METHODS

New Specific Marker of Cytochrome P450 1A2 Activity

A. A. Filimonova, L. E. Ziganshina, A. U. Ziganshin*, and A. A. Chichirov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 12, pp. 712-714, December, 2010
Original article submitted December 22, 2009.

Various methods were proposed for phenotyping of patients by activity of cytochrome P450 1A2, each has some advantages and disadvantages. However, no reference parameters were developed for measuring CYP1A2 activity that could be used as a unified standard for phenotyping of patients. We propose a mathematic model of caffeine metabolism allowing calculation of rate constants for the formation of its primary metabolites. First-order rate constant of paraxanthine formation was tested as a new specific marker of isoenzyme 1A2 in healthy volunteers.

Key Words: *caffeine; cytochrome P450 isoenzyme 1A2; metabolism; phenotyping*

Standard methods of studying metabolism include measurements of the concentrations of the test substrate, *i.e.* model substance primarily metabolized by the test isoenzyme and its metabolites in a biological material (plasma, urine, saliva, exhaled air, *etc.*) [3,5,8,11]. Caffeine is the test substrate for cytochrome P450 1A2 (CYP1A2). In caffeine metabolism (1,3,7-trimethyl xanthine, 1,3,7X), CYP1A2 primarily participates in 3N-demethylation of caffeine to paraxanthine [2,6]. Theobromine and theophylline are mainly formed by CYP2E1 [7]. Isoenzyme 3A4 metabolizes caffeine to 1,3,7-trimethyluric acid [15].

Methods of measuring CYP1A2 activity with caffeine as the substrate can be divided into two large groups. The first group includes calculation of caffeine elimination constant and all related pharmacological parameters (caffeine clearance, half-elimination period, area under pharmacokinetic curve, *etc.*) [3,8,10]. These parameters reflect all processes of caffeine elimination, including caffeine metabolism, inter-compartment exchange, and excretion in unchanged form. For precise phenotyping by CYP1A2 activity, the reaction of paraxanthine formation from caffeine should be singled out from the entirety of caffeine biotransformation reactions. Therefore, simple description of caffeine pharmacokinetic parameters cannot specifically reflect CYP1A2 activity.

Another method of phenotyping by CYP1A2 activity is calculation of concentration ratios for metabolite of the paraxanthine pathway [2,12,13]. The main drawback of this method is that not all products of paraxanthine metabolism are taken into account. It should be also noted that this parameter depends on the rates of formation and elimination of paraxanthine and its metabolites, *i.e.* is a complex integral parameter.

Analysis of published data showed that there is no consensus about the choice of the parameter for describing CYP1A2 activity.

The aim of the present study was to develop and test the mathematic model of *in vivo* pharmacokinetics of caffeine after its extravascular administration with

Department of Clinical Pharmacology and Pharmacotherapy, Kazan' State Medical Academy; *Department of Pharmacology, Pharmaceutical Faculty, Kazan' State Medical University; **Department of Chemistry, Kazan' State Power Engineering University, Russia. ***Address for correspondence:*** aachichirova@mail.ru. A. A. Chichirov

0007-4888/11/1506-0762

calculation of a criterion explicitly related to CYP1A2 activity.

MATERIALS AND METHODS

We examined 96 healthy volunteers (58 women and 38 men, mean age 36.4±16.7 years, mean body weight 68.8±15.8 kg), citizens of Kazan. Of them, 18 individuals (5 women and 13 men) were smokers, 11 women took oral contraceptives (including 2 smokers).

The samples for HPLC were collected and HPLC was carried out as described previously [1].

Comparison of the empirical distribution with theoretical one was performed using Kolmogorov–Smirnov test and χ^2 test (Statistica 7.0).

RESULTS

We used a scheme of caffeine metabolism in the form of a set of successive and parallel first-order reactions in a two-compartment model with absorption and singled out the reactions of paraxanthine formation and metabolism (Fig. 1).

The paraxanthine formation constant ($k_{1,7X}$) characterizes the reaction of paraxanthine formation from caffeine (Fig. 1), *i.e.* is a specific marker of CYP1A2 activity.

For calculation of first-order rate constant for paraxanthine formation from caffeine we solved a system of differential equations describing the kinetics of successive reactions of paraxanthine formation.

In general, the kinetics of caffeine metabolism is described by a system of first-order differential equations:

$$\frac{d[1,3,7X(0)]}{dt} = -k_{01}[1,3,7X(0)]; \quad \textbf{(1)}$$

$$\frac{d[1,3,7X(1)]}{dt} = k_{01}[1,3,7X(0)] - k_{1,7X}[1,3,7X(1)] - k_{el1,3,7X}[1,3,7X(1)] - k_{12}[1,3,7X(1)] + k_{21}[1,3,7X(2)]. \quad \textbf{(2)}$$

The kinetics of a fragment of the paraxanthine pathway of caffeine metabolism is described by an equation (**3**):

$$\frac{d[1,7X]}{dt} = k_{1,7X}[1,3,7X(1)] - k_{el1,7X}[1,7X]. \quad \textbf{(3)}$$

Solution of system **(1)-(3)** yields complex transcendental equations that cannot be solved by analytical methods. Therefore we calculated only the kinetic parameters of the reactions of paraxanthine formation and elimination.

If $k_{1,7X} \neq k_{el1,7X}$, integration of equation **(3)** at t=0: [1,3,7X(1)]=0, [1,7X]=0 yields:

$$\frac{[1,7X]}{[1,3,7X(1)]} = \frac{k_{1,7X}}{k_{el1,7X} - k_{1,7X}}(1 - e^{(k_{1,7X} - kel_{1,7X})t}). \quad \textbf{(4)}$$

Numerical solution of equation **(4)** for experimental concentrations [1,7X] and [1,3,7X(1)] makes it possible to directly find $k_{1,7X}$.

As is seen from equation **(4)**, the paraxanthine to caffeine concentration ratio is a variable that depends exponentially on the time and individual peculiarities of metabolism ($k_{1,7X}$ and $k_{el1,7X}$).

Analysis of caffeine pharmacokinetics in healthy volunteers revealed lognormal distribution by $k_{1,7X}$. In light of this, first-order rate constant $pk_{1,7X}$ equal to $-lnk_{1,7X}$ was chosen as the criterion of caffeine metabolism by the paraxanthine pathway, *i.e.* CYP1A2 activity ($pk_{1,7X}$ is positive due to minus sign before $lnk_{1,7X}$).

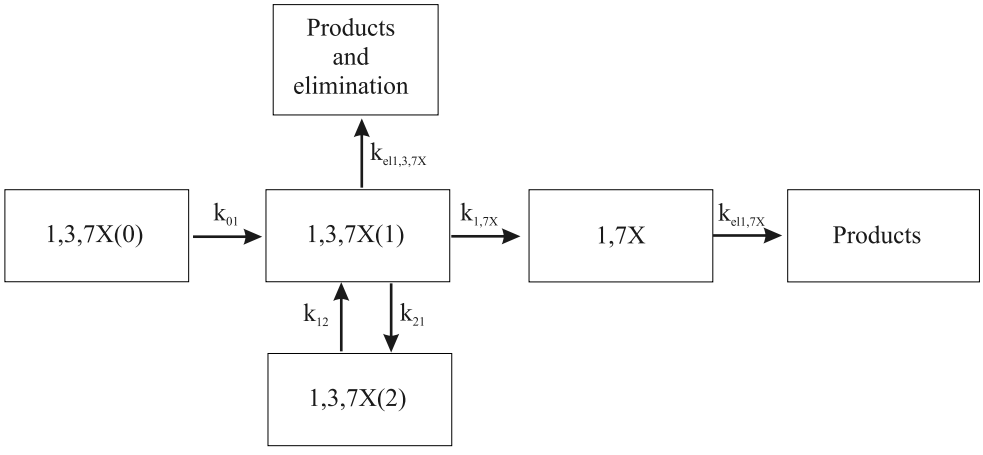

Fig. 1. Scheme of two-compartment model of caffeine pharmacokinetics after its extravascular administration. 1,3,7X(0): instant caffeine concentration at the site of injection, 1,3,7X(1): caffeine concentration in the central compartment (blood and tissues rapidly exchanging with plasma), 1,3,7X(2): in peripheral compartment, 1,7X: paraxanthine; k_{01}: caffeine absorption constant, $k_{el1,3,7X}$: sum of rate constants of parallel reactions of caffeine elimination except paraxanthine formation ($k_{1,7X}$) and intercompartment exchange (k_{12} and k_{21}); $k_{el1,7X}$ sum of all rate constants of all parallel reactions of paraxanthine elimination.

The higher is $pk_{1,7X}$, the lower is the rate of metabolic transformation. Normal distribution of healthy donors by the negative logarithm of paraxanthine formation rate constant was observed (Fig. 2).

Published reports on phenotyping of different populations by CYP1A2 activity demonstrate different variants of distributions: normal or lognormal [4], unimodal [14], bimodal and trimodal [9]. These differences are related to the absence of reference parameters for measuring CYP1A2 activity.

New method for calculation of first-order rate constant for the reaction of paraxanthine formation from caffeine for evaluation of specific activity of CYP1A2 is correct from mathematical point of view, because it characterizes activity of this particular isoenzyme. Measurement of the concentrations of all subsequent paraxanthine metabolites is obviously unnecessary. An important advantage of the proposed method is the fact that rate constant does not depend on the initial caffeine concentration, *i.e.* we can compare the data from different patients without concern about improper caffeine intake. The proposed algorithm of measuring CYP1A2 activity can be used for any cytochrome P450 isoenzymes.

Thus, a new specific marker pf CYP1A2 activity, rate constant of paraxanthine formation, can be used as a unified standard for phenotyping of patients by isoenzyme 1A2 activity.

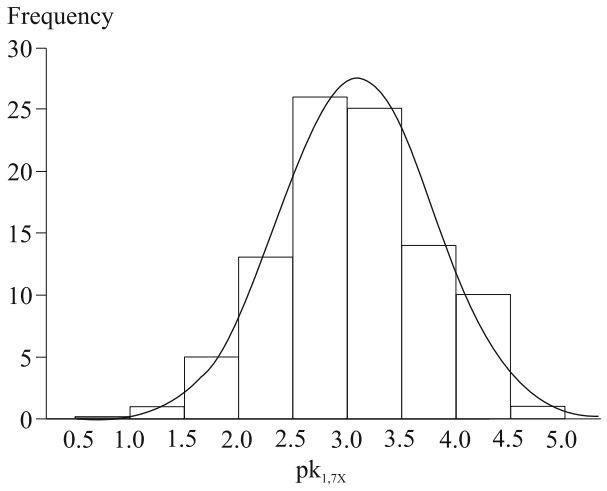

Fig. 2. Distribution of volunteers by $pk_{1,7X}$. Bars: empirical frequencies; curve: normal distribution. Kolmogorov–Smirnov test: d=0.05758, p=n.s., χ^2=1,6393, number of degrees of freedom=3, p=0.65051.

REFERENCES

1. A. A. Filimonova, *Klin. Lab. Diagn.,* No. 10, 34-36 (2008).
2. M. A. Butler, N. P. Lang, J. F. Joung, *et al., Pharmacogenetics,* **2**, No. 3, 116-127 (1992).
3. J. A. Carrillo, M. Christensen, S. I. Ramos, *et al., Ther. Drug. Monit.,* **22**, No. 4, 409-417 (2000).
5. L. M. Christensen, *Clin. Pharmacol. Ther.,* **74**, No. 2, 144 (2003).
4. A. Catteau, Y. C. Bechtel, N. Poisson, *et al., Eur. J. Clin. Pharmacol.,* **47**, 423-430 (1995).
6. M. L. Dahl, *Clin. Pharmacokinet.,* **41**, No. 7, 453-470 (2002).
7. W. A. Daniel, M. Kot, and J. Wojcikowski, *Pol. J. Pharmacol.,* **55,** No. 6, 1045-1053 (2003).
8. M. S. Faber, A. Jetter, and U. Fuhr, *Basic Clin. Pharmacol. Toxicol.,* **97**, No. 3, 125-134 (2005).
9. U. Fuhr and K. L. Rost, *Pharmacogenetics*, **4**, No. 3, 109-116 (1994).
10. J. Jodynis-Liebert, J. Flieger, A. Matuszewska, and J. Juszczyk, *J. Clin. Pharmacol.,* **44**, No. 4, 338-347 (2004).
11. A. C. Parker, P. Pritchard, T. Preston, and I. Choonara, *Br. J. Clin. Pharmacol.,* **45**, No. 2, 176-178 (1998).
12. K. L. Shirley, Y. Y. Hon, S. R. Penzar, *et al., Neuropsychopharmacology*, **28**, No. 5, 961-966 (2003).
13. B. Sinués, M. A. Sáenz, J. Lanuza, *et al., Cancer Epidemiol. Biomarkers Prev*., **8**, No. 2, 159-166 (1999).
14. B. K. Tang, Y. Zhou, D. Kadar, and W. Kalow, *Pharmacogenetics,* **4**, No. 3, 117-124 (1994).
15. W. Tassaneeyakul, D. J. Birkett, M. E. McManus, *et al., Biochem. Pharmacol*., **47**, No. 10, 1767-1776 (1994).