Absence of interaction between erythromycin and a single dose of clozapine

Abstract Objective: To study the suggested pharmacokinetic interaction between erythromycin, a strong inhibitor of CYP3A4, and clozapine.

Methods: Twelve healthy male volunteers received a single dose of 12.5 mg of clozapine alone or in combination with a daily dose of 1500 mg erythromycin in a randomised crossover study. Clozapine and its metabolites clozapine-N-oxide and desmethyl-clozapine were measured in serum samples which were collected during a 48 h period and in a sample of the urine secreted over the interval 0–12 h.

Results: There were no significant differences in mean area under the serum concentration time curves (1348 (633) nmol h$^{-1}$ in the control phase and 1180 (659) nmol h$^{-1}$ in the erythromycin phase), terminal half-lives (19 (13) h and 15 (6) h, respectively), peak serum concentrations (92 (53) nmol h$^{-1}$ and 77 (40) nmol h$^{-1}$, respectively), time to peak serum concentrations (1.4 (0.7) h and 1.5 (1.0) h, respectively) or apparent oral clearances of clozapine (34 (15) l h$^{-1}$ and 46 (37) l h$^{-1}$, respectively), time to peak serum concentrations (1.4 (0.7) h and 1.5 (1.0) h, respectively) or apparent oral clearances of clozapine (34 (15) l h$^{-1}$ and 46 (37) l h$^{-1}$, respectively), or in renal clearances of clozapine (0.8 (0.5) l h$^{-1}$ and 1.0 (0.7) l h$^{-1}$, respectively) between the two phases.

Conclusion: These results demonstrate that erythromycin at a clinically relevant dosage does not inhibit the metabolism of clozapine. Hence, CYP3A4 seems to be of minor importance in the disposition of clozapine in humans at least when clozapine is taken at a low single dose.

Key words Clozapine · Erythromycin · CYP3A4 · Pharmacokinetics · Drug interaction

Introduction

The atypical antipsychotic agent clozapine is widely used in treatment of resistant schizophrenia and in schizophrenic patients intolerant to conventional neuroleptics. Clozapine is metabolised by the enzyme CYP1A2 [1, 2], but not CYP2D6 [3]. There are in vitro data suggesting that the enzyme CYP3A4 also is involved in the metabolism of clozapine [4, 5].

Two case reports [6, 7] have recently been published suggesting that the macrolide antibiotic erythromycin, which is a strong inhibitor of CYP3A4 [8, 9], can cause an increase in clozapine serum concentrations, hereby resulting in clozapine-related toxic symptoms. Moreover, concomitant use of carbamazepine, which is known to induce CYP3A4 [10], but not CYP1A2 [11], has been reported to decrease serum levels of clozapine [2, 12]. The present study was performed to investigate whether erythromycin influences the pharmacokinetics of clozapine.
sampling period. The subjects had previously been characterised as extensive metabolisers of drugs catalysed by CYP2D6 and CYP2C19 tested by means of dextromethorphan and mephenytoin [13]. It was calculated that, with a power of 80% and a significance level of 0.05, 12 subjects were needed to detect a 20% change in clozapine AUC between the phases.

Study design

A randomised crossover design was used and the phases were separated by a period of 2–17 weeks. In the control phase the study subjects received a single dose of 12.5 mg of clozapine (Lepoxin, Sandoz, Basel, Switzerland) at 8 AM after an overnight fast. In the erythromycin phase the study subjects received 500 mg of erythromycin (Erymax, Astra, Södertälje, Sweden) orally 9 h before intake of 12.5 mg of clozapine at 8 AM and thereafter a daily dose of 1500 mg throughout the sampling phase. On the day of clozapine intake erythromycin was given at six occasions: 250 mg was given 2 h before, together with, and 2, 6, 10, 15 h after intake of clozapine. Thereafter 500 mg of erythromycin was given 24, 32 and 39 h after clozapine intake.

As clozapine is not available in dose units less than 25 mg, half a tablet (12.5 mg) of clozapine was given. Administration of correct doses was controlled by weighing each half tablet. The mean weight of the half tablet used was 79.3 (SD 1.3) mg in the control phase and 78.9 (SD 1.1) mg in the erythromycin phase. No food intake, except standardised meals at noon and at 5 PM, was allowed during the first 12 h after intake of clozapine.

Sample collection

Venous blood samples (10 ml) for analysis of clozapine and metabolites were collected before and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 32 and 48 h after intake of clozapine. Venous blood samples for analysis of serum concentrations of erythromycin (trough values) were collected 10, 24 and 32 h after intake of clozapine. Serum was separated within 45 min and stored at −70 °C until clozapine assay and at −20 °C until erythromycin assay.

All urine secreted over the 0–12 h interval after the morning dose of clozapine dose was collected. At the end of the collection interval the urine volume was measured and an aliquot of 10 ml was stored at −70 °C until analysis.

Analytical methods

Serum levels of clozapine and metabolites were determined at our laboratory by modification of a previously described reversed phase high performance liquid chromatography technique [14]. In brief, to 2.0 ml serum were added 20 μl of a 20 μM solution of the internal standard Lundbeck N-7084, [S]pyrrolidinylpropyliden]-10, 11-dihydro-5-H-dibenzo (a,d) cyclohepten; Lundbeck A/S, Copenhagen, Denmark) and 2.0 ml of a 0.1 M sodium acetate buffer (pH 6.5). After mixing and centrifuging for 5 min at 3000 × g the supernatant was applied to an Isolute HCX solid phase column (International Sorbent Technology, Mid-Glamorgan, UK). After passing the sample at a slow rate, the column was washed with 1 ml 1 M acetic acid and 2 ml methanol and the compounds were then eluted with 2 × 0.75 ml of 2% NH₃ in methanol. After evaporation to dryness at 40 °C the compounds were dissolved in 100 μl of an eluent consisting of 58% acetonitrile, 22% methanol and 20% K₂HPO₄. Then 40 μl of the eluate was injected onto an Apex CN RP analytical column (particle size 5 μm; length 150 mm; inner diameter 4.6 mm) (Jones Chromatography, Mid-Glamorgan, UK) with an Apex CN RP precolumn (particle size 5 μm; length 10 mm) (Jones Chromatography, Mid-Glamorgan, UK). The separation was performed with a mobile phase flow of 2.0 ml·min⁻¹. The limit of quantitation was 2 nmol·l⁻¹ for clozapine as well as for the metabolites desmethyl-clozapine and clozapine-N-oxide. The method was linear for all analytes up to at least 3000 nmol·l⁻¹. The intra-assay coefficient of variation ranged between 3.5% and 5.2% for clozapine, between 3.3% and 7.8% for desethyl-clozapine and between 1.8% and 4.5% for clozapine-N-oxide at 10, 50 and 150 nmol·l⁻¹. The inter-assay coefficient of variation ranged between 2.4% and 10.7% for clozapine, between 1.9% and 9.4% for desethyl-clozapine and between 4.8% and 14.8% for clozapine-N-oxide at 10, 50 and 150 nmol·l⁻¹. The ultraviolet detector was set at a wavelength of 254 nm and the retention times were approximately 2 min for clozapine-N-oxide, 3 min for clozapine and 4 min for desmethyl-clozapine. The recovery for the different analytes varied between 81% and 90%. Erythromycin did not interfere with the assay method for clozapine.

The urine concentrations of clozapine and the metabolites desethyl-clozapine and clozapine-N-oxide were analysed by using a modified assay as for serum clozapine. In short, 0.1 ml of urine were incubated at 65 °C for 2 h at pH 6.5 with β-glucoronidase (Sigma Chemical, Mo, USA) containing 2 100 000 units·gram⁻¹ of β-glucuronidase and 36 800 units·gram⁻¹ of sulfatase. Then, 50 μl of 40 μM solution of the internal standard and 1.0 ml of 0.1 M sodium acetate buffer were added. After mixing and centrifuging, the supernatant was applied to the solid phase column as described above. After the sample had passed at a slow rate, the column was washed as above. The eluted compounds were then dissolved in 500 μl of the same eluent that was used for analysis of clozapine in serum. The eluate was injected into the analytical column. The limit of quantitation was 25 nmol·l⁻¹ for clozapine, desmethyl-clozapine and clozapine-N-oxide. The method was linear for the analytes up to at least 3000 nmol·l⁻¹. For clozapine and its two major metabolites, the intra-assay and inter-assay coefficient of variation ranged between 0.8% and 3.4% and 0.9% and 8.4%, respectively, at 500 nmol·l⁻¹. The recovery for the different analytes varied between 82% and 102%.

Concentrations of erythromycin in serum were measured by a microbiologic assay using Micrococcus luteus ATCC 9341 as test organism [15]. The samples and standard erythromycin solutions were added to agar wells in PDM-Antibiotic Sensitivity Medium (Biodisc, Solna, Sweden). The inhibition zone diameters were measured after incubating the plates for 18 h at 37 °C. The intra-assay coefficient of variation was 6.6% and the interassay coefficient of variation was 7.8% at 1.0 mg·l⁻¹. The limit of quantitation was 0.25 mg·l⁻¹.

Pharmacokinetic analysis

Peak concentrations (Cₘₚₓ) and concentration peak times (tₘₚₓ) were taken directly from the original data. Other pharmacokinetic parameters were calculated with the pharmacokinetic program package Siphar/Win, version 1.13 (Simated SA, Créteil, France), by using a two-compartment model. Data from the erythromycin phase in two subjects did not fit in the two-compartment model, and a one-compartment model was therefore used instead. Areas under the serum concentration curve (AUC) were calculated by use of the linear trapezoidal rule with extrapolation to infinity. Mean residual areas for clozapine made up 15% and 19% of the total areas for the erythromycin and the control phase, respectively. Elimination half-lives (t₁/₂) for clozapine was calculated as ln2/t₁/₂, in which t₁/₂ is a parameter describing the linear terminal slope of the log concentrations of clozapine. Apparent oral clearance of clozapine was calculated as dose/AUC and renal clearance of clozapine was calculated as difference between total amount secreted 0–12 h/clozapine AUC₀–₁₂. Partial metabolic clearances (Clₘ) of clozapine was calculated as total amount secreted in urine 0–12 h for clozapine-N-oxide/clozapine AUC₀–₁₂ and desmethy1-clozapine/clozapine AUC₀–₁₂.

Statistical analysis

The results are expressed as mean values (SD). Statistical evaluations were performed with the program package Statistica version, 5.0 (Statsoft, Ok, USA). Wilcoxon test for paired data and Spearman’s rank correlation test were used for statistical analysis. A P value of less than 0.05 was considered statistically significant.