Bull. Environ. Contam. Toxicol. (1993) 51:901–908

Environmental Contamination and Toxicology

Pharmacokinetic Model for the Disposition of 3-Methylcholanthrene in Channel Catfish after Slow Intraaortic Infusion

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Oxidative metabolism of xenobiotics is often the first step in biotransformation. Hepatic microsomal oxygenases, in both mammalian and non-mammalian groups, are the dominant catalysts for oxidative transformation of xenobiotics (Lake and Gangoli 1981; James 1986). This enzyme system is regulated by various environmental and biological variables, and by chemical compounds known as inducers. 3-Methylcholanthrene (3MC) is a potent inducer of hepatic microsomal oxygenases in mammals and fish (Poland and Glover 1974; Addision et al., 1978). The degree of induction depends on the concentration of 3MC in the blood/liver of the animal as well as on the time of exposure. Administration of 3MC increases the contents of the 7-ethoxycoumarin O-deethylase (ECOD) and 7-ethoxyresorufin O-deethylase (EROD) enzymes, and also the content of cytochrome P-450 in channel catfish (Lech et al., 1982; Ankley et al., 1987; Tate 1988).

Pharmacokinetic modeling has been proven useful for understanding drug and toxicant dynamics in animals and can provide information that is important for toxicological hazard assessment studies (Karara and Hayton 1984; Barron et al., 1987; Karara and Hayton 1989). To date, no information describing the disposition of 3MC in either mammals or fish is available in the scientific literature. The primary objective of this study was to develop a pharmacokinetic model to describe the disposition of 3MC in channel catfish following slow intraaortic infusion. The model was developed in the course of our study of the effect of 3MC on the enzyme induction and the correlation between the pharmacokinetics and the degree of enzyme induction.

MATERIALS AND METHODS

Channel catfish were obtained locally (Franklin Catfish Growers, Wisner, LA) and transported in cool aerated water containing sodium chloride (10 g/10 L) and oxytetracycline (0.15 g/10 L). On reaching the

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laboratory, the fish were treated to control fungal growth and prevent bacterial diseases (Kulkarni and Karara 1990). They were then acclimated at 23 ± 1°C, for at least two weeks (wash out period), in a constant temperature aquarium. During this period the fish received tablet fish food.

A cannula was fitted into the dorsal aorta using a modification of the Smith and Bell (1964) procedure details of which was published previously (Kulkarni and Karara 1990).

About 24 hours after the cannulation the fish were infused with 3MC. 3MC was administered as a solution (10 mg/ml) in a mixture of water and dimethylacetamide (10:90) at a rate of 200 µl/h for a specific period of 2.2 to 2.7 hours, depending on the weight of the fish, to deliver a dose of 10 mg/kg body weight. At the end of the infusion the cannula was flushed several times with heparinized saline to remove any trace of 3MC. Blood samples (350 µl) were collected at various time intervals and placed into microcentrifuge tubes. No more than 4 samples were withdrawn from each fish. Blood samples were centrifuged immediately and the plasma was separated. Plasma samples were stored at -4°C until analyzed for 3MC by an HPLC method (Choudhury et al., 1990). During the experiment 50% of the exposed water of the tank was replaced with fresh water every 12 hours.

The pharmacokinetics of 3MC were determined following the method of Loo and Reigelman (1970). According to this method, the plasma concentration-postinfusion time curve, after intravenous infusion, is describable by Equation 1:

$$(C_p)_{post} = \sum_{i=1}^{n} R_i e^{-k_i t'} \qquad (1)$$

where $(C_p)_{post}$ represents the postinfusion plasma concentration, t' is the postinfusion time, R_i is the intercept at postinfusion zero-time, k_i is the corresponding first-order rate constant and n is the number of compartments required to describe the compartmental model. The relationship between zero-time intercept after i.v. bolus administration (A_i) and the postinfusion zero-time intercept after i.v. infusion (R_i) of the same dose can be described by Equation 2:

$$A_i = \frac{k_i \cdot \tau}{(1 - e^{-k_i \tau})} R_i \qquad (2)$$

where τ is the time of infusion. Since it is possible to

determine R_i form Equation 1, the pharmacokinetic parameters after intravenous bolus administration may be obtained from Equation 2 without really giving the bolus dose.

The mean plasma concentration-postinfusion time course indicated that channel catfish could be best modeled as two compartment open model(Figure 1), a central

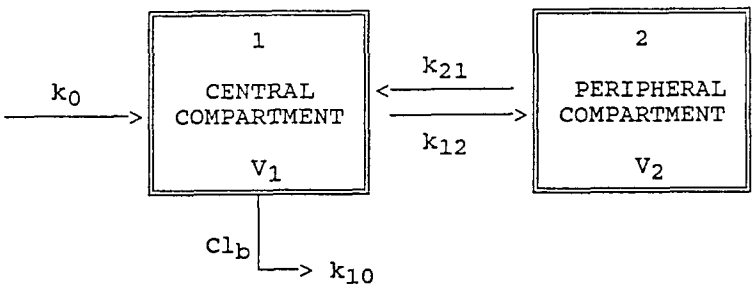

Figure 1. A two-compartment open model for 3MC in catfish

compartment and a peripheral compartment. In the model, V_1 and V_2 are the apparent volume of distribution of central and peripheral compartments, respectively; k_{12} and k_{21} are the intercompartmental first-order transfer rate constants between central and peripheral compartments, respectively; and k_{10} is the overall first-order elimination rate constant of 3MC from central compartment. Curve fitting of the data was performed by using a nonlinear least squares regression computer program, PCNONLIN, (Statistical Consultants Inc., Lexington, KY). Thus the following biexponential function (Equation 3) was fitted to the plasma concentration-postinfusion time data (mean of six fish) using PCNONLIN:

$$C_p = A_1 \cdot e^{-k_1 \cdot t} + A_2 \cdot e^{-k_2 \cdot t} \qquad (3)$$

where R_1 and R_2 are the coefficients of distribution and k_1 and k_2 are the hybrid rate constants. The coefficients and hybrid rate constants in Equation 3 can be expressed in terms of the model parameters (Gibaldi and Perrier 1982).

RESULTS AND DISCUSSION

Following intraarterial infusion of 3MC, the plasma concentration-postinfusion time course showed a biexponential decline (Figure 2). Attempts were made to fit the data to two and three compartment based models.

The partial F test (Boxenbaum et al. 1974), the Akaike's Information Criterion (AIC) (Yamaoka et al. 1978), and the coefficient of determination (R^2) between observed and predicted plasma concentration of 3MC indicated that a two compartment model resulted in a significantly

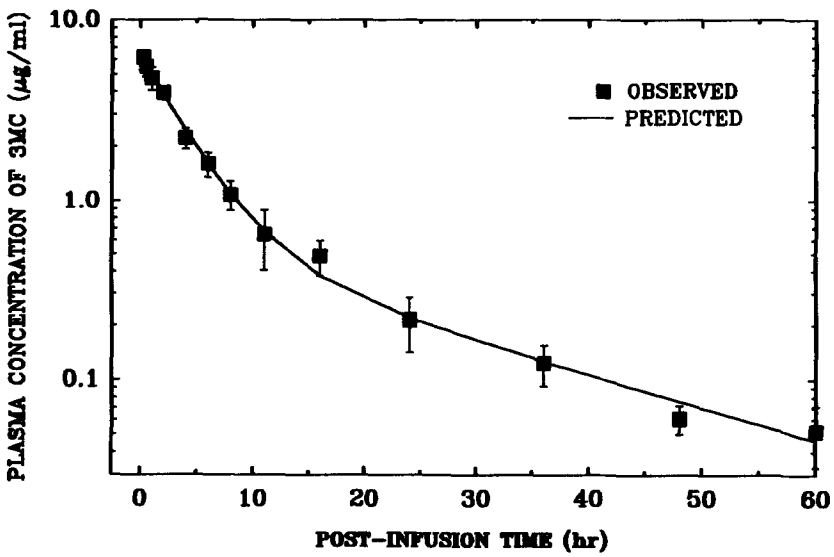

Figure 2. Plasma concentration of 3MC in catfish as a function of time after infusion of 10 mg/kg body weight. Each point is the mean ± SD of data from six fish.

better fit compared to a three compartment model. The model predicted parameters are presented in Table 1. The parameters had small standard error values indicating greater confidence in the obtained estimates and the suitability of the proposed compartmental model. The parameters obtained after compartmental analysis of the postinfusion data were converted using Equation 2 into the parameters as if obtained from a single intravenous bolus injection. The derived pharmacokinetic parameters are listed in Table 2.

The terminal half-life ($t_{½\beta}$), the volume of distribution of the central compartment (V_1), of the peripheral compartment (V_2) and also at steady state (V_{ss}), and the whole body clearance (Cl_b) of 3MC in catfish were calculated using the following equations:

$$t_{1/2\beta} = \frac{0.693}{k_2} \quad (4)$$

$$V_1 = \frac{dose}{A_1 + A_2} \quad (5)$$

$$V_2 = \frac{k_{12}}{k_{21}} V_1 \quad (6)$$

$$V_{ss} = V_1 + V_2 \quad (7)$$

$$Cl_b = k_{10} V_1 \quad (8)$$

Table 1. Model predicted pharmacokinetic parameters for 3MC in catfish.

Parameter	Least Square Estimates
R_1, μg/ml	5.744 (0.117)[a]
R_2, μg/ml	0.586 (0.107)
k_1, hr^{-1}	0.267 (0.0112)
k_2, hr^{-1}	0.0426 (0.0054)

[a]Standard Error of Mean (n = 6)

Table 2. Derived pharmacokinetic parameters for 3MC in catfish.

Parameter	Derived Value
A_1, μg/ml	7.87
A_2, μg/ml	0.619
k_{10}, hr^{-1}	0.193
k_{12}, hr^{-1}	0.0577
k_{21}, hr^{-1}	0.0589
V_1, ml/kg	1178.4
V_2, ml/kg	1154.4
V_{ss}, ml/kg	2332.8
Cl_b, ml/hr/kg	227.4
$t_{(½)\beta}$, hr	16.26

In case of a two-compartment system, as is the case here, the larger the ratio of the zero-time intercepts (A_1/A_2) following intravenous bolus injection the more readily one can distinguish the multicompartment characteristics of the time-course profile. As A_1 approaches zero, the ratio A_1/A_2 approaches zero and the plasma concentration-time curve becomes monoexponential (Gibaldi and Perrier, 1982). On the other hand, if A_1 is exceedingly large relative to A_2, the plasma concentration-time curve may again appear to reflect a one-compartment model. The ratio of zero-time intercepts R_1/R_2 after intraaortic infusion of 3MC was 9.83. As this value is not too large or too small, use of intraaortic infusion to determine pharmacokinetic parameters of 3MC was justified and two phases of distribution had been observed distinctly.

Compartmental pharmacokinetic analysis of the data indicated that the channel catfish could be modeled into two compartments (Figure 1). The central compartment is assumed to consist of the blood pool and highly perfused tissues such as the liver and the peripheral compartment is assumed to consist of poorly perfused tissues such as the adipose tissue, the muscle and the skin. In this study, during the earlier time samples there was a rapid loss of 3MC from the blood and although no other tissues were sampled, the initial phase appears to reflect distribution to tissues outside the blood pool.

Presently, there are no reports in the scientific literature on <u>in vivo</u> pharmacokinetic studies of 3MC in any species. Tierrney et al. (1978) investigated the metabolism of 3MC both by hepatic microsomal fractions from rats pretreated with 3MC or untreated. They observed that 3MC-pretreated rats metabolized 3MC faster and to a greater extent than untreated rats. This indicates that 3MC may be appreciably metabolized *in vivo* in catfish and may have a shorter elimination half-life. In fact, the half-life of 3MC (16.3 hr) in catfish was almost one-fourth of that of PCB (Kulkarni and Karara 1990). This may be possible because of the high metabolism of 3MC in catfish due to autoinduction.

The values for the apparent volume of distribution of the central and peripheral compartments are 1178.4 and 1154.4 ml/kg body weight, respectively. These values reflect the capacity of each compartment to hold or accumulate 3MC, which is approximately the same for both the compartments. This indicates that 3MC has the same affinity for both compartments.

Polycyclic aromatic hydrocarbons have a high affinity for liver cells (Abraham et al., 1988). The liver, being the

well perfused tissue, is considered as part of the central compartment and this may be one of the reasons for the relatively large volume of distribution of the central compartment for 3MC in catfish. PCBs, which have high binding affinity to adipose tissues, have a volume of distribution of 1000 ml/kg for the adipose tissues compartment in catfish (Kulkarni and Karara 1990).

In the pharmacokinetic compartment model, elimination was assumed to take place from the central compartment. The total body clearance of 3MC in catfish was found to be 227.4 ml/hr/kg body weight. The high total body clearance indicated the ability of the eliminating organs, mainly the liver and kidney, to efficiently clear the body of 3MC through metabolism and excretion. In contrast, the total body clearance of PCBs, which are resistant to metabolism, was only 19.7 ml/hr/kg in catfish (Kulkarni and Karara 1990). The intercompartment clearances (Cl_1 and Cl_2) were also almost equal indicating a quick equilibration between the two compartments.

The model described in this study provides an understanding of the mechanisms involved in the accumulation and disposition of 3MC in the channel catfish. Such an approach may be useful in making predictions regarding persistence and toxicity of 3MC in fish.

Acknowledgement. The authors would like to acknowledge the support received from the U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

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Received February 21, 1993; accepted May 10, 1993.