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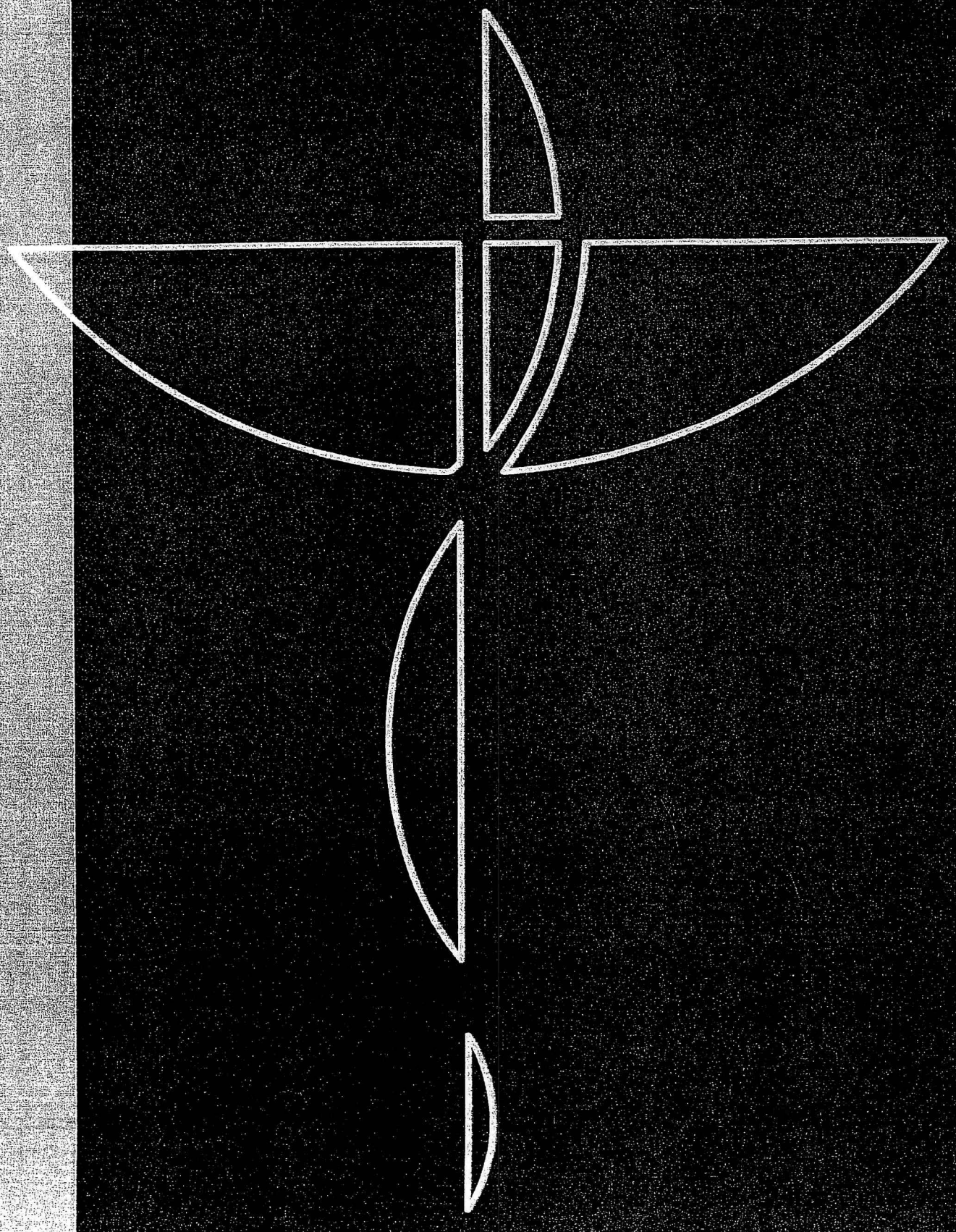

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CYP2C9 GENOTYPE AND PHARMACOKINETICS OF LOSARTAN AND ITS METABOLITE E-3174

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Abstract

Objective: Comparison of pharmacokinetic properties of losartan and its metabolite E-3174 in healthy subjects in relation to CYP2C9 genotype (phenotype status).

Methods: A pharmacokinetic study of 9 extensive (EM) and 4 intermediate (IM) metabolisers using losartan at a dose of 100 mg was conducted. In a group of 124 healthy Macedonian volunteers, genotypes of these subjects were obtained by determining allelic variants. CYP2C9 genotype was determined by PCR technique.

Results: By comparing the pharmacokinetic parameters (Cmax, AUC_{0-inf}) for losartan and its metabolite E3174, a difference was found in the metabolism of the drug in extensive and intermediate metabolisers.

Conclusion: CYP2C9 genotype is the main factor which affects the conversion of losartan to E-3174, carboxylic acid metabolite. The results suggest differences in the pharmacokinetics of losartan and its pharmacologically active metabolite E-3174 in healthy subjects with different genotypes. These findings have presented a difference between groups of subjects with genotype CYP2C9*1/*1 and CYP2C9*1/*2*, *1/*3,*2/3*.

Keywords: CYP2C9, polymorphism, losartan, E-3174, pharmacokinetics

CYP2C9 ГЕНОТИПОТ И ФАРМАКОКИНЕТИКАТА НА ЛОСАРТАН И НЕГОВИОТ МЕТАБОЛИТ Е-3174

Апстракт

Цел: Споредба на фармакокинетските карактеристики на лосартан и неговиот метаболит Е-3174 кај здрави субјекти во поврзаност со CYP2C9 генотипот (фенотипскиот статус).

Методи: Изведена е фармакокинетска студија на 9 екстензивни (EM) и 4 (IM) метаболизери со примена на лосартан во доза од 100 mg. Генотипот на овие субјекти е добиен по одредувањето на алеличните варијанти кај група од 124 здрави Македонски доброволци, каде CYP2C9 генотипот е одреден со PCR техника.

Резултати: Со споредување на фармакокинетските параметри (C_{max}, AUC_{0-inf}) за лосартан и неговиот метаболит Е3174 добиени од фармакокинетска студија која е изведена на Институтот, најдено е дека постои разлика во метаболизирањето на лекот кај екстензивните и интермедиерните метаболизери.

Заклучок: CYP2C9 генотипот е главниот фактор кој има влијание врз претворбата на лосартан во Е-3174, карбоксиличниот киселински метаболит. Добиените резултати укажуваат на разлики во фармакокинетиката на лосартан и неговиот фармаколошки активен

метаболит Е-3174 кај здрави субјекти со различни генотипови. Наодите индицираат на постоење на разлика помеѓу групите на субјекти со генотип *CYP2C9*1*1* и *CYP2C9*1/*2, *1/*3, *2/*3*.

Клучни зборови: CYP2C9, полиморфизам, лосартан, Е-3174, фармакокинетика

Introduction

Losartan is a selective antagonist of the angiotensin II receptor that is used in treatment of hypertension and heart failure (Timmermans et al., 1993). It is subject to CYP-mediated oxidative metabolism (Stearns RA.et al., 1995; Dzun CH. Et al., 1995). About 14% of the dose of losartan is metabolized into E-3174, an active metabolite of losartan which significantly contributes to the antihypertensive effect of losartan, a longer elimination half-life and greater AUC (Lo et al., 1995; Ohtava et al., 1993). E-3174 is created by *CYP2C9* and *CYP3A4* in vitro.

Aim

The aim of this study was to examine the influence of polymorphisms of *CYP2C9* (through allelic variants **1, *2, *3*) on the pharmacokinetics of losartan and E-3174 metabolite by comparing the results of pharmacokinetic study in 13 healthy subjects.

Materials and methods

Subjects

Data from 13 healthy subjects who participated in the pharmacokinetic study performed in 2005 using the dose of 100 mg losartan were analyzed. The subjects have signed the informed consent for participation in the study, which was approved by the Ethics Committee of the Medical Faculty. The study was performed in accordance with the Declaration of Helsinki.

As a segment of an extensive examination (doctoral thesis), blood samples (3 ml) from healthy subjects (n = 124) were taken, which were used for genotyping of the most common polymorphic allelic variants. The study has been approved by the Scientific Committee of the Medical Faculty. The subjects have signed informed consent for genotyping.

Tests for genotyping

Blood samples were collected after signing the informed consent. DNA was isolated from peripheral blood and polymorphic alleles of *CYP2C9 (*1, *2, *3)* were determined. Genotyping was performed at the Institute of Immunology and Human Genetics, Medical Faculty with commercially available kits (GeneID GmbH, Strasberg, Germany, AID Diagnostica), which are based on PCR (polymerase chain reaction) with consequent hybridization.

Method for determination of losartan and E-3174 in plasma

The method used to determine losartan and its active metabolite E-3174 in plasma was developed and validated at the Department of Toxicology of Drugs at the Institute of Physiology of the Bulgarian Academy, which is based on a modified procedure described by Williamson et al. (1998).

A small amount of plasma was stirred after thawing, with a small amount of internal standard and double volume of MTBE (tert-Methyl butyl ether). Samples were shaken

for 30 seconds, centrifuged for 5 minutes at 3500 rpm. After centrifuging, the organic phase was transferred into clean tubes containing sodium hydroxide reversible extraction.
Equal amount of 80% 0.2 M phosphoric acid was added and the sample was transferred into the auto sample vial. The vial was briefly stirred and 100 µl of the aqueous phase was injected into the HPLC.
Plasma content of losartan and E-3174 metabolite in control and unknown samples were determined using the method of internal standard with calibration curves of chromatographic peaks and comparing them with known amounts of losartan and metabolite according to the internal standard.

Pharmacokinetic analysis
Pharmacokinetic parameters of losartan and E-3174 were assessed with non-compartment methods in the program Kinetica, ver. 5.0. All parameters, $AUC_{0\text{-}INF}$ (area under the curve) for plasma concentrations, K_{el}, C_{max}, T_{max}, $T_{1/2}$, were obtained from the program, while CL/F (oral clearance) was obtained by the mathematical formula CL/F = dose/$AUC_{0\text{-}INF}$.

Results

Calculated (obtained) allele frequencies of *CYP2C9 (*1, *2* and **3*) in tested population of the Republic of Macedonia are given in Table 1. Frequency distribution of genotypes did not deviate from Hardy-Weinberg-'s equilibrium (Guo, Sw; Thompson, Ea. 1992). *CYP2C9*1* was the most common allele with a frequency of 0831, while **2* had a frequency of 0.101; consequently **3* had a frequency of 0.068.

Table 1 Frequency of allelic *CYP2C9* variants in a particular group of tested subjects (n = 124)

Allele	n (124)	Frequency
**1*	103	0.831
**2*	13	0.101
**3*	8	0.068

Table 2 Distribution of subjects by genotypes (phenotypes) by allelic variants of CYP2C9

Genotype	Number of subjects	Observed frequency	Predicted phenotype
*CYP2C9*1/*1*	85	0.685	EM
*CYP2C9*1/*2*	20	0.161	IM
*CYP2C9*1/*3*	14	0.113	IM
*CYP2C9*2/*2*	2	0.016	PM
*CYP2C9*2/*3*	3	0.024	IM

Table 2 shows subjects according to genotype, and they were divided into groups by extensive, intermediate and slow metabolisers.

Out of the 13 subjects who were included in the mentioned pharmacokinetic study, 9 were EM *(*1/*1)* and 4 were IM *(*1/*2, *1/*3, *2/*3)*. The study did not include any subject with allelic variant **2/*2* (PM).
Table 3 and 4 give the results of pharmacokinetic data obtained for losartan and its metabolite for entities subject to evaluation.

Table 3 Pharmacokinetic parameters of losartan in subjects with *CYP2C9*1/*1* and *CYP2C9*1/*2, *1/*3* and **2/*3* genotypes

Parameter	***CYP2C9*1/*1* (n=9)**	***CYP2C9*1/*2, *1/*3 и *2/*3* (n=4)**	**Significancy (p)**
C_{max} (ng/ml)	383.71±90.36	505±82.48	p<0.05
$AUC_{0\text{-}inf}$ (ng*h/ml)	791.12±99.56	1113.67±141.28	p<0.01
CL/F (l/h)	0.064±0.009	0.0454±0.005	p<0.01

Table 4 Pharmacokinetic parameters of E-3174 in subjects with *CYP2C9*1/*1* and *CYP2C9*1/*2, *1/*3* and **2/*3* genotypes

Parameter	*CYP2C9*1/*1* (n=9)	*CYP2C9*1/*2, *1/*3 и *2/*3* (n=4)	Significancy (p)
C_{max} (ng/ml)	606.4±71.06	518.5±37.19	p<0.05
$AUC_{0\text{-}inf}$ (ng*h/ml)	4100.25±562.102	3911.967±479.85	p>0.05
CL/F (l/h)	0.013±0.001	0.01±0.0018	p>005

For better visibility, only the parameters for losartan and its metabolite E-3174 ($AUC_{0\text{-}inf}$, C_{max}, and CL / F) were shown.
The mean value of $AUC_{0\text{-}inf}$ for losartan in extensive metabolisers was 791.12±99.56, while in the group of intermediate metabolizers it was 1113.67±141.28 ng*h/mL. The mean value of $AUC_{0\text{-}inf}$ for E-3174 in extensive metabolizers was 4100.25±562.102, while in the group of intermediate metabolisers it was 3911.967±479.85 ng*h/mL.
Mean C_{max} value for losartan in extensive metabolisers was 383.71± 90.36, while in the group of intermediate metabolizers it was 505±82.48 ng/ml. Mean C_{max} value for E-3174 in extensive metabolisers was 606.4±71.06, while in the group of intermediate metabolizers it was 518.5±37.19 ng/ml.

Discussion

*CYP2C9*2* and **3* allelic variants are found in about 12% and 7% of Caucasians. In more than 95% of the population *CYP2C9*1/*1* (~65%) (in our study 68.5%), **1/*2* (~20%) (in our study 16.1%), and **1/*3* (~11%) (in our study 11.3%) genotype was expressed (Buzoianu AD et al., 2012).

The presence of these alleles is important for determination of the optimal dose of *CYP2C9* substrates, including losartan. In vitro and in vivo studies have shown that expression of *CYP2C9*2* or **3* allele significantly affects the conversion of losartan to E-3174.

The effect of genomic variability of *CYP2C9* on losartan metabolism has been examined in a number of studies. The degree of oxidation of losartan was decreased in liver microsomes of individuals who are hetero- or homozygous for *CYP2C9*3* (rs1057910) allele or homozygous for *CYP2C9*2* (rs1799853) allele (Yasar U. et al., 2001). Our results are in agreement with those presented in studies reporting minimal or reduced conversion of losartan to E-3174 in subjects who were homozygous for the *CYP2C9*3* allele (McCrea JB. et al., 1999; BAE. JW et al, 2011).

CYP2C9 allelic **3* variant is associated with a reduced formation of metabolite E-3174, while *CYP2C9*2* allele has a smaller impact on the function of the enzyme (Babaoglu MO. et al., 2004; Sekino K. et.al, 2003).

Most studies are focused on the effect of *CYP2C9* variations only on the pharmacokinetics of losartan in healthy volunteers. *CYP2C9*2* and *CYP2C9*3* allelic variations do not affect the antihypertensive effect of losartan in Finnish and Japanese population with essential hypertension and normal renal function (Donner KM. et al., 2009; Yin T. et al., 2008).

This suggests that *CYP2C9*1/*2* and **1/*3* and **2/*3* individuals can have significant differences in the pharmacokinetics of losartan and E-3174 compared with individuals with *CYP2C9*1/*1*.

For the first time in our conditions assessment of pharmacokinetic parameters of a substrate in relation with the *CYP2C9* genotype in healthy subjects was made.

It is noteworthy that in this investigation a significant difference ($p < 0.01$) was found in $AUC_{0\text{-}inf}$ only for losartan between individuals with *1/*1 and those with **1/*2, 1*/*3, *2/*3*. Significant reduction among the tested groups has also been shown in oral clearance, which indicates reduced cleaning of the body from the drug in intermediate metabolisers. Our results are in accordance with data reported by other authors (Yasar U. et al., 2002; BAE. JW et al, 2011; Lee CR. et al, 2003). Consequently, the values of C_{max} in both groups showed a significant ($p < 0.05$) difference (383.71 vs. 505 ng/ml).

In terms of the difference between the pharmacokinetic parameters of metabolite E-3174, specifically C_{max}, there was a significant difference in the group of extensive metabolisers (**1/*1*), while $AUC_{0\text{-}inf}$ in spite of the expected reduced values due to lower conversion of losartan in active metabolite, almost equal values were obtained for this parameter with no significant difference between the two groups ($p > 0.05$).

Such insignificant statistical differences were reported by Lee CR. et al. (2003), who explained that the small number of samples instead of the possible conversion of losartan to the active metabolite might have influenced the obtained results.

Our evaluation is in line with results published over the last years, where differences in pharmacokinetic parameters between extensive and intermediate (spores) metabolisers of losartan have been presented (Z. Li. et al., 2009; Yang L. et al., 2012).
The obtained results indicate the possibility of preventing exposure of subjects in bioequivalence studies to substrates that are metabolized, specifically by *CYP2C9*, to protect "poor" metabolisers (by NOT including them in the study) from the increased incidence and severity of adverse events of the drug.

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