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Association between CYP1A2 activity and riluzole clearance in patients with amyotrophic lateral sclerosis

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Aims
Riluzole is used in a fixed dosing schedule of 50 mg twice daily to treat patients with amyotropic lateral sclerosis (ALS), one form of motor neurone disease. The large variability in the pharmacokinetics of riluzole may be a factor contributing to its limited therapeutic benefit. Riluzole is assumed to be mainly metabolized by the cytochrome P450 enzyme 1A2 (CYP1A2). The aim of the study was to investigate the relationship between CYP1A2 activity and riluzole clearance with a view to optimize drug treatment.

Methods
A group of 30 ALS patients participated in the study. In each patient the CYP1A2 activity was determined using caffeine as a metabolic probe. Riluzole clearance was estimated from serum drug concentration measurements followed by Bayesian fitting.

Results
Riluzole clearance and the serum paraxanthine : caffeine (P/C) ratio showed a positive correlation ($r = 0.693$; $P = 0.0002$). Linear regression analysis identified the P/C ratio (beta: 1.16) and height (beta: 0.027) as independent predictors of riluzole clearance (adjusted $r^2 = 0.369$).

Conclusions
The P/C ratio, used as measure of CYP1A2 activity, significantly correlated with the riluzole clearance, although only 37% of the observed variability could be explained.

Introduction

Riluzole (Rilutek®) is the only licensed drug on the market for treatment of amyotrophic lateral sclerosis (ALS). From the time of diagnosis it is prescribed to almost all patients with this neurodegenerative disease in a fixed regimen of 50 mg twice daily. Riluzole has been shown to be effective by improving survival, and has a mild profile of adverse events. A dose-effect relationship was found for patients treated with 50, 100 and 200 mg [1, 2]. The mechanism of action of riluzole remains unclear, but the effect of the drug is partially mediated through inhibition of the deleterious effects of an overload of glutamic acid and other neurotransmitters in the central nervous system [3–5].

After oral administration, riluzole is rapidly absorbed with a maximum concentration 1–2 h after intake and a mean bioavailability of 60% [6–9]. Its elimination is assumed to depend mainly on oxidative metabolism by

the cytochrome P450 isoform 1A2 (CYP1A2) to N-hydroxyriluzole [10].

Earlier work of our group has shown that in patients with ALS, who receive the same dose of riluzole, 2 h and trough serum concentrations of riluzole are highly variable [11]. We hypothesized that this large variability may be a factor contributing to the limited efficacy of riluzole in ALS patients. To test this hypothesis we have studied the relationship between CYP1A2 activity and riluzole clearance in ALS patients. Using caffeine as a probe drug, the paraxanthine : caffeine molar (P/C) ratio has been accepted as a reliable index of CYP1A2 activity [12, 13].

Methods

Patients

A total of 30 ALS patients from the outpatient clinic of the University Medical Centre Utrecht (UMCU) were recruited after approval of the study protocol by the UMCU medical ethics committee. Patients all gave written informed consent before participation. Inclusion criteria were a probable, probable/laboratory-supported or definite diagnosis of ALS (revised El Escorial criteria), use of riluzole 50 mg twice daily for at least 2 weeks, age between 18 and 75 years, symptoms of ALS for at least 6 months and no longer than 60 months prior to the study [14]. Patients on medication interacting with CYP1A2 were excluded. Participants were asked to take riluzole tablets at exactly 10.00 h and 22.00 h during the study and to write the time of ingestion in a drug diary. Age, gender, body weight, height and smoking status were recorded for each patient.

Determination of CYP1A2

To avoid interference with dietary caffeine, patients were asked to refrain from coffee, tea and other caffeine containing products for a period of 48 h before assessment of CYP1A2 activity. Each patient was given a dose of 200 mg caffeine as an oral solution at 12.00 h. Six hours later, a blood sample was taken and serum caffeine and paraxanthine (1,7-dimethylxanthine) concentrations were measured using a validated liquid chromatographic assay with ultraviolet detection (quantification range: 0.45–20 μg ml^{-1} and 0.16–20 μg ml^{-1}; between-run coefficient of variation: 6–9% and 7–11%, respectively) [15]. CYP1A2 activity was expressed as the P/C molar serum concentration ratio. To exclude recent caffeine intake a pretest blood sample was also analyzed.

Estimation of riluzole clearance

To estimate riluzole clearance, 2 h post dose and trough serum concentrations of riluzole were measured using a validated liquid chromatographic assay with ultraviolet detection in venous blood samples collected at weekly intervals (quantification range: 0.020–2.00 μg ml^{-1}; between-run coefficient of variation: 1.8–9.7%) [16]. From at least one 2 h and one trough concentration per patient, riluzole clearances were estimated using a Bayesian fitting procedure (Marquardt algorithm) performed with MW/Pharm v3.50 software (MediWare, Zuidlaren, the Netherlands) [17]. The *a priori* pharmacokinetic data were taken from the pharmacokinetic population model as described by Bruno *et al.* [6] (1-compartment open model, without covariates; ($k_e = 0.124 \pm 0.174$ h^{-1}; $V_d = 4.83 \pm 3.66$ l kg^{-1}; $k_a = 5$ h^{-1}, fixed). Data for oral bioavailability ($F = 0.60 \pm 0.18$) were taken from the product information on Rilutek® [9].

Data analysis

The relationship between riluzole clearance and the P/C ratio was determined using Pearson correlation analysis. Multipolynomial curve fitting was also used to determine the type of relationship giving the best fit to the data. To find predictors of riluzole clearance, multivariate linear regression analyses were performed with the P/C ratio, age, gender, body weight, height and smoking status (yes/no) as independent variables. The final regression model was obtained by following a stepwise backward procedure with a removal criterion of $P > 0.10$. To meet the normal distribution requirement for linear regression, riluzole clearance was logarithmically transformed. The adjusted r^2 was used as a measure of goodness-of-fit and explained variance. All statistical calculations were performed using SPSS software version 11.0.1 (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics are summarized in Table 1. For 20 patients three 2 h and three trough concentrations of riluzole were obtained at weekly intervals. For the other subjects 2 h and trough concentrations were measured only once. Caffeine was not detected in any of the pretest samples.

A significant Pearson correlation was found between riluzole clearance and the P/C ratio (see Figure 1; $r = 0.632$, $P = 0.0002$). Multipolynomial curve fitting indicated that a linear relationship best fitted the data. Multivariate linear regression analysis showed that the P/C ratio (beta: 1.16; 95% CI: 0.50, 1.82) and height (beta: 0.027; 95% CI: –0.003, 0.057) were positively associated with riluzole clearance at the 90% significance level (final model; adjusted $r^2 = 0.369$). Thus 37% of variability in riluzole clearance was explained by the

Table 1
Patient characteristics

Number of patients	30
Age (years; mean (SD))	56 (12)
Gender (male/female)	23/7
Body weight (kg; mean (SD))	81 (12)
Height (cm; mean (SD))	176 (8)
Smoking status (yes/no)	11/19

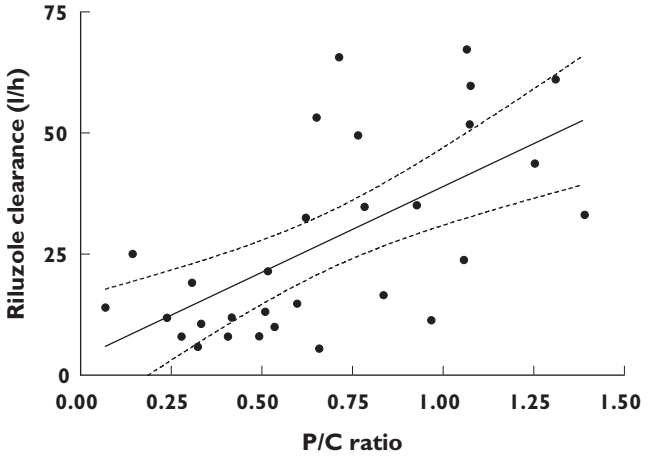

Figure 1
Scatter plot and the linear regression line showing the relationship between P/C ratio and riluzole clearance. The dotted lines represent the 95% confidence boundaries of the regression line

P/C ratio and height. At the 95% significance level, the P/C ratio was the only predictor of clearance (beta: 1.26; 95% CI: 0.58, 1.93; adjusted $r^2 = 0.319$). Age, weight and smoking only slightly reduced the variability of the final model and did not significantly predict riluzole clearance.

Discussion

The aim of this study was to examine the relationship between CYP1A2 activity expressed as the P/C ratio and the clearance of riluzole. We found a significant positive correlation between the two parameters. In the final linear regression model only the P/C ratio and height were found to be significant independent predictors. We have no explanation of why height and not body weight was predictive in our model. We expected to find that smokers have higher P/C ratios reflecting induced CYP1A2 activity, but this was not the case in this relatively small study group (one-way ANOVA, $P = 0.178$) [18]. Serum total bilirubin and albumin concentrations, which were found as predictors of riluzole clearance in the population pharmacokinetic study of Bruno *et al.* [6] were not evaluated in our study due to a lack of sample material.

Both correlation and linear regression analysis showed a large unexplained variability in riluzole clearance. The Bayesian approach we used to estimate it may have introduced more variability than the calculation of clearance from a full concentration-time curve, although Mahmood *et al.* [19] have recently illustrated the reliability of the Bayesian method. We chose to use a limited number of concentration measurements of riluzole, caffeine and paraxanthine because the study was carried out in normal clinical practice, and to prevent burdening in ALS patients with frequent blood sampling.

Another explanation for the unaccounted variability in riluzole clearance is the possible contribution of other metabolic routes such as glucuronidation or extrahepatic CYP1A2 activity which may also play a role in the elimination of riluzole. The only published data on the metabolic pathways involved in the elimination of riluzole are from *in vitro* experiments with liver microsomes [10], and thus further studies are merited.

Other possible causes of variability include the quality of the pharmacokinetic model, nonadherence to the study protocol and random variability. In addition, an interaction between riluzole and caffeine during CYP1A2 phenotyping cannot be fully excluded, but results from *in vitro* studies do not support this [10].

In conclusion, although riluzole clearance correlated significantly with the P/C ratio, this measure of CYP1A2 activity explained only 37% of the variability in riluzole clearance. A better insight into the determinants of riluzole pharmacokinetics might help to design studies aimed at pharmacokinetic-pharmacodynamic modelling, dose optimization and improvement in therapeutic outcome.

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