Intra-individual variability in urinary losartan oxidation ratio, an *in vivo* marker of CYP2C9 activity

Cytochrome P450 2C9 (CYP2C9) is a polymorphic enzyme that catalyses the metabolism of many clinically used drugs [1]. Prediction of CYP2C9 activity *in vivo* might prove to be of clinical importance in patients treated with CYP2C9 substrates with a narrow therapeutic index, such as warfarin and phenytoin [2–4]. We recently proposed that losartan, a selective angiotensin II receptor antagonist, might be used as a specific probe drug for CYP2C9 *in vivo* and *in vitro* [5, 6]. The carboxylic acid metabolite of losartan, E-3174, is produced specifically by CYP2C9 [6, 7]. Losartan oxidation *in vivo* was found to be decreased 2-, 3-, and 40-fold, in individuals genotyped as *CYP2C9*1/*3, *2/*3* or **3/*3*, respectively, compared with individuals genotyped as *CYP2C9*1/*1* [5]. Moreover, there was a good correlation between the plasma $AUC_{losartan}/AUC_{E-3174}$ ratio and the losartan/E-3174 recovery ratio in urine collected for 8 or 24 h after drug intake [5]. In the present study, the intraindividual variability of this ratio was investigated.

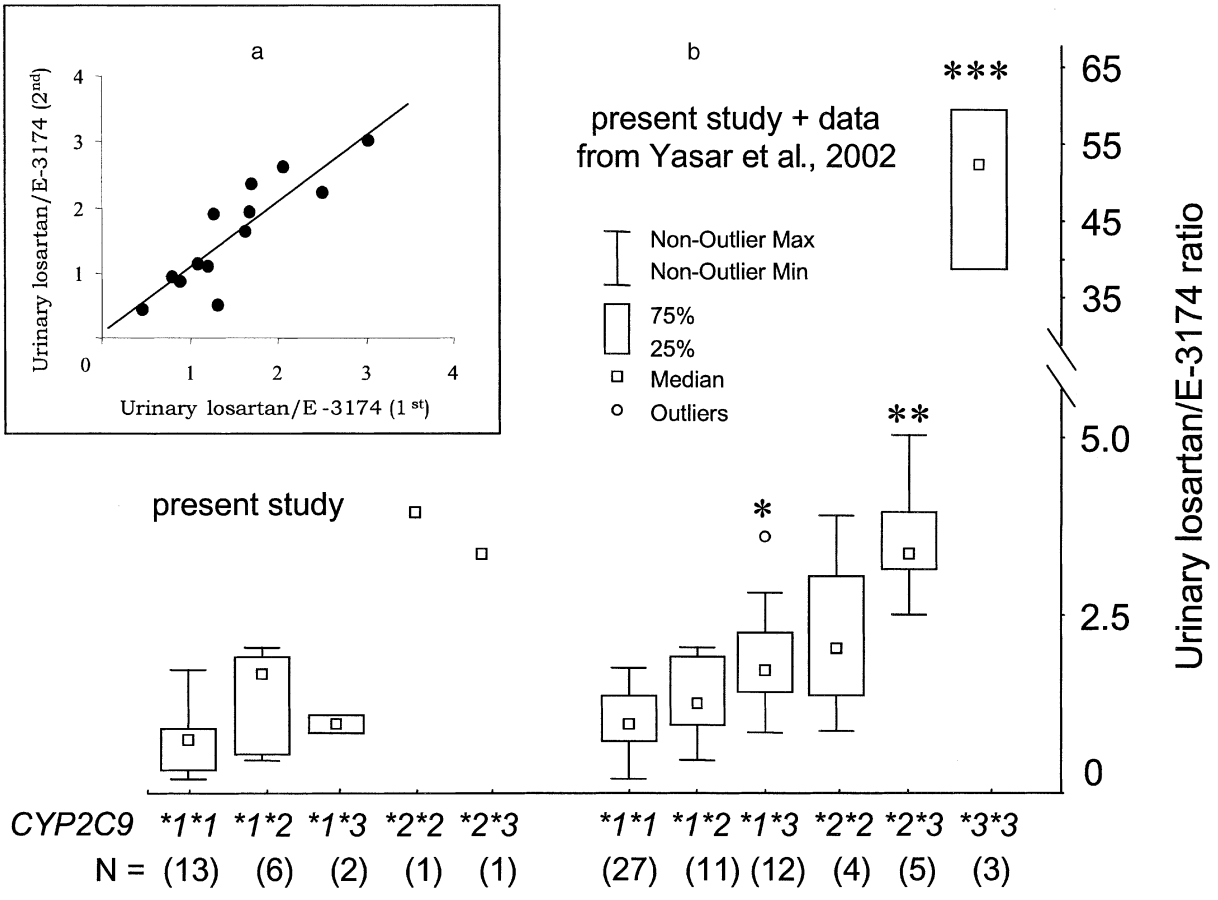

Figure 1 (a) The 0–8 h urinary losartan/E-3174 ratio in the same subjects on two different occasions. (b) Box plot of 0–8 h urinary losartan/E-3174 ratios in 23 subjects in the present study and additional to the present data, 39 subjects (17 subjects after 25 mg and 22 subjects after 50 mg single oral dose of losartan) from Yasar *et al.* 2002 [5]. *$P<0.01$ compared with **1*1*; **$P<0.01$ compared with **1*1* and **1*2*; ***$P<0.0001$ compared with all other groups.

Twenty-three healthy Swedish subjects (female/male: 12/11) with a mean age of 29 years (24–47 range) and 70 kg mean body weight (49–84 range) participated in the study. The subjects were genotyped with respect to *CYP2C9*1, *2,* and **3* [8]. Experimental conditions followed the protocol by Yasar *et al.* [5]. In brief, the procedure included collection of urine samples for 8 h following intake of a single 25 mg oral dose of losartan (Cozaar^{R}, Merck Sharp & Dohme). Analysis of losartan and E-3174 in urine was performed as described previously [5]. The coefficients of variation (CVs) of the losartan and E-3174 assays were less than 10% and 8%, respectively. The losartan/E-3174 ratio was calculated from the molar recoveries of losartan and E-3174 in the 0–8 h urine collection. The pH of urine samples was measured with Metrohm 691 pH Meter (Herisau, Switzerland). The procedure was repeated in 13 of the subjects with genotypes CYP2C9*1/*1 (6 subjects), *1/*2 (3), *1/*3 (2), *2/*2 (1) and *2/*3 (1) after 9–12 months. The correlation between urinary losartan/E-3174 ratios after losartan administration on these two separate occasions was determined using linear regression (STATISTICA 4.3 (StatSoft. Inc. Tulsa, OK, USA). A paired *t*-test was applied for comparison of the two urinary recovery ratios in the same subjects. CV was defined as standard deviation over mean obtained from two separate observations. For the comparison of different genotype groups (if one way ANOVA was significant) the Tukey HSD posthoc test in STATISTICA 4.3 was applied, and P values of <0.05 were accepted as statistically significant.

The results show a highly significant correlation ($r=0.88$, $P<0.0001$, $n=13$, slope 1.03) between the individual losartan/E-3174 urinary ratio at two different occasions (Figure 1a). No significant difference between the two measurements was found ($P=0.59$). There was one outlier (genotype *CYP2C9*1/*2*) that differed as much as 61% between the two measurements. The CVs were 13% and 9% when the outlier subject was included and excluded, respectively. The average difference was $\pm 10\%$ (8%, 12%, 95% confidence interval). There was no correlation between the urinary losartan/E-3174 ratio and urinary pH ($r=0.23$, $P=0.25$).

Figure 1b shows the losartan/E-3174 ratios in different genotypes of *CYP2C9*. The data from the present subjects in Figure 1a are in accordance with our previous data from 39 healthy Caucasian subjects [5]. Figure 1b also contains a summary of all individuals pheno- and genotyped for CYP2C9 in our laboratory to date, including data from the original study [5]. Importantly, a statistically significant difference in urinary losartan/E-3174 ratios between the different genotypes was found (Figure 1b), where the ratios within each genotype group were very similar in the two studies [5].

In conclusion, the observed good correlation between the metabolic ratios of losartan on two different occasions indicates that individual CYP2C9 activity is stable over time. This suggests that genetic factors governing gene expression and enzyme activity are major determinants of the CYP2C9 phenotype. Furthermore, the low intra-individual variability of this *in vivo* assay of CYP2C9 makes it suitable for additional studies in larger populations.

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Ümit Yasar,[1] Marja-Liisa Dahl,[2] Magnus Christensen[1] & Erik Eliasson[1]

[1]*Department of Medical Laboratory Sciences & Technology, Division of Clinical Pharmacology, Karolinska Institutet at Huddinge University Hospital, Stockholm, and* [2]*Department of Medical Sciences, Clinical Pharmacology, University Hospital, Uppsala, Sweden*

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Correspondence: Erik Eliasson MD, PhD, Karolinska Institutet, Department of Medical Laboratory Sciences & Technology, Division of Clinical Pharmacology, Huddinge University Hospital, SE-141 86 Stockholm, Sweden. Tel.: + 468 58587899; Fax: + 468 58581070; E-mail: erik.eliasson@labtek.ki.se

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