Eur J Clin Pharmacol (1994) 46: 551–555

European Journal of Clinical Pharmacology

A pharmacokinetic interaction between roxithromycin and midazolam

J. T. Backman[1], K. Aranko[1], J.-J. Himberg[1], K. T. Olkkola[2]

[1] Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland
[2] Department of Anaesthesia, University of Helsinki, Helsinki, Finland

Received: 15 October 1993 / Accepted in revised form: 23 March 1994

Abstract. The interaction between roxithromycin and midazolam was investigated in a double-blind, randomised crossover study of two phases. Ten healthy volunteers were given roxithromycin (300 mg) or placebo once daily for 6 days. On the sixth day they ingested 15 mg midazolam. Plasma samples were collected and psychomotor performance measured for 17 h.

Roxithromycin administration significantly increased the area under the plasma midazolam concentration-time curve from 8.3 to 12.2 $\mu g \cdot ml^{-1} \cdot min$ and the elimination half-lives from 1.7 to 2.2 h. In psychomotor performance only minor differences were seen between the treatments in one of the measured psychomotor parameters.

Thus, in contrast to the strong interaction between erythromycin and midazolam, the interaction between roxithromycin and midazolam appears less likely to be clinically significant.

Key words: Midazolam, Roxithromycin; drug interaction, pharmacokinetics

Midazolam is a 1,4-benzodiazepine that is widely used as a short-acting hypnotic. Although it has proved to be a safe drug, special attention has been paid to its profound sedative effects (Editorial 1988). Midazolam has an extensive first-pass metabolism with oral bioavailability less than 50% (Allonen et al. 1981). The macrolide antibiotic erythromycin inhibits the metabolism of midazolam in vitro and also in humans (Byatt et al. 1984; Gascon and Dayer 1991; Hiller et al. 1990). In a previous study erythromycin treatment increased the area under the midazolam concentration-time curve more than four-fold and peak midazolam concentration almost three-fold after a 15-mg oral dose of midazolam, which caused more profound and prolonged psychomotor effects (Olkkola et al. 1993).

Roxithromycin, a newer macrolide antibiotic, is an alternative to erythromycin. As a group, macrolide antibiotics are associated with multiple pharmacokinetic drug interactions (Periti et al. 1992). Roxithromycin, too, has been thought to produce drug interactions. Studies report that the coadministration of roxithromycin with theophylline increases theophylline plasma concentrations in humans (Houin et al. 1992; Saint-Salvi et al. 1987). However, some reports have questioned this interaction (Hashiguchi et al. 1992). Because of the strong interaction between erythromycin and midazolam it was important to evaluate the effects of the newer macrolide derivative on the pharmacokinetics and pharmacodynamics of midazolam in this study.

Materials, methods and subjects

The study protocol was approved by the ethics committee of the Department of Clinical Pharmacology, University of Helsinki. Five male and five female volunteers aged 19–26 years and weighing 52–81 kg participated in the study after giving their written informed consent. They were ascertained to be healthy by medical history and a clinical examination before entering the study. None received continuous medication except for two subjects who were using contraceptive steroids.

Study design

The study was carried out using a randomised, double-blind, crossover study design of two phases at an interval of 10 days. The subjects were given orally 300 mg roxithromycin (Surlid 150-mg tablets, Roussel, Paris, France) or matched placebo once a day for 6 days. On day 6 they ingested 15 mg midazolam (Dormicum 15-mg tablets, Hoffmann-La Roche, Basel, Switzerland) with 150 ml water at 3.00 p.m., 2 h after the administration of roxithromycin. The volunteers fasted 3 h before midazolam was administered and had a light standard meal 4 h afterwards. They were not allowed to smoke or ingest coffee, tea, alcohol and cola during the test days.

Presented in part at the 18th International Congress of Chemotherapy, Stockholm, Sweden, June 27–July 2, 1993

Correspondence to: J. T. Backman, Department of Clinical Pharmacology, University of Helsinki, Paasikivenkatu 4, SF-00250 Helsinki, Finland

Blood sampling

Blood samples (10 ml) were taken into lithium-heparin tubes. Samples were taken once before and once during the pretreatments. On day 6 a forearm vein was cannulated with a plastic cannula and timed samples were drawn immediately before the administration of midazolam and 0.5, 1, 1.5, 2, 3, 4, 6, and 17 h after it. Plasma was separated within 30 min and stored at –40 °C until analysis.

Determination of plasma midazolam and roxithromycin

Plasma midazolam concentration was determined by a specific gas chromatographic method with electron capture detection using methoxydiazepam as an internal standard (Aaltonen et al. 1985). The sensitivity of the method was 1 ng/ml and the inter-day coefficient of variation was 5 % (mean 35 ng · ml^{-1}; $n = 10$).

Plasma roxithromycin concentrations were determined once during the pretreatments, immediately before and 120 min after the administration of midazolam. The method was a microbiological agar diffusion assay with agar wells 4 mm in diameter as diffusion centers. The medium used was Isosensitest agar pH 8.0 and the test strain was *Sarcina lutea* ATCC 9341. The concentrations were calculated from standard curves made from known concentrations of roxithromycin prepared in pooled human serum. The sensitivity of the method was 1.0 mg · l^{-1} and the linear correlation coefficient was 0.999. Inter-day variation of the method was tested in duplicate using roxithromycin standards (1 to 32 µg · ml^{-1}) in 10 days. The maximum standard deviation of the results was 6.7 % of the mean inhibition zone diameter.

Pharmacokinetics of midazolam

The areas under the midazolam concentration-time curves [AUC (0-∞)] were calculated using the trapezoidal rule with extrapolation to infinity. The terminal log-linear phase of the plasma midazolam concentration-time curve was identified visually for each subject. The elimination rate constant (k_{el}) was determined by regression analysis of the log-linear part of the curve. The elimination half-life of midazolam ($t^1/_2$) was calculated from $t^1/_2 = \ln 2/k_{el}$. Peak midazolam concentrations (C_{max}) and concentration peak times (t_{max}) were derived directly from original data (Gibaldi and Perrier 1982).

Psychomotor tests

The effect of midazolam on psychomotor performance was assessed at the time of each blood sampling (except the one during the pretreatment) using a test battery. The volunteers were trained in the psychomotor tests before the study. *Saccadic eye movements* were recorded and analysed by the Cardiff Saccade Generation and Analysis System (Griffiths et al. 1984). Peak saccadic velocity and reaction time at a saccadic angle of 35 degrees were used as primary results. The discrimination of the fusion of a flickering red light was determined in the *critical flicker fusion test* (Smith and Misiak 1976). Standard pupil diameter was achieved by wearing special spectacles. In the *digit symbol substitution test* the number of digits correctly substituted by simple symbols in 3 min was recorded (Stone 1984). The *Maddox wing test* was used to measure the coordination of extraocular muscles (Hannington-Kiff 1970). Subjective effects were recorded on seventeen 100-mm-long horizontal *visual analogue scales* (VAS). The opposing ends of the scales were (in the subject's own native language): alert–drowsy, calm–excited, strong–feeble, muzzy–clear-headed, well-coordinated–clumsy, lethargic–energetic, contented–discontented, troubled–tranquil, mentally slow–quick-witted, tense–relaxed, attentive–dreamy, incompetent–proficient, happy–sad, antagonistic–friendly, interested–bored, withdrawn–sociable, very good–very bad performance (Bond and Lader 1974). Subjective drowsiness (alert–drowsy) was used as the primary result. Adverse effects were noted on an item questionnaire.

For the pharmacodynamic test results, differences from baseline (the time point before midazolam administration) values were calculated at each time point for each subject. Areas under the effect-time curves were determined by the trapezoidal rule for 0–6 h [AUC(0–6 h)] and 0–17 h [AUC(0–17 h)] using these differences from baseline results. The maximum effects (E_{max}) over 0–17 h were also registered.

Statistical analysis

The pharmacokinetic parameters, the areas under the effect-time curves and the maximum effects of midazolam during the pretreatments were compared using the Student's *t*-test for paired data. The correlation between plasma roxithromycin concentration and the change in the pharmacokinetic parameters of midazolam was evaluated using Pearson's correlation coefficient. All the data were analysed by the Systat System for Statistics (Wilkinson 1988). Results are expressed as mean values (SEM). The chosen significance level was $P < 0.05$.

Results

All subjects completed the study. Roxithromycin administration resulted in a 47 % increase in the mean AUC(0-∞) of midazolam ($P < 0.05$) compared to administration of placebo (Fig. 1, Table 1). The mean elimination half-life

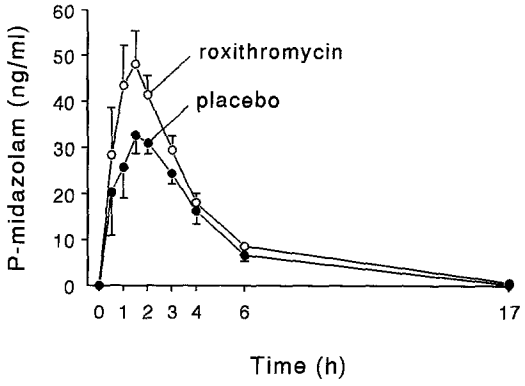

Fig. 1. Plasma concentrations [mean (SEM)] of midazolam after an oral dose of 15 mg following pretreatment with oral roxithromycin (300 mg) or placebo once daily for 6 days in 10 healthy volunteers. *Open circles* concentrations of midazolam after roxithromycin; *solid circles* concentrations of midazolam after placebo

Table 1. The pharmacokinetic parameters [mean (SEM)] of a 15-mg oral dose of midazolam after pretreatment with oral roxithromycin (300 mg) or placebo daily for 6 days in 10 healthy volunteers

Parameter	After placebo	After roxithromycin	*P* value
C_{max} (ng · ml^{-1})	43 (7.2)	59 (7.2)	0.14
t_{max} (h)	1.6 (0.3)	1.5 (0.2)	0.52
AUC (0-∞) (µg · ml^{-1} · min)	8.3 (1.1)	12.2 (1.3)	0.018
$t^1/_2$ (h)	1.7 (0.2)	2.2 (0.3)	0.046

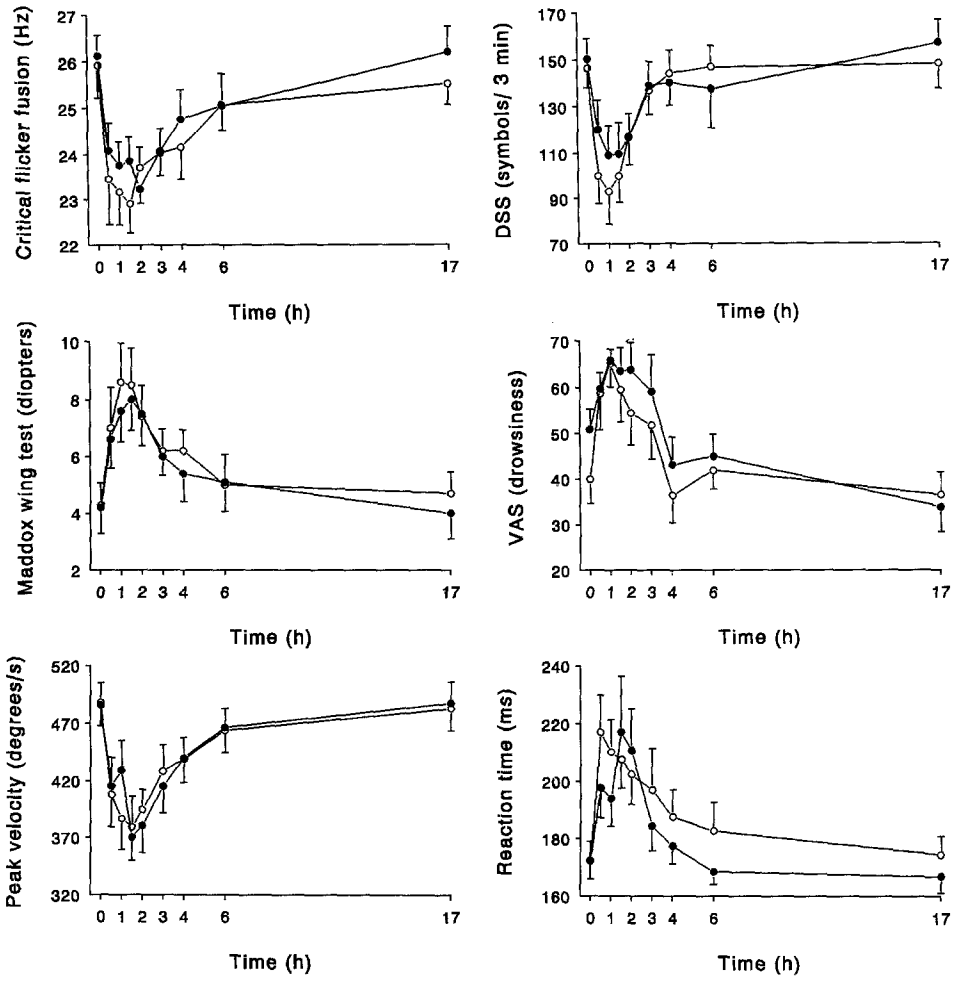

Fig. 2. Results of critical flicker fusion, digit symbol substitution (DSS) and Maddox wing tests, as well as recordings of subjective drowsiness (VAS), peak saccadic velocities and saccadic reaction times after a 15-mg oral dose of midazolam following pretreatment with oral roxithromycin (300 mg) or placebo once daily for 6 days in 10 healthy volunteers [mean (SEM)]. *Open circles* psychomotor effects of midazolam after roxithromycin; *solid circles* psychomotor effects of midazolam after placebo

Table 2. Midazolam maximum effects (E_{max}) and areas under the effect-time curves (AUC(0–6 h), AUC(0–17 h)) for differences from baseline results in psychomotor tests [mean (SEM)]: critical flicker fusion test (CFF), digit symbol substitution test (DSS) and Maddox wing test, subjective drowsiness (VAS), peak saccadic velocity and saccadic reaction time, after a single oral 15-mg dose of midazolam following pretreatment with oral roxithromycin (300 mg) or placebo daily for 6 days in 10 healthy volunteers

Test	Parameter	Treatment		
		Placebo	Roxithromycin	*P* value
CFF	E_{max} [Hz]	–36 (4)	–44 (6)	0.128
	AUC(0–6 h) [Hz·h]	–107 (23)	–113 (25)	0.761
	AUC(0–17 h) [Hz·h]	–163 (56)	–180 (69)	0.757
DSS	E_{max} [symbols]	–57 (8)	–68 (11)	0.103
	AUC(0–6 h) [symbols·h]	–110 (15)	–109 (22)	0.929
	AUC(0–17 h) [symbols·h]	–88 (34)	–101 (45)	0.831
Maddox wing test	E_{max} [diopters]	4.8 (1.3)	4.8 (1.1)	1.000
	AUC(0–6 h) [diopters·h]	11.8 (3.9)	13.4 (3.6)	0.510
	AUC(0–17 h) [diopters·h]	15.6 (8.1)	19.4 (5.7)	0.552
VAS (drowsiness)	E_{max} [mm]	27 (5)	35 (6)	0.291
	AUC(0–6 h) [mm·h]	18 (27)	50 (33)	0.325
	AUC(0–17 h) [mm·h]	–108 (76)	41 (109)	0.308
Peak velocity	E_{max} [deg·s^{-1}]	–130 (7)	–143 (7)	0.238
	AUC(0–6 h) [deg·s^{-1}·h]	–370 (35)	–380 (25)	0.806
	AUC(0–17 h) [deg·s^{-1}·h]	–467 (97)	–543 (94)	0.544
Reaction time	E_{max} [ms]	62 (13)	78 (11)	0.302
	AUC(0–6 h) [ms·h]	86 (37)	144 (20)	0.140
	AUC(0–17 h) [ms·h]	35 (86)	215 (70)	0.163

of midazolam was prolonged from 1.7 h to 2.2 h ($P < 0.05$) by roxithromycin. However, there were no statistically significant differences between the treatments in mean C_{max} and mean t_{max}. The maximum changes of psychomotor performance occurred 1–1.5 h after the administration of midazolam and they coincided fairly well with the respective t_{max} values (Fig. 2). One of the subjects was so drowsy at the 0.5, 1 and 1.5 h time-points during the roxithromycin phase that she could not do all the psychomotor tests. This subject also had the biggest increase in the AUC(0-∞) of midazolam. In the psychomotor tests, roxithromycin administration caused no statistically significant changes in the AUC(0–6 h), the AUC(0–17 h) or the E_{max} values of differences from baseline results (Table 2). However, the paired comparison of the effects at different time points showed significant differences between the treatments in the digit symbol substitution test 0.5 ($P < 0.05$) and 1 h ($P < 0.05$) after the administration of midazolam, but not in the other psychomotor tests.

Compliance was documented by plasma roxithromycin concentrations, which were 1.8 (0.4) $\mu g \cdot ml^{-1}$ on the fifth day of the pretreatment, 4.0 (0.6) $\mu g \cdot ml^{-1}$ immediately before the administration of midazolam on the sixth day and 4.3 (0.4) $\mu g \cdot ml^{-1}$ 2 h after the administration of midazolam. The difference in the pharmacokinetic parameters between the roxithromycin and placebo phases did not correlate with plasma roxithromycin concentrations.

Discussion

In this controlled study roxithromycin clearly affected the pharmacokinetics of a single oral dose of midazolam in young adult subjects. Pretreatment with 300 mg roxithromycin daily resulted in a statistically significant increase in the AUC(0-∞) and the elimination half-life of midazolam. Because midazolam was given orally we could not calculate the clearance and the volume of distribution of midazolam, but we believe that the observed increase in the $t^1/_2$ of midazolam was due to decreased clearance of midazolam, as it is unlikely that roxithromycin would have changed the volume of distribution. Since roxithromycin increased AUC(0-∞) to a greater extent than $t^1/_2$, it is probable that roxithromycin also increased the oral bioavailability of midazolam.

Roxithromycin administration caused a statistically significant change in only one pharmacodynamic parameter. The change in the digit symbol substitution test results was small and occurred only at two time-points. Roxithromycin administration did not alter any other pharmacodynamic parameters. The missing values in psychomotor tests at time-points of high midazolam concentrations during roxithromycin administration in one subject reduced the power of the statistical analysis and increased the possibility of a false negative result. However, from 2 h onwards the pharmacodynamic parameters were available in all subjects. Our results demonstrate that if there was any extra deterioration of psychomotor performance because of coadministration of roxithromycin, it was over within 4 h after the intake of midazolam. The effect of roxithromycin on the pharmacokinetics of midazolam appears to be too small to cause clinically significant changes in the pharmacodynamics of midazolam.

Many macrolide antibiotics have been reported to interact pharmacokinetically with drugs that are mainly metabolised by cytochrome P450 IIIA enzymes. These macrolides inhibit cytochrome P450 IIIA-mediated drug metabolism by forming complexes with the enzymes (Larrey et al. 1983; Periti et al. 1992). Roxithromycin forms complexes with cytochrome P450 in rats, but this only happens after induction of cytochrome P450 enzymes by dexamethasone or troleandomycin (Tinel et al. 1989). It is not yet known which specific cytochrome P450 forms are responsible for the metabolism of roxithromycin. Roxithromycin has been reported to interact with theophylline, leading to a small but statistically significant increase in the C_{max} and the AUC of theophylline (Houin et al. 1992; Saint-Salvi et al. 1987). However, it has no clinically significant interaction with cyclosporin, carbamazepine, oral contraceptives, ranitidine or warfarin (Billaud et al. 1990; Meyer et al. 1990; Paulsen et al. 1988; Saint-Salvi et al. 1987; Young et al. 1989).

The changes in the pharmacokinetics of midazolam caused by roxithromycin could be the result of enzyme inhibition by roxithromycin. The metabolism of midazolam is predominantly mediated by cytochrome P450 IIIA (Kronbach et al. 1989). Cimetidine and ranitidine have been shown to increase the bioavailability of midazolam (Fee et al. 1987). In vitro data indicate that midazolam metabolism is inhibited by many other drugs (Gascon and Dayer 1991). We have previously shown that erythromycin distinctly alters the pharmacokinetics of midazolam and potentiates the psychomotor actions of midazolam hazardously (Olkkola et al. 1993). Now, using the same study design, the effect of roxithromycin on the pharmacokinetics of midazolam proved to be clearly smaller than the effect of erythromycin and appeared less likely to lead to clinically significant changes in the pharmacodynamics of midazolam. Our findings are in good agreement with a previous study, in which an oral dose of roxithromycin clearly increased the effects of midazolam less than a dose of erythromycin (Mattila et al. 1993).

This pharmacokinetic interaction between roxithromycin and midazolam caused some statistically significant changes in the pharmacodynamics of midazolam. These changes are probably not clinically relevant and the interaction should not jeopardise the safe therapeutic use of midazolam. However, to be on the safe side, the lowest effective dose of midazolam is recommended during treatment with 300 mg roxithromycin daily. The number of patients in the study was too small to draw conclusions on the clinical significance of the interaction of roxithromycin with midazolam in specific groups in the population, such as the elderly.

Acknowledgement. The study was supported by Roussel Nordiska Co. Ltd.

References

Aaltonen L, Himberg J-J, Kanto J, Vuori A (1981) The usefulness of radioreceptor assay and chromatography in pharmacokinetic studies. Int J Clin Pharmacol 23: 247–252

Allonen H, Ziegler G, Klotz U (1981) Midazolam kinetics. Clin Pharmacol Ther 30: 653–661

Billaud EM, Guillemain R, Fortineau N, Kitzis MD, Dreyfus G, Amrein C, Kreft-Jais C, Husson JM, Chretien P (1990) Interaction between roxithromycin and cyclosporin in heart transplant patients. Clin Pharmacokinet 19: 499–502

Bond A, Lader MH (1974) The use of visual analogue scales in rating subjective feelings. Br J Med Psychol 47: 211–218

Byatt CM, Lewis LD, Dawling S, Cochrane GM (1984) Accumulation of midazolam after repeated dosage in patients receiving mechanical ventilation in an intensive care unit. BMJ 289: 799–800

Editorial (1988) Midazolam – is antagonism justified? Lancet II: 140–142

Fee JPH, Collier PS, Howard PJ, Dundee JW (1987) Cimetidine and ranitidine increase midazolam bioavailability. Clin Pharmacol Ther 41: 80–84

Gascon M-P, Dayer P (1991) In vitro forecasting of drugs which may interfere with the biotransformation of midazolam. Eur J Clin Pharmacol 41: 573–578

Gibaldi M, Perrier D (1982) Pharmacokinetics. Dekker, New York

Griffiths AN, Marshall RW, Richens A (1984) Saccadic eye movements analysis as a measure of drug effects on human psychomotor performance. Br J Clin Pharmacol 18: 73–82

Hannington-Kiff JG (1970) Measurements of recovery from outpatient general anaesthesia with simple ovular test. BMJ 3: 132–135

Hashiguchi K, Niki Y, Soafima R (1992) Roxithromycin does not raise serum theophylline levels. Chest 102: 653–654

Hiller A, Olkkola KT, Isohanni P, Saarnivaara L (1990) Unconsciousness associated with midazolam and erythromycin. Br J Anaesth 65: 826–828

Houin G, Montastruc JL, Lequellec A, Bonnat C, Lenfant B (1992) A study of interaction of roxithromycin with theophylline. The Vth World Conference on Clinical Pharmacology and Therapeutics, Yokohama, 26–31 July 1992

Kronbach T, Mathys D, Umeno M, Gonzales FJ, Meyer UA (1989) Oxidation of midazolam and triazolam by human liver cytochrome P450 IIIA4. Mol Pharmacol 36: 89–96

Larrey D, Funck-Brentano C, Briel P, Vitaux J, Theodore C, Babany G, Pessayre D (1983) Effects of erythromycin on hepatic drug-metabolizing enzymes in humans. Biochem Pharmacol 32: 1063–1068

Mattila MJ, Idänpään-Heikkilä JJ, Törnwall M, Vanakoski J (1993) Oral single doses of erythromycin and roxithromycin may increase the effects of midazolam on human performance. Pharmacol Toxicol 73: 180–185

Meyer B, Muller F, Wessels P, Maree J (1990) A model to detect interactions between roxithromycin and oral contraceptives. Clin Pharmacol Ther 47: 671–674

Olkkola KT, Aranko K, Luurila H, Hiller A, Saarnivaara L, Himberg J-J, Neuvonen PJ (1993) A potentially hazardous interaction between erythromycin and midazolam. Clin Pharmacol Ther 53: 298–305

Paulsen O, Nilsson LG, Saint-Salvi B, Manuel C, Lunell E (1988) No effect of roxithromycin on pharmacokinetic or pharmacodynamic properties of warfarin and its enantiomers. Pharmacol Toxicol 63: 215–220

Periti P, Mazzei T, Mini E, Novelli A (1992) Pharmacokinetic drug interactions of macrolides. Clin Pharmacokinet 23: 106–131

Saint-Salvi B, Tremblay D, Surjus A, Lefebure MA (1987) A study of the interaction of roxithromycin with theophylline and carbamazepine. J Antimicrob Chemother 20 [Suppl B]: 121–129

Smith JM, Misiak H (1976) Critical flicker frequency (CFF) and psychotropic drugs in normal human subjects – A review. Psychopharmacology 47: 175–182

Stone BI (1984) Pencil and paper tests – sensitivity to psychotropic drugs. Br J Clin Pharmacol 18: 15–20

Tinel M, Descatoire V, Larrey D, Loeper J, Labbe G, Letterton P, Pessayre D (1989) Effects of clarithromycin on cytochrome P-450. Comparison with other macrolides. J Pharmacol Exp Ther 250: 746–751

Wilkinson L (1988) Systat: system for statistics. Systat, Evanston, Illinois

Young RA, Gonzalez JP, Sorkin EM (1989) Roxithromycin: a review of it's antibacterial activity, pharmacokinetic properties and clinical efficacy. Drugs 37: 8–41