Activity of Cytochrome P450 in Children

V. A. Vavilin, E. G. Shchepotina, N. A. Manankin*, L. F. Kaznacheeva**, and V. V. Lyakhovich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 9, pp. 272-274, September, 2004
Original article submitted April 26, 2004

The 6β-hydroxycortisol/cortisol ratio was measured in 52 children aging 1.1-14.0 years. The maximum increment in this ratio occurred in the age interval of 1.1-2.0 years. During this period, the regression coefficients in the linear (r=0.57; p=0.044) and nonlinear logarithmic models (r=0.56; p=0.049) were similar. At the age of 10-14 years, the examined ratio attained 19.17±17.79.

Key Words: *cytochrome P450 3A; activity; age-related changes; polymorphism*

In living organisms, xenobiotics (*e.g.* drugs) undergo biotransformation by multiple isoforms of cytochrome P450 and phase II enzymes [5]. In this variety, the cytochrome P450 3A (CYP3A) subfamily, whose members CYP3A4, 3A5, 3A7, and 3A43 are presented in humans, is of particular importance due to their involvement in the metabolism of about 50% drugs with established metabolic pathways [4] and due to their pronounced content (about 30% of the total hepatic P450 cytochrome content) [2]. During human ontogeny, activity of xenobiotic metabolism changes in a wide range, which affects the efficiency of drug therapy and tolerance to the toxic action of xenobiotics [6]. Our aim was to assess activity of CYP3A in children by 6β-hydroxycortisol/cortisol (6β-OHCl/Cl) ratio, since 6-hydroxylation of cortisol is highly specific for cytochromes of this subfamily [14].

MATERIALS AND METHODS

We examined 52 patients admitted into First Municipal Children Hospital for acute medical drug intoxication. CYP3A activity was determined by 6β-OHCl/Cl ratio measured in nocturnal urine during one-month follow-up after discharge from the hospital, since the episode of drug intoxication and the following drug therapy could distort the constitutive level of enzyme activity. The advantage of measuring 6β-OHCl/Cl ratio in comparison with assessment of 6β-OHCl activity alone can be explained by the fact that this ratio does not depend on circadian rhythms of glucocorticoids [1], but is sensitive to processes modulating P450 3A activity [3]. Cortisol and its metabolite were extracted from 1 ml urine diluted with ethyl acetate-ether mixture (4:1, 5 ml) as described elsewhere [10]. Methyl-prednisolone was used as an internal standard. Solid residue was dissolved in 50 μl ethanol (50%) and applied to a column (20 μl). Gradient reverse-phase high-performance liquid chromatography was carried out in Altex chromatograph equipped with Nucleosil C_{18} analytic column (5 μ, 250×2 mm). During the first 7 minutes, 16% buffer B was eluted. In the following 20 minutes, the percentage of buffer B was increased to 70, after which the elution proceeded for the next 18 minutes. Starting from minute 45, the system returned to the initial conditions over 10 minutes, and the column was equilibrated for the following 15 minutes. The elution rate was 0.2 ml/min. Buffer A consisted of 10 mM KH_2PO_4, 10 mM CH_3COOH, 10% acetonitrile, pH 4.4. Buffer B consisted of 50% (v/v) water solution of acetonitrile. The samples were analyzed on Spectromonitor 3100 using ultraviolet absorbance at 254 nm. The data were processed statistically using Statistica 5.0 and SPSS 9.0 software.

Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences; *First Municipal Children Hospital; **State Medical Academy, Novosibirsk. ***Address for correspondence:*** drugmet@soramn.ru. V. A. Vavilin

0007-4888/04/1383-0240

RESULTS

In children, the distribution of measured 6β-OHCl/Cl ratio was polymorphous (Fig. 1). The histogram revealed three modes corresponding to low, medium, and high values of 6β-OHCl/Cl ratio or, in other words, to slow, intermediate, and rapid metabolizers. The boundaries between these groups were determined by antimodes corresponding to the minimum values of the examined ratio: ≤12.5, >12.5 and ≤32.5, and >32.5 for slow, intermediate, and rapid metabolizers, respectively. The distribution of 6β-OHCl/Cl ratio differed significantly from the normal one according to Lilly-force ($p<0.01$) and Shapiro—Wilk ($p<0.001$) tests. This is also corroborated by other statistical indices: large variability of the examined ratio in the range from 0.276 to 85.000, close values of the mean and standard deviation ($M\pm SD$=18.79±19.86), asymmetry of distribution (1.65±0.33), and kurtosis (2.499± 0.650).

Comparison of the mean values of 6β-OHCl/Cl ratio in boys ($M\pm SD$=25.33±27.38) and girls ($M\pm SD$= 14.28±13.48) revealed no significant difference (p=0.069), therefore the analysis of the age-related changes in CYP3A activity was carried out for the whole group (Fig. 2). To analyze the effect of age on CYP3A activity, the group was divided into several age intervals (Table 1, Fig. 2). The minimum and maximum mean values were observed in the intervals of 1.1-1.5 and 10-14 years, respectively. However, the difference between these groups was insignificant due to small number of patients in the young group. Comparison of other age interval with the oldest group revealed no differences. The greatest difference in 6β-OHCl/Cl ratio (almost 6-fold) was observed between the intervals of 1.1-1.5 and 1.5-2.0 years. The mean value of 6β-OHCl/Cl ratio in the interval of 1.5-2.0 years was 76.6% of the corresponding ratio in the interval of 10-14 years. According to published data, CYP3A activity in children measured at the end of the first year of life attains 50% of the corresponding value in the adult persons [2]. By contrast, our data showed that this moment takes place a half-year later. The results of quantitative immunochemical assessment of CYP3A4 content in liver microsomes are contradictory. According to [9], it attains the adult level to the age of 4 years. During the first year of life, CYP3A4 content increases significantly, but remains below the adult level for 15 years [13].

The increase in activity during 1.5-2.0 year interval surpassed the increment during the following 12 years (Table 1). This dynamics of 6β-OHCl/Cl ratio necessitates analysis of the age-dependence of this ratio using linear and nonlinear (logarithmic) regression models in various age intervals. Only in the interval of 1-2 year, the regression coefficients and their significance were similar in the linear and logarithmic models, and the age-dependence was significant (Ta-

TABLE 1. Descriptive Statistics of 6β-OHCl/Cl Ratio in Children of Various Ages ($M\pm SD$)

Age interval, years	6β-OHCl/Cl	p
1.1-1.5 (n=4)	2.49±2.38	0.086
>1.5 and ≤2.0 (n=9)	14.66±12.56	0.506
>2 and ≤3 (n=11)	15.5±12.5	0.557
>3 and ≤5 (n=7)	13.80±8.67	0.458
>1 and ≤5 (n=31)	13.19±11.3	0.47
>10 and ≤14 (n=18)	19.15±17.79	
Total group (n=49)	15.38±14.15	

Note. Parameter p was calculated by comparing the data to the values obtained in 10-14-year age interval.

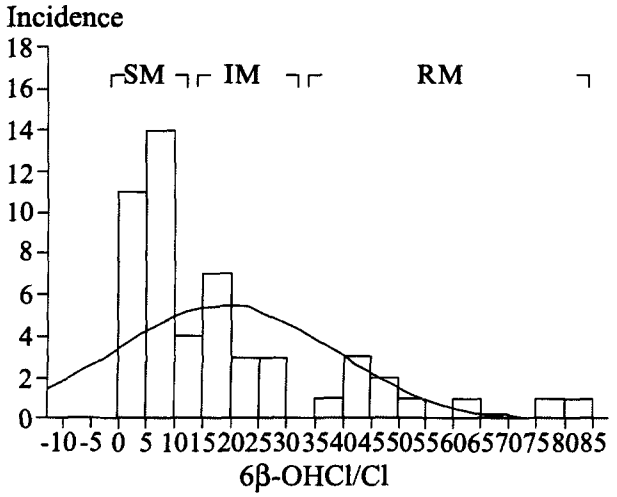

Fig. 1. Amplitude distribution of 6β-OHCl/Cl ratio. The curve describes the corresponding normal distribution. SM, IM, and RM mark slow, intermediate, and rapid metabolizers, respectively.

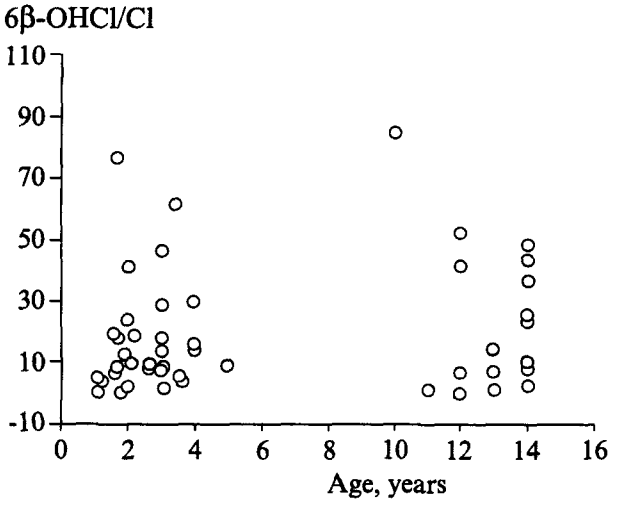

Fig. 2. Distribution of 6β-OHCl/Cl ratio as a function of age.

TABLE 2. Regression Analysis of Dependence of 6β-OHCl/Cl Ratio on Age

Age interval, years	Linear Regression		Non-linear Regression	
	equation	*r*, (*p*)	equation	*r*, (*p*)
>1 and ≤2	$y=-19.01+0.63x$	0.565 (0.044)	$y=-1.25+27.38\times lnx$	0.556 (0.049)
>1 and ≤3	$y=-0.52+6.33x$	0.359 (0.085)	$y=3.54+13.44\times lnx$	0.38 (0.062)
>1 and ≤4	$y=4.21+3.70x$	0.287 (0.131)	$y=5.46+9.51\times lnx$	0.326 (0.084)
>1 and ≤5	$y=7.012+2.38x$	0.228 (0.216)	$y=6.72+7.49\times lnx$	0.288 (0.116)
>1 and ≤14	$y=11.27+0.63x$	0.236 (0.1)	$y=9.21+4.13\times lnx$	0.263 (0.067)

Note. *y*) 6β-OHCl/Cl, *x*) age.

ble 2). The dependence of 6β-OHCl/Cl ratio on age was insignificant in all other age intervals. The nonlinear model described the dependence of 6β-OHCl/Cl ratio on age better than the liner model.

The genes of subfamily *CYP3A* (*3A4*, *3A5*, *3A7*, and *3A43*) are characterized by complex regulation, which determines marked individual peculiarities. These genes are strongly regulated by age-related factors [13], the nuclear transcriptional factors — pregnane (PXR), retinoid (RXR), constitutive androstane receptor (CAR), *etc* [11]. Molecular genetic studies revealed numerous mutations in structural and regulatory sites of genes of this subfamily; however, only few papers consider their functional manifestations [8,12]. Functionally significant *in vitro* mutations of gene PXR were found [7]. The data obtained are important for choosing safe pharmacotherapy in yearly juvenile age, because metabolic clearance of drugs in children cannot be correctly extrapolated from body weight and expression data [13]. The effect of age on CYP3A activity decreases in children older than 2 years, which attests to the possibility of using this parameter for *in vivo* assessment of functional manifestations of mutations in cytochrome genes and the genes of their regulator factors.

REFERENCES

1. T. Bienvenu, E. Rey, G. Pons, *et al.*, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **29**, No. 11, 79-85 (1991).
2. S. N. de Wildt, G. L. Kearns, J. S. Leeder, J. N. van der Anker, *Clin. Pharmacokinet*, **37**, No. 6, 485-505 (1999).
3. C. Ged, J. M. Rouillon, L. Pichard, *et al.*, *Br. J. Clin. Pharmacol.*, **28**, No. 4, 373-387 (1989).
4. F. P. Guengerich, *Annu. Rev. Pharmacol. Toxicol.*, **39**, 1-17 (1999).
5. F. P. Guengerich, *Mol. Interv.*, **3**, No. 4, 194-204 (2003).
6. R. N. Hines and D. G. McCarver, *J. Pharmacol. Exp. Ther.*, **300**, No. 2, 355-360 (2002).
7. E. Hustert, A. Zibat, E. Presecan-Siedel, *et al.*, *Drug. Metab. Dispos.*, **29**, No. 11, 1454-1459 (2001).
8. P. Kuehl, P. Zhang, Y. Lin, *et al.*, *Nat. Genet.*, **27**, No. 4, 383-391 (2001).
9. D. Lacroix, M. Sonnier, A. Moncion, *et al.*, *Eur. J. Biochem.*, **247**, No. 2, 625-634 (1997).
10. J. Lykkesfeldt, S. Loft, and H. E. Poulsen, *J. Chromatogr. B. Biomed. Appl.*, **660**, No. 1, 23-29 (1994).
11. L. C. Quattrochi and P. S. Guzelian, *Drug Metab. Dispos.*, **29**, No. 5, 615-622 (2001).
12. G. R. Robertson, J. Field, B. Goodwin, *et al.*, *Mol. Pharmacol.*, **64**, No. 1, 42-50 (2003).
13. J. C. Stevens, R. N. Hines, C. Gu, *et al.*, *Drug. Metab. Dispos.*, **307**, No. 2, 573-582 (2003).
14. P. B. Watkins, *Pharmacogenetics*, **4**, No. 4, 171-184 (1994).