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SHORT COMMUNICATION

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Comparison of chlorzoxazone one-sample methods to estimate CYP2E1 activity in humans

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Abstract *Objective*: Comparison of a one-sample with a multi-sample method (the metabolic fractional clearance) to estimate CYP2E1 activity in humans.
Methods: Healthy, male Caucasians ($n = 19$) were included. The multi-sample fractional clearance (Cl_{fe}) of chlorzoxazone was compared with one-time-point clearance estimation (Cl_{est}) at 3, 4, 5 and 6 h. Furthermore, the metabolite/drug ratios (MRs) estimated from one-time-point samples at 1, 2, 3, 4, 5 and 6 h were compared with Cl_{fe}.
Results: The concordance between Cl_{est} and Cl_{fe} was highest at 6 h. The minimal mean prediction error (MPE) of Cl_{est} as a percentage of actual mean Cl_{fe} was −4.2% at 6 h. Furthermore, regarding Cl_{fe}, there was a negligible difference ($P = 0.56$) of bias between Cl_{est} at 3 h (MPE = −8.9%) and 6 h (MPE = −4.2%). The best concordance between MR and Cl_{fe} was found at 3 h ($r = 0.74$; $P < 0.001$).
Conclusion: All three single-dose-sample estimates, Cl_{est} at 3 h or 6 h, and MR at 3 h, can serve as reliable markers of CYP2E1 activity. The one-sample clearance method is an accurate, renal function-independent measure of the intrinsic activity; it is simple to use and easily applicable to humans.

Keywords Chlorzoxazone · Single sample clearance · CYP2E1

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Introduction

Chlorzoxazone (CZX) can be used as a probe of cytochrome P_{450} 2E1 (CYP2E1) activity in humans [1]. It is metabolised by CYP2E1 to 6-hydroxychlorzoxazone (6-OHCZX) [2], which is glucuronidated and excreted in the urine (~80%) [3]. CZX has been used to characterise CYP2E1 activity by time-consuming multi-sample methods [4]. Attempts have, therefore, been made to develop a single-sample method.

The CZX concentration 6 h after a single dose was sufficient to estimate the total oral clearance [5]. Drug/metabolite plasma ratios 1.5–5 h after dosing could be used as activity markers, although they do not provide an accurate quantitative measure [6].

The aim of the present study was to estimate CYP2E1 activity by a CZX single-sample method and to compare this method with an optimal method for CYP2E1 activity estimation, the fractional CZX clearance.

Materials and methods

Subjects

Male Caucasians ($n = 20$) aged 21–30 years with a body mass index (BMI) of 19–27 kg/m^2 (10 non-smokers and 10 smokers, 0 alcoholics) participated in the study. One subject was excluded due to violation of the inclusion criteria (fasting). Time to reach peak plasma concentration (t_{max}) of chlorzoxazone was extended, precluding estimation of the elimination rate constant. Although peak plasma concentration (C_{max}) was comparable, urinary recovery was under the lower confidence limit, suggesting an altered absorption rate, not completed absorption. The study was approved by the regional ethics committee.

Drug administration

After overnight fasting, 250 mg CZX was given orally with 200 ml water. Food was withheld until 2 h after drug administration. Blood samples were collected at 10, 20, 30, 40, 50, 60, 90, 120 min and hourly for the next 4 h into heparinised tubes, stored on ice, centrifuged, and the separated plasma was stored at

Table 1 Chlorzoxazone clearance in 19 healthy male volunteers after an oral 250-mg dose. The mean prediction error method was used. *Cl_{fe}* multi-sample fractional clearance calculated as fe dose/$AUC_{0-\infty}$, *fe* fraction of the dose recovered as 6-hydroxychlorzoxazone in 0–24 urinary collection, *Cl_{est}* clearance estimated from the single sample at time 3, 4, 5 and 6 h was used. All Cl, Cl_{fe}, Cl_{est} are in units of ml/min/kg. *MPE* mean prediction error, *MPEa* mean prediction error as a percentage of the mean multi sample clearance, *CI* confidence interval

	Cl_{fe}	Cl_{est} 3 h	Cl_{est} 4 h	Cl_{est} 5 h	Cl_{est} 6 h
Mean	1.50	1.37	1.38	1.39	1.44
(95% CI)	(1.18, 1.82)	(1.15, 1.58)	(1.26, 1.80)	(1.17, 1.61)	(1.26, 1.61)
Mean Cl_{est}/Cl_{fe}		0.98	1.02	1.02	1.09
(95% CI)		(0.82, 1.14)	(0.87, 1.12)	(0.87, 1.17)	(0.92, 1.27)
MPE		−0.13	−0.12	−0.11	−0.06
(95% CI)		(−0.37, 0.10)	(−0.38, 0.14)	(−0.36, 0.15)	(−0.32, 0.20)
MPEa		−8.9%	−8.1%	−7.0%	−4.2%

−20°C. Urine was collected, and the volume was recorded 0–6 h and 6–24 h after dosing. Aliquots of each sample were frozen at −20°C.

Analytical methods

CZX and 6-OHCZX were measured using a modified high-performance liquid chromatography method [7].

Pharmacokinetics

The area under the concentration–time curve $(AUC)_{CZX(0-6)}$ and $AUC_{6\text{-}OHCZX(0-6)}$ were determined trapezoidal. $AUC_{CZX(0-\infty)}$ was calculated by adding $AUC_{(0-6)}$ and $C_{last}/\lambda_{z.}$ C_{last} is the 6-h concentration, and λ_z is the elimination rate constant estimated from the slope of the terminal portion of the disappearance curve. The ratio $AUC_{(6-\infty)}/AUC_{(0-\infty)}$ was recorded. A $t_{1/2}$ was obtained as the ratio: $0.693/\lambda_z$. The oral CZX clearance, Cl, was determined from the ratio: $D/AUC_{(0-\infty)}$. The fractional clearance of the 6-hydroxylation (Cl_{fe}) was estimated from the product of the fraction recovered and Cl, f_e*$D/AUC_{(0-\infty)}$. An apparent volume of distribution, $V_{\lambda z}$ was determined as: Cl_{fe}/λ_z. Single sample clearance, Cl_{est}, was calculated:

$$CI_{est} = [\ln(D/V_{\lambda z}) - \ln C_t] * (V_{\lambda z}/t)$$

[1] where C_t is the concentration of CZX at time t (2, 3, 4, 5 or 6 h). To get a more independent measure, $V_{\lambda z}$ was calculated as a product of the sample mean (174.4 ml/kg) and the individual weight. The plasma ratio of 6-OHCZX and CZX (metabolic ratio) was calculated every hour from 1–6 h. The f_e was estimated as the amount in the 0- to 24-h sample. The ratio 0–6 h/0–24 h urinary recovery was recorded.

Statistical methods

The Cl_{fe} was evaluated by the prediction error method (MPE) [8]. The metabolic ratio versus Cl_{fe} was evaluated by correlation/regression analyses. Student *t*-test was used where appropriate. $P = 0.05$ was considered significant.

Results

Mean $AUC_{(6-\infty)}$ of CZX was 10% lower than $AUC_{(0-\infty)}$. Total urinary recovery was 74 ± 19%; almost 90% of this amount was excreted within 0–6 h. In accordance with other studies, the parent compound was not excreted [3].

The Cl_{fe} and the metabolic ratio after 2 h were similar in smokers and non-smokers ($P = 0.81$ and $P = 0.54$, respectively). Samples taken before 3 h after dosing could not be used to estimate Cl_{est} (late C_{max}).

In Table 1, the mean ratios of Cl_{est}/Cl_{fe} and the respective MPEs are shown. The mean ratio of Cl_{est}/Cl_{fe} was almost 1 at 6 h, and the Cl_{est} compared with Cl_{fe} exhibited a negative bias of 4.2%. There was no difference ($P = 0.56$) between the bias at 6 h and 3 h. In Fig. 1, a plot of each set of Cl_{est}/Cl_{fe} is shown. The best correlation between metabolic ratio and Cl_{fe} was after 3 h ($r = 0.74$), second best after 5, 4 or 2 h (not shown). There was correlation between Cl_{est} after 3 h and the metabolic ratio after 2 h ($r = 0.75$, $P < 0.001$).

Discussion

This study compares for the first time one-sample CZX clearance with fractional clearance and indicates that the 6-h post-dose estimation reflects 6-hydroxylation best. It may, therefore, serve as a surrogate measure of CYP2E1 activity.

Assuming complete hydroxylation of CZX and excretion of the metabolite, the urinary recovery reflects bioavailability. According to a one-sample principle [9], we have estimated CZX clearance (Cl_{est}) from CZX plasma concentrations.

The total oral CZX clearance (2 ml/min/kg) was lower than reported in other studies after the same dose (4–5 ml/min/kg) [10], probably due to reported shorter half-life (1.0–1.2 h) and lower urinary recovery (< 65%), suggesting reduced absorption.

The similar disposition of CZX in smokers and non-smokers is consistent with other studies [4, 11]. CYP1A activity, which can be induced by tobacco, plays a minor role in CZX hydroxylation [12].

The metabolic ratio was related to Cl_{fe} after 1.5 h and 2 h [4], and 2–4 h [13]; in this study, best after 3 h (2–5 h). Such ratios, although they are likely to reflect changes in the hydroxylation capacity, are absorption dependent early after drug administration and the low determination coefficients [4, 6] in relation to Cl_{fe} preclude them as accurate measures. Moreover, decreased elimination of the metabolite in uraemia may reduce the accuracy [13].

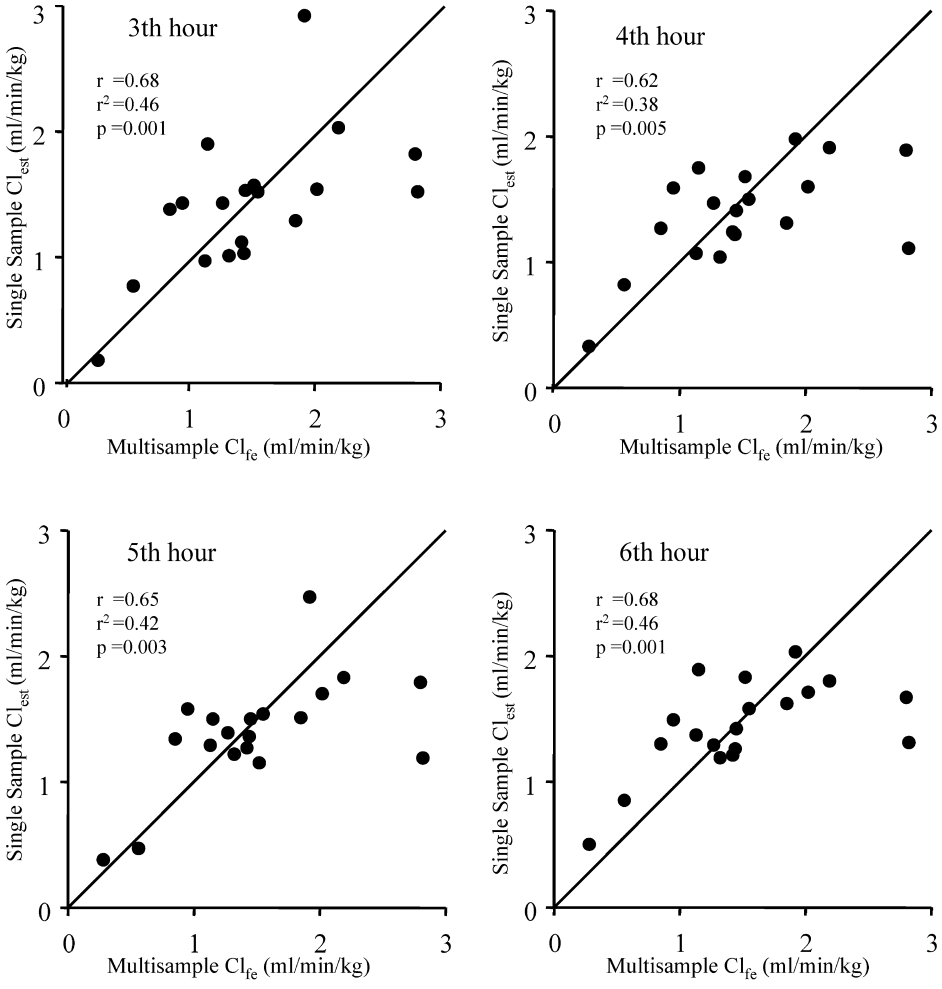

Fig. 1 Single sample clearance (Cl_{est}) versus multi-sample fractional clearance (Cl_{fe}) at four different time-points in 19 healthy male volunteers after an oral 250-mg chlorzoxazone dose. *Diagonal lines* are identity lines. The mean prediction error method was used, and the correlation coefficients are shown for comparison

One-sample CZX clearance is a more accurate measure of the process and does not need the metabolite measurement. Furthermore, it saves time and money in the laboratory by avoiding time-consuming enzymatic methods and purchase of expensive standards.

In six volunteers, the best agreement between Cl_{est} and Cl after a 500-mg CZX dose [5] was observed after 6 h, comparable with the present study, in which a dose of 250 mg was used. However, the lower dose is appropriate due to the dose-dependent disposition of CZX [13].

A distribution volume (Vd) of ~13 l suggests that the drug would be confined in the extracellular fluid. However, obesity increased Vd by ~50% [10]. Neither enzyme induction nor fasting could account for the differences in Vd [4, 10, 11]. Gender differences have modest clinical consequences, and age does not affect CZX disposition [6].

Since the difference between the 6-h and 3-h samples is negligible, the use of the 3-h estimate is recommended to save time (correcting for the bias to get a more accurate estimate). It is important, however, to secure a rapid CZX absorption to avoid bias if you use the 3-h estimate. Fasting and normal gastric function (normal emptying) is therefore crucial. A Vd of 174.4 ml/kg seems to be a sufficient measure of the parameter in a white, not extensively obese, population.

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