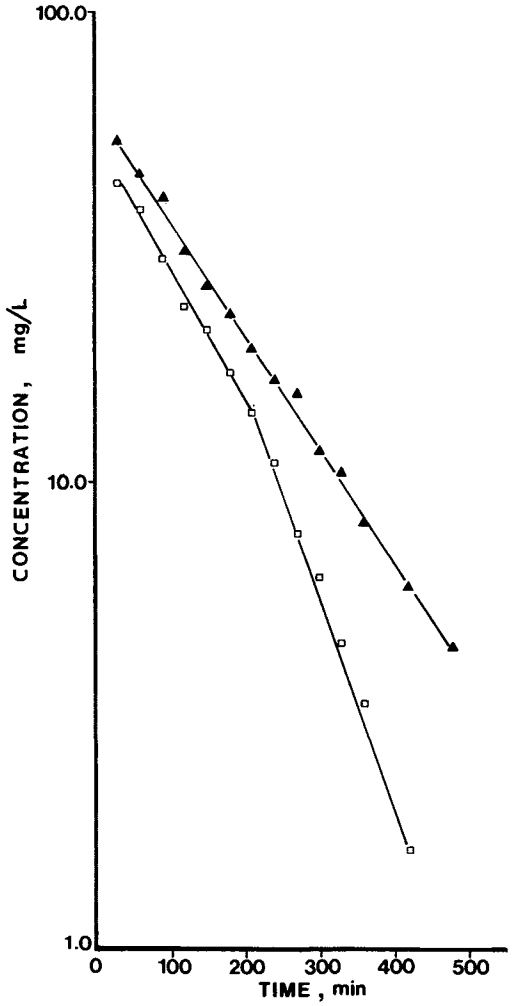

Fig. 4. Typical antipyrine plasma concentration–time profiles from rats following either antipyrine and BNF (□) or antipyrine and vehicle (▲) concomitantly by intravenous bolus administration.

dicted concentrations because the real clearance at this stage is lower than the average calculated from the single-dose study. Similarly the volume of distribution is a time-averaged parameter and inaccuracies in the calculation of this parameter may also contribute to the discrepancies in the accrual phase.

The infusion studies carried out at three rates and over varying lengths of time all consistently demonstrate that BNF clearance is enhanced with time. Between 1 and 4 hr after commencing the infusion of BNF, the nonlinearity is evident. Even allowing for the inaccuracies in estimating BNF clearance, this parameter must be close to the limiting value of hepatic blood flow. Thus to accommodate the degree of change indicated in the infusion studies, extrahepatic metabolism must contribute substantially to BNF clearance.

Simulation of Time-Dependent Clearance

In view of the well-known cytochrome P450 induction properties of BNF, autoinduction is an obvious mechanism to explain the time-dependent clearance. Pharmacokinetic models for the induction process have been proposed, and one of these models (18) has been modified in order to simulate the autoinduction of BNF. A time-dependent clearance generates a log plasma concentration–time profile which is convex. For example curve 1 in Fig. 6 illustrates the case where the clearance of 0.1 L/min/kg is doubled and hence the terminal phase has a half-life of 20 min rather than 40 min, which results when clearance is constant. Two additional important parameters [see Eq. (1)] are the rate constant for and the time lag prior to induction. Increasing the latter delays the change in kinetics but, as expected, without affecting the terminal half-life. When the induction rate constant is altered, the time to reach the new higher clearance is affected. Hence when the rate constant is small, long experimental times are required to see the change in kinetics. Once again, the final clearance determines the kinetics in the final stage.

Table III. Pharmacokinetic Parameters[a] Describing the Disposition of Antipyrine Following Its Administration Alone and Concomitantly with BNF

Parameter	Control (n = 6)	BNF bolus[b] (n = 7)	BNF infusion[c] (n = 7)	Statistical significance between control and BNF groups[d]
First half-life (min)[e]	107 ± 18	110 ± 22	113 ± 21	NS
Second half-life (min)[f]	107 ± 18	70 ± 19	71 ± 8	$P < 0.01$
Volume of distribution (L/kg)	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	NS
Area under curve (mg/L · hr)	9080 ± 1071	7924 ± 657	7756 ± 103	$P < 0.05$

[a] Mean ± SD.
[b] BNF intravenous dose, 10 mg/kg.
[c] BNF intravenous infusion, 6 mg/hr/kg over 2 hr.
[d] By one-way ANOVA with range test.
[e] Half-life over 0–4 hr.
[f] Half-life over 4–8 hr.

In the infusion simulations the importance of these same parameters is evident. Figures 7A and B illustrate the effects of altering final clearance (hence the extent of induction) and the time lag prior to the onset of induction respectively. The effect of the induction rate constant (simulations not shown) is qualitatively similar to that of changing the time lag. There is a close similarity between the simulated (Fig. 7A) and the observed (Fig. 2) profiles. Both show an early maximum concentration and a subsequent decrease toward a final plateau. The maximum concentration reached and the time when the concentration starts to fall are a function of the time lag and the plateau concentration dependent on the final (induced) clearance.

Table V summarizes the half-lives which describe the concentration–time decline following short infusions of varying times. Once again, the model produces profiles consistent with those observed experimentally. For a given infusion rate, the length of infusion time dictates the dose of BNF entering the body and hence the degree of induction. Longer infusion times produce shorter half-lives. Also shown in Table V is the effect of infusion rate and induction rate constant, which influence the degree of induction evident in the postinfusion plasma half-life.

Antipyrine–BNF Interaction

Support for enzyme induction as the mechanism responsible for the time-dependent pharmacokinetics of BNF is supplied by the antipyrine interaction studies. Antipyrine is well documented as a sensitive *in vivo* probe for cytochrome P450 activity (24), and the sensitivity of antipyrine clearance to BNF induction established (25).

Convex log plasma concentration–time profiles for antipyrine result when BNF is administered concomitantly. Both bolus and infusions of approximately equivalent doses of BNF generate a similar response, where a charge in antipyrine clearance is identified within 4 hr of BNF administration. In a second series of experiments allowing a 4-hr time period between BNF and subsequent antipyrine administration, the enhanced clearance and faster half-lives for antipyrine are confirmed and no significant effect on volume of distribution is seen.

Proposed Mechanism

After intravenous administration, BNF disposition has been characterized and time-dependent features identified. Time dependency in BNF clearance would explain the unusual features described for BNF. Since BNF is extensively metabolized and its P450IA induction properties are well-known, autoinduction would appear a likely mechanism to explain the enhanced clearance. The antipyrine interaction studies indicate that the phenomenon is not limited to BNF *per se* but is evident in the disposition of other substrates which share the P450IA enzymes. The computer simulation studies are also consistent with the data reflecting enhanced/induced enzyme activity.

The limitations of the assay prevented characterization of BNF disposition after the most commonly employed intraperitonial route of administration. Despite administration

Table IV. Effect of BNF on Antipyrine Disposition[a]

Parameter	Control (n = 6)	BNF pretreated[b] 1 mg/kg (n = 6)	BNF pretreated[b] 10 mg/kg (n = 6)	Statistical significance between groups[c]
Clearance (ml/min/kg)	6.5 ± 1.8	9.2 ± 1.6	9.6 ± 0.9	$P < 0.05$
Volume of distribution (L/kg)	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	NS
Half-life (min)	92 ± 15	61 ± 9	53 ± 4	$P < 0.001$

[a] Mean ± SD.
[b] BNF administered 4 hr prior to antipyrine.
[c] By one-way ANOVA.

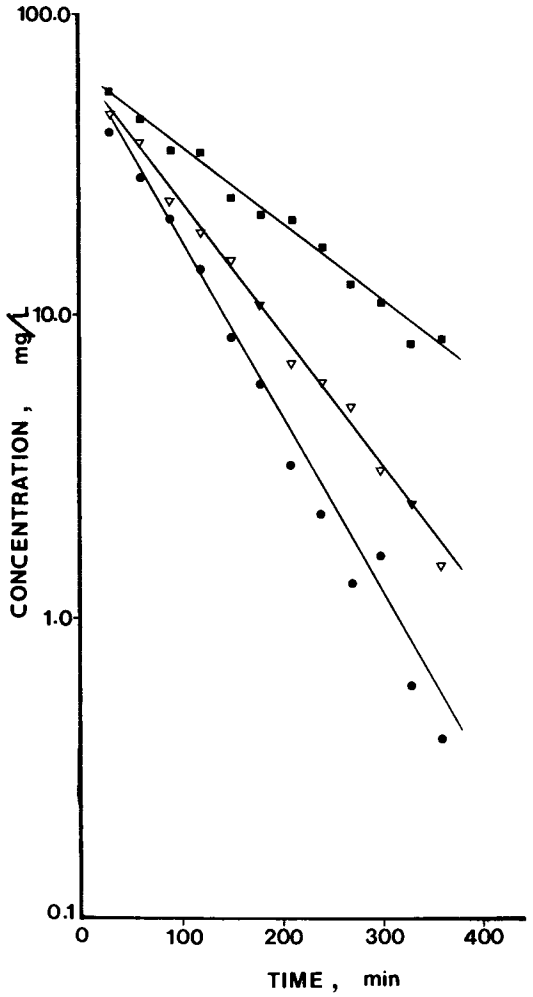

Fig. 5. Typical antipyrine plasma concentration–time profiles from rats following antipyrine intravenous bolus administration 4 hr after either vehicle (■) or 1 mg/kg (▽) or 10 mg/kg (●) BNF intravenous bolus dosing.

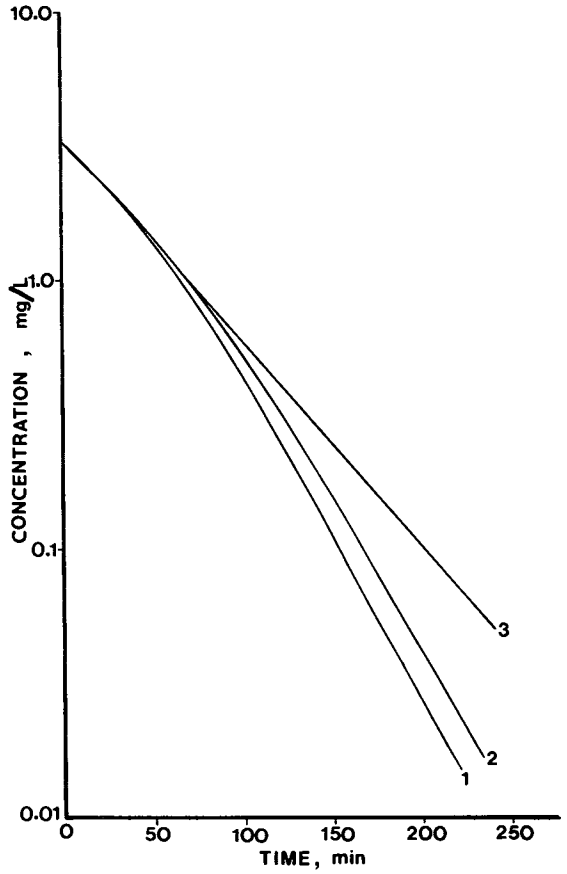

Fig. 6. Simulated drug plasma concentration–time profiles in rats following an intravenous bolus dose (10 mg/kg) of drug. Equations (1), (2), and (3) were used; V = 6 L/kg, CL = 100 ml/min/kg, θ = 45 min, S = 2 mg/kg, and CL'_{max} = 200 ml/min/kg. Different k values are shown: 0.1 (1), 0.02 (2), and 0.001 (3) min^{-1}. The terminal half-lives for these profiles are 20 min (not achieved within the time of the simulation for curve 3), whereas the control half-life is 40 min.

of high doses (100 mg/kg), slow and erratic absorption (9) would explain the failure of these experiments. BNF was dispersed in corn oil, the vehicle routinely used in induction studies. Slow absorption for this formulation may be a major reason why lengthy time periods (12–24 hr) are allowed (9,26) between BNF administration and preparation of microsomes to quantify the induction effect. Induction studies in cell culture (27,28) demonstrate dramatic increases in transcription rates of P450IA genes and protein synthesis within 3–4 hr after exposure to inducer. Intravenous administration would result in rapid and high exposure of BNF to all cells in the body, and hence the rapid response reported here is not surprising.

P450IA induction has been reported to occur in a wide variety of extrahepatic tissues (3) including the lung (29,30). The initial clearance of BNF is high and of the order of hepatic blood flow, yet considerable enhancement/induction of BNF clearance occurs. This apparent disparity may be explained by the role of induced extrahepatic tissues. It has been demonstrated in benzo(*a*)pyrene-induced rats that 3-methylcholanthene clearance is substantially extrahepatic,

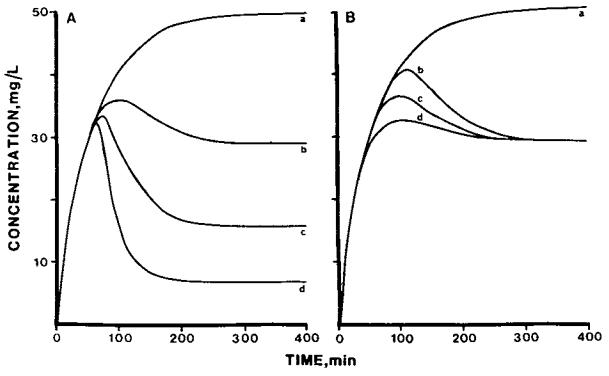

Fig. 7. Simulated drug plasma concentration–time profiles in rats receiving a continuous intravenous infusion (5 mg/min/kg) of a drug which enhances its own clearance. Equations (1), (2), and (4) were used. (A) Effect of different degrees of enhancement. Curve a, constant clearance (100 ml/min/kg); curves b, c, and d, final clearance of 200, 400 and 1000 ml/min/kg, respectively, and lag time of 60 min. (B) Effect of different lag times. Curve a, constant clearance; curves b, c, and d, lag times of 30, 60, and 90 min, respectively, and final clearance of 200 ml/min/kg. The following parameters were constant in all infusion simulations: V = 6 L/kg, CL = 100 ml/min/kg, S = 2 mg/kg, k = 0.02 min^{-1}, and R = 5 mg/min/kg.

Table V. Effect of Infusion Rate (R), Length of Infusion, and Induction Rate Constant (k) on the Postinfusion Elimination Half-Life of β-Naphthoflavone

Length of infusion (min)	Elimination half-life (min)[a]			
	k = 0.001 min^{-1}		k = 0.02 min^{-1}	
	R = 20 mg/hr	R = 1 mg/hr	R = 20 mg/hr	R = 1 mg/hr
60	25.4	32.6	9.4	15.2
120	21.9	29.3	6.8	11.9
180	19.2	26.8	5.9	10.8
240	17.3	24.8	5.7	10.5
300	15.6	23.2	5.6	10.4

[a] Calculated from simulated postinfusion data by regression of concentration data above 0.1 mg/L (lower limit of BNF assay).

whereas in control rats the liver is essentially the sole site of metabolism (23). Induction of pulmonary P450IA would have particular marked effects on BNF clearance in our studies since the intravenous route of administration was used and this would result in the potential of pulmonary first-pass metabolism (31). Therefore the full impact of any induction of pulmonary P450IA enzymes would be evident, in contrast to induction of hepatic P450IA enzyme activity, which would be masked by the upper limit for hepatic clearance of hepatic blood flow. Antipyrine has widely differing pharmacokinetic properties to BNF, in particular its low clearance, which results from hepatic P450 activity. For this compound, as anticipated, a BNF-induced change in hepatic P450IA activity is fully expressed, as there is no blood flow limitation operating.

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