Integrated pharmacokinetics and pharmacodynamics of Ro 48–8684, a new benzodiazepine, in comparison with midazolam during first administration to healthy male subjects

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Aims This study aimed to investigate the pharmacodynamics and pharmacokinetics of ascending doses of Ro 48–8684, compared with midazolam, in healthy subjects during first administration to man.

Methods The study was double-blind and five-way crossover (three ascending doses, placebo, fixed midazolam dose), performed in two groups of five males. Ro 48–8684 was infused in doses of 0.1–0.3–1 mg in the first group, and 1–3–10 mg in the second, with different infusion rates (expressed as mg min^{-1}) among doses. Midazolam was infused at 0.1 mg^{-1} kg. Infusions were stopped after 20 min or if sedation became too strong for proper performance of saccadic eye movements. Pharmacokinetics and pharmacodynamics and their relationships were evaluated as described in the companion article.

Results Ro 48–8684 caused dose-dependent sedation. No serious adverse events occurred. The volume of distribution and clearance of Ro 48–8684 were larger than of midazolam (337 ± 114 *vs* 50 ± 12 l and 2.4 ± 0.5 *vs* 0.47 ± 0.11 l min^{-1}, resp). The recovery of saccadic eye movements from equal levels of sedation was on average almost half an hour faster for Ro 48–8684 than for midazolam, with considerable interindividual differences (range 2, 55 min). The doses of Ro 48–8684 leading to the same clinical endpoint as midazolam were comparable, but the corresponding predicted effect compartment concentrations of Ro 48–8684 were on average 2.6 times lower (range 1.5, 4.9 times). The slope of the linear concentration–effect-relationship for saccadic peak velocity was on average 2.2 times steeper for 10 mg Ro 48–8684 than for midazolam (range 1.3, 3.3). The slope decreased on average 4.4-fold (range 1.6, 7.3 times), with doses of Ro 48–8684 increasing from 1 to 10 mg. The metabolite Ro 61–2466 had a longer half-life than the parent compound Ro 48–8684. The influence of this metabolite during prolonged administration should be further investigated.

Conclusions These results show that Ro 48–8684 has a considerably shorter duration of action than midazolam. There may be a reduction of sensitivity to Ro 48–8684 with repeated administration of rising doses due to as yet undetermined factors.

Keywords: pharmacokinetics, pharmacodynamics, benzodiazepines, Ro 48–8684, midozolam

Introduction

Ro 48–8684, 3-(5-dipropylaminomethyl-oxazol-2-yl)-8-fluoro-5-methyl-5, 6-dihydro-4H-imidazo [1, 5-a] [1, 4] benzodiazepin-6-one, is a new water soluble full agonist at the benzodiazepine receptor which is being developed for induction of sleep and conscious sedation (Figure 1). The present study represents the counterpart of the entry-into-man-study of Ro 48–6791, which is described elsewhere in this issue of the journal [1]. Both new benzodiazepines were investigated according to the same basic study design and methodology, but with different doses and in different subjects. The aim of the present study was to determine the tolerability, pharmacodynamics, and pharmacokinetics of Ro 48–8684 during its first administration in humans, compared with midazolam, using saccadic eye movements as the primary pharmacodynamic effect measure.

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Methods

Subjects

Ten healthy male subjects (age 22–32 years, weight 67–89 kg) were selected. In- and exclusion criteria were the same as in the counterpart of this study [1]. No subject participated in both studies. All subjects gave written

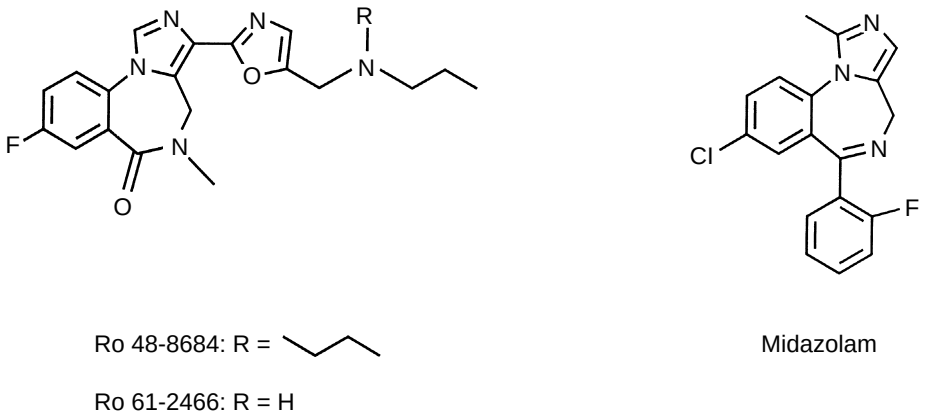

Figure 1 Chemical structure of Ro 48–8684 and its monopropyl metabolite Ro 61–2466.

informed consent. The study was approved by the Ethics Committee of Leiden University Hospital.

Design

The design of the study was described previously [1]. Briefly, ascending doses of Ro 48–8684 were studied in two separate groups, using a double-blind five-way crossover design, with treatment on 5 consecutive days (Ro 48–8684 0.1–0.3–1 mg and 1–3- 10 mg, with randomized placebo and midazolam 0.1 mg^{-1} kg in each block). The study drugs were infused over 20 min, or until sedation was too strong to measure saccadic eye movements adequately.

Assessments

Assays Blood samples of 4.5 ml were obtained for determination of Ro 48–8684 and its monopropyl metabolite Ro 61–2466 (Figure 1) or midazolam. Sampling frequency and storage conditions pending analysis were the same as for the Ro 48–6791 study [1]. A sensitive and specific assay was used for simultaneous quantification of Ro 48–8684 and Ro 61–2466 in plasma samples, using liquid-liquid extraction with n-butylchloride/4% dichloromethane for isolation of the analytes from the matrix, and reversed phase h.p.l.c. for separation combined with ion spray tandem mass spectrometry for detection in the selected reaction monitoring mode. The mean precision and accuracy for Ro 48–8684 and Ro 61–2466 in the concentration range 0.05–50 $\mu g l^{-1}$ were 4.5% and 101%, and 5.8% and 100%, respectively. The lower limits of quantification for Ro 48–8684 and Ro 61–2466 were 50 $ng l^{-1}$ and 100 $ng l^{-1}$, respectively, using a 0.5 ml aliquot of plasma. Midazolam concentrations were determined as described previously [2].

Pharmacodynamics Saccadic peak velocity and EEG β-power were assessed at the same time intervals as blood samples for drug assays were obtained. The measurement scheme and the methodology were described in detail in the companion article [1].

Evaluation

Pharmacokinetics From the concentration-time-profiles of Ro 48–8684, Ro 61–2466 and midazolam, maximum plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC), systemic plasma clearance (CL), volume of distribution at steady state (V_{ss}), and terminal elimination half-life ($t_{1/2,z}$) were derived by model-dependent and model-independent methods, using the programme TOPFIT [3]. Plasma concentrations of Ro 48–8684 and midazolam were fitted to an open two-compartment pharmacokinetic model with a constant weight-factor ($w = 1$).

Pharmacodynamics The same definitions were used as in the Ro 48–6791 study for maximum drug effect (E_{min} for saccadic peak velocity, E_{max} for β-power), threshold of action (below 50% of E_{min} for saccadic peak velocity, and above 20% of E_{max} for β-power with a minimum of 25% above baseline, determined from the individually largest response on all occasions), onset and duration of action (relative to the threshold), and total treatment effect (area under the effect curve (AUE) corrected by subtraction of baseline values).

Pharmacokinetic/pharmacodynamic relationships The same methods as described previously [1] were used to decide for each subject whether a linear or E_{max}-type pharmacological model would be most appropriate. In brief, both models were applied to the individual data, and the Extra Sum of Squares principle [4] with *t*-statistics was used to determine whether the variance of the fit was significantly reduced by the more complicated model. All models included an effect compartment. Individual concentration/effect parameters were estimated with NONMEM software (Version IV, NONMEM Project Group, UCSF, San Fransisco, CA), tuned to calculate ordinary least squares estimates (weight factor = 1) using previously determined pharmacokinetic parameter estimates.

Equipotent doses of Ro 48–8684 and midazolam were identified by comparison of individual effect-time profiles with respect to saccadic peak velocity. The predicted effect compartment concentrations calculated at the timepoint when subjects lost the ability to perform saccadic eye movements were used for a comparison of potency between Ro 48–8684 and midazolam.

Statistical analyses The statistical methods were the same as described in the companion article of this study, described elsewhere in this issue of the journal [1].

Results

Clinical events

All 10 subjects completed the study according to the protocol, without any serious adverse events. Three of ten subjects reported adverse events with placebo, and all with midazolam. Manifestations of a central depressant effect of Ro 48–8684 (mostly somnolence, fatigue and anterograde amnesia) increased with dose. The clinical effects of midazolam were similar to those of the highest dose of midazolam. All events resolved completely, but sedation reported to the question 'how do you feel' disappeared somewhat sooner after Ro 48–8684 10 mg (32–95 min) than after midazolam (70–163 min). There were no evident effects on vital signs, ECG, pulse oximetry or routine laboratory tests.

Drug doses

The mean dose of midazolam administered was 6.1 mg (range 3.4, 8.4 mg). Ro 48–8684 was fully administered to all subjects up to and including the 3 mg dose. The infusion of Ro 48–8684 10 mg was prematurely terminated in four of five subjects. The mean administered dose of Ro 48–8684 10 mg was 7.5 mg (range 5.5, 10.0) mg.

Pharmacokinetics

Assessment of the pharmacokinetic parameters by model-independent analysis and model-dependent compartmental analysis yielded very similar results. Table 1 summarizes the pharmacokinetic parameters of Ro 48–8684 and midazolam determined by compartmental analysis. Figure 2 presents the average plasma concentration-time profiles of Ro 48–8684 and Ro 61–2466, after the scheduled dose of 10 mg Ro 48–8684. Regression analysis indicated a linear relationship between AUC and administered dose ($r^2 = 0.93$). Analysis of variance did not reveal statistically significant differences in dose-normalized C_{max} and AUC values. The metabolite Ro 61–2466 appeared simultaneously with the parent drug in plasma. Peak concentrations were reached in 2.8 h on average. The concentrations of the metabolite surpassed those of the parent compound within approximately 90 min after the start of the infusion. The ratio of C_{max} of Ro 61–2466 to the dose of Ro 48–8684 was independent of the administered dose (ratio $1.9 \pm 0.6\ \mu g\,l^{-1}\,mg^{-1}$). Due to insufficiently long blood sampling, the elimination half-life of the metabolite could not be estimated.

Table 1 Pharmacokinetic parameters of Ro 48–8684 and midazolam.

	Ro 48–8684	*Midazolam*
CL ($l\,min^{-1}$)	2.4 ± 0.5	0.47 ± 0.11
V_{ss} (l)	337 ± 114	50 ± 12
$t_{1/2,\lambda_1}$ (min)	4.2 ± 1.6	3.2 ± 1.0
$t_{1/2,z}$ (min)	121 ± 38	88 ± 18

Data are mean values ± s.d. ($n = 30$ for Ro 48–8684, $n = 10$ for midazolam). CL, systemic plasma clearance; V_{ss}, volume of distribution at steady state;
$t_{1/2,\lambda_1}$ and $t_{1/2,z}$, half-life of distribution and elimination, respectively.

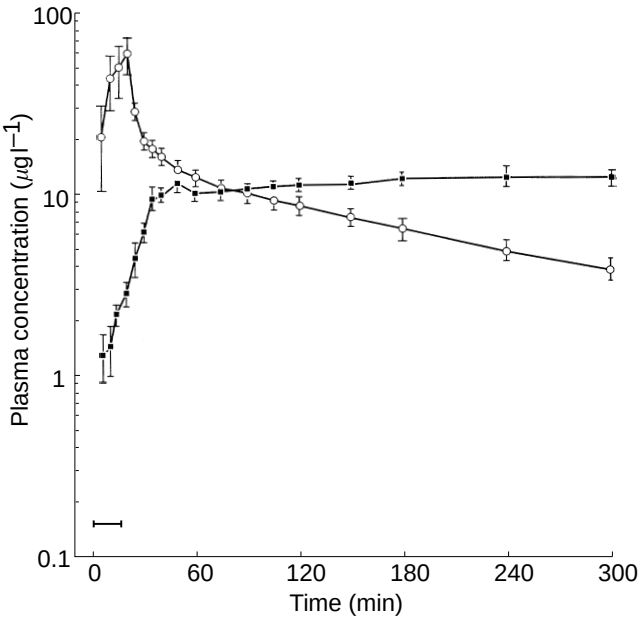

Figure 2 Plasma concentration–time profiles of Ro 48–8684 (○) and Ro 61–2466 (■) for the scheduled 10 mg dose. Data are represented as the mean ± s.e.mean ($n = 5$). The average duration of i.v. infusion was 15.0 min (range 11, 20 min).

Pharmacodynamics

Saccadic peak velocity Ro 48–8684 caused a dose-related decrease in saccadic peak velocity, as illustrated by the average effect-time profiles (Figure 3) and by the changes in E_{min}, AUE and onset and duration of effect with rising doses (Table 2). Within subjects, Ro 48–8684 was administered in 1.2 times the amount of midazolam (range 0.8, 1.6) until sedation became too severe for proper performance of saccadic eye movements. The mean amount that was infused during these occasions was 7.5 mg (range 5.5, 10.0 mg) of Ro 48–8684 and 6.1 mg (4.0, 8.7 mg) of midazolam. At

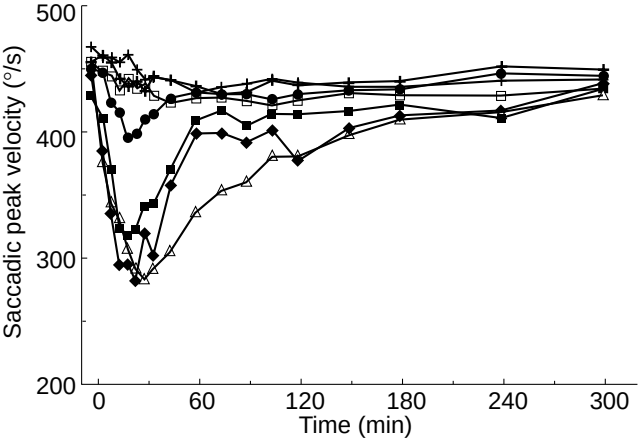

Figure 3 Average profiles of saccadic peak velocity per treatment: Ro 48–8684 *vs* midazolam and placebo. Δ midazolam ($n = 10$); □ placebo ($n = 10$); + Ro 48–8684 0.1 mg ($n = 5$); + Ro 48–8684 0.3 mg ($n = 5$); ● Ro 48–8684 1 mg ($n = 10$); ■ Ro 48–8684 3 mg ($n = 5$); ◆ Ro 48–8684 10 mg ($n = 5$).

Table 2 Pharmacodynamic effect parameters (mean ± s.d.) of saccadic peak velocity for Ro 48–8684 compared with midazolam.

	Ro 48–8684 1 mg	n	*Ro 48–8684 3 mg*	n	*Ro 48–8684 10 mg*	n	*Midazolam*	n	*Difference Ro 48–8684 10 mg—Midazolam* *Mean*	*95% CI*
Onset of action (min)	16	1	15 ± 6	5	7 ± 1	5	6 ± 4	5	2	−2, 6
Duration of action (min)	16	1	17 ± 18	5	35 ± 11	5	64 ± 20	5	−29	−56, −2
E_{min} ($^{o}\cdot s^{-1}$)	363 ± 68	5	308 ± 33	5	269 ± 43	5	249 ± 10	5	20	−35, 75
AUE ($\cdot 10^{3}\ ^{o}\ s^{-1}$ min)	5.7 ± 8.0	5	6.8 ± 3.7	5	14.3 ± 3.5	5	19.8 ± 5.9	5	−5.5	−11.0, 0.1

these equipotent doses, the duration of action of Ro 48–8684 was 41% (range 5, 66%) shorter than of midazolam.

EEG β-power Ro 48–8684 caused a dose-related increase in EEG β-power, as shown in Figure 4 and Table 3 (E_{max} and AUE). Maximum β-power (E_{max}) was on average 44% (range 6, 94%) higher with midazolam than with the highest dose of Ro 48–8684, even though the maximum effects on saccadic peak velocity were comparable. The more pronounced effect of midazolam on β-power was also evident from the significantly longer duration of action and the larger total treatment effect (AUE).

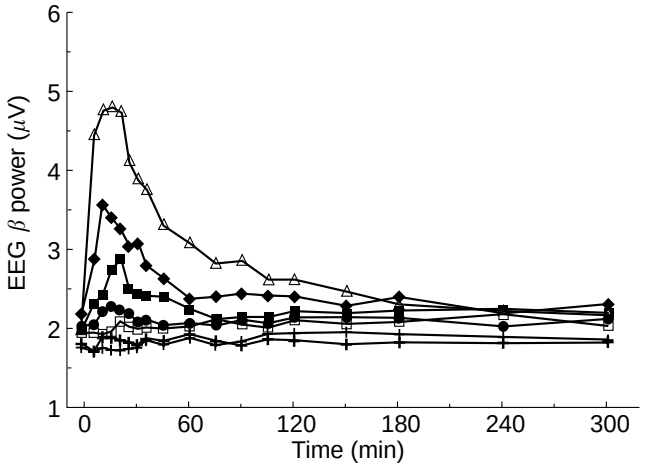

Figure 4 Average profiles of β-power per treatment: Ro 48–8684 *vs* midazolam and placebo. Δ midazolam ($n=10$); □ placebo ($n=10$); + Ro 48–8684 0.1 mg ($n=5$); + Ro 48–8684 0.3 mg ($n=5$); ■ Ro 48–8684 1 mg ($n=10$); ■ Ro 48–8684 3 mg ($n=5$); ◆ Ro 48–8684 10 mg ($n=5$)

Pharmacokinetic/pharmacodynamic relationships

Saccadic peak velocity Figure 5 illustrates the average saccadic peak velocity *vs* the average predicted plasma and effect compartment concentrations for midazolam and the three highest doses of Ro 48–8684. The concentration–effect parameters for the individually best fitting pharmacological models are shown in Table 4. Only one of the ten concentration–effect profiles of midazolam was better described by an E_{max} than a linear model, with a value for E_{max} of $-159^{o}\ s^{-1}$, and for EC_{50} of 35 $\mu g\ l^{-1}$. All Ro 48–8684 treatments yielded linear profiles. Indications of hysteresis were obtained clearly in four and less clearly in three of the ten midazolam profiles. This is demonstrated by the hysteresis loop of the average midazolam plot (Figure 5, left panel); this figure also shows the increasingly evident hysteresis loops with rising doses of Ro 48–8684.

The slopes of the concentration–effect-relationships were steeper with Ro 48–8684 10 mg than with midazolam in all cases. Mean slopes (and ranges) were −4.9 (−8.9, −2.5) and −2.3 (−2.9, −1.0) $^{o}\ s^{-1}\ \mu g^{-1}\ l$, respectively. Within subjects, slopes differed on average by a factor 2.2 (range 1.3, 3.3; $P=0.043$). Similar differences were found between the predicted effect compartment concentrations of the two drugs at the time when voluntary saccadic eye movements were first lost. These concentrations were 34.6 $\mu g\ l^{-1}$ (range 17.9, 56.0 $\mu g\ l^{-1}$) for the highest dose of Ro 48–8684, and 75.2 $\mu g\ l^{-1}$ (range 60.4, 86.9 $\mu g\ l^{-1}$) for midazolam. Within subjects, the predicted effect compartment concentrations at the end of the infusion were on average 2.6 times higher (range 1.5, 4.9 times) for midazolam than for Ro 48–8684 ($P=0.008$).

In all subjects except one, the slope of the concentration–effect relationship decreased systematically with ascending

Table 3 Pharmacodynamic effect parameters (mean ± s.d.) of EEG β-power for Ro 48–8684 and midazolam.

	Ro 48–8684 1 mg	n	*Ro 48–8684 3 mg*	n	*Ro 48–8684 10 mg*	n	*Midazolam*	n	*Difference Ro 48–8684 10 mg—Midazolam* *Mean*	*95% CI*
Onset of action (min)	12	1	10 ± 4	3	10 ± 7	5	2 ± 0	5	−8	−1, 16
Duration of action (min)	1	1	25 ± 2	3	19 ± 17	5	78 ± 41	5	−59	−113, −6
E_{max} (μV)	3 ± 1	5	3 ± 1	5	4 ± 1	5	5 ± 1	5	−1.5	−3, −0
AUE (μV min)	20 ± 27	5	87 ± 48	5	70 ± 33	5	173 ± 70	5	−103	−169, −37

Table 4 Parameters of the linear concentration–effect relationships for saccadic peak velocity.

		Linear model slope ($^{\circ} \cdot s^{-1}\ \mu g^{-1}\ l^{-1}$)		$t_{1/2k_{eo}}$ (min)	
Treatment	n/*total*	*Median*	*Range*	*Median*	*Range*
Midazolam	9/10	−2.1	−2.9, −1.0	1.5	0.0, 8.6
Ro 48–8684 1 mg	10/10	−18.2	−43.2, −1.9	1.1	0.0, 17.4
Ro 48–8684 3 mg	5/5	−12.3	−19.6, −5.3	0.1	0.0, 3.3
Ro 48–8684 10 mg	5/5	−4.1	−8.9, −2.5	1.1	0.0, 5.1

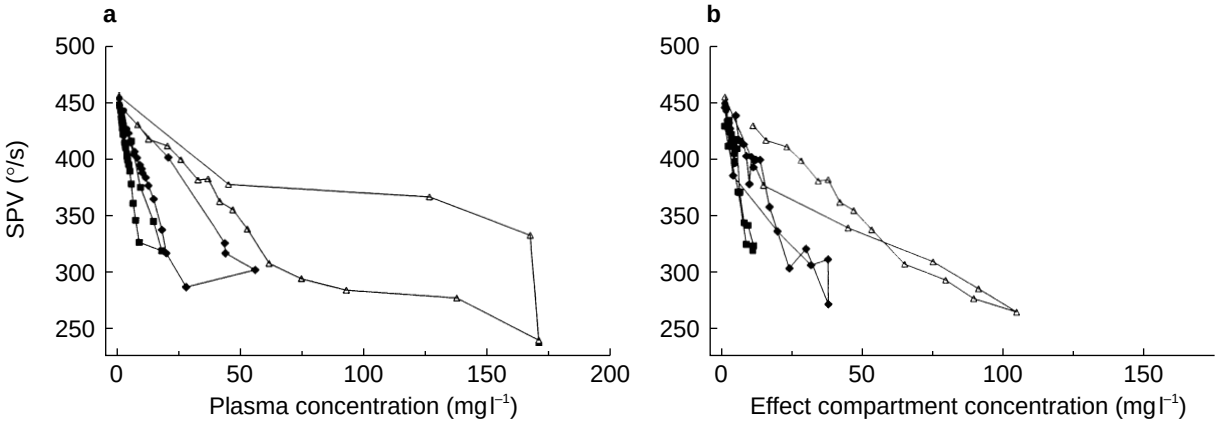

Figure 5 Average saccadic peak velocity (SPV) *vs* average corresponding predicted plasma (a) and effect compartment (b) concentration for Ro 48–8684 1 mg (●), 3 mg (■), 10 mg (◆), and midazolam Δ.

doses of Ro 48–8684 ($P=0.015$). Within subjects, slopes were on average 4.4 times (range 1.6, 7.3 times) steeper for the 1 mg than for the 10 mg dose of Ro 48–8684. This is also illustrated by the steeper average slope for Ro 48–8684 1 mg and 3 mg, compared with the highest dose (*c.f.* Figure 5, right panel). The intercept showed no dose-dependency.

β-power Figure 6 illustrates the average plots for β-power *vs* predicted plasma and effect compartment concentrations for midazolam and the three highest doses of Ro 48–8684. The concentration–effect-parameters for the individually determined pharmacological models are shown in Table 5. Neither Ro 48–8684 nor midazolam showed hysteresis for β-power. The apparent proteresis loop for midazolam in the right panel is due to averaging and was not shown by any individual subject; all other plots are reflective of the individual results. Two of ten subjects showed indications of a maximal effect with midazolam, with E_{max}-values of 3.3 and 5.2 μV and EC_{50} of 65 and 74 $\mu g\, l^{-1}$. All subjects treated with the highest dose range of Ro 48–8684 displayed linear concentration–effect-relationships with both benzodiazepines. The slope of the highest dose of Ro 48–8684 did not differ significantly from that of midazolam (Table 5; $P=0.22$).

Discussion

The present study compared the pharmacokinetic and pharmacodynamic properties of rising doses of the new benzodiazepine receptor agonist Ro 48–8684, with those of a single dose of midazolam. This comparison was possible,

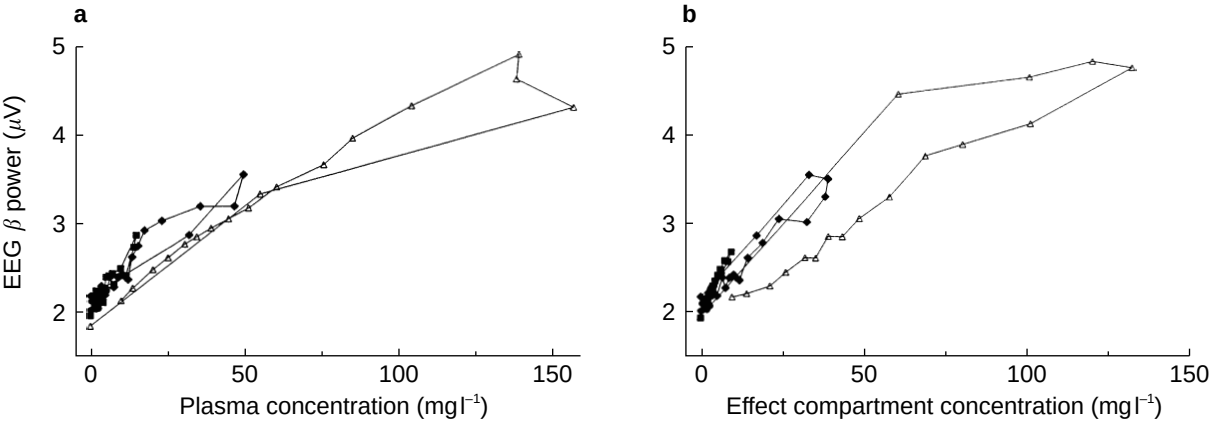

Figure 6 Average β-power *vs* average corresponding predicted plasma (left panel) and effect compartment (right panel) concentration of Ro 48–8684 1 mg (●), Ro 48–8684 3 mg (■), Ro 48–8684 10 mg (◆), and midazolam Δ.

Table 5 Parameters of the linear concentration–effect relationships for EEG β-power.

		Linear model			
		Slope ($\mu V\,ng^{-1}\,ml$)		$t_{1/2,k_{eo}}$ (*min*)	
Treatment	n/*total*	*Median*	*Range*	*Median*	*Range*
Midazolam	8/10	0.03	0.01, 0.06	0.0	0.0, 0.1
Ro 48–8684 1 mg	10/10	0.04	0.00, 0.15	1.0	0.1, 30.2
Ro 48–8684 3 mg	5/5	0.11	0.03, 0.18	0.1	0.0, 205
Ro 48–8684 10 mg	5/5	0.03	0.01, 0.10	0.0	0.0, 1.9

because in all subjects the same clinical endpoint was reached with the highest dose of Ro 48–8684 and midazolam. This allowed a comparison of the potencies of the two drugs, and of the onset and duration of their effects. Loss of voluntary saccadic eye movements was reached on average after 15 min of infusion for both midazolam and the highest dose of Ro 48–8684. Onset of action, individually defined as the time needed to reach half the maximum effect on saccadic peak velocity, also differed little between the two benzodiazepines. The recovery, however, was on average almost 0.5 h faster with Ro 48–8684 than with midazolam. This difference is likely to be of clinical significance.

The pharmacokinetics of Ro 48–8684 were linear, and could be adequately described with a two-compartment open model. Volume of distribution and clearance were five to seven-fold larger than for midazolam, whereas variability was comparable. With an *ex vivo* blood/plasma ratio of 1.36, a mean blood clearance of 1.7 $l\,min^{-1}$ was calculated for Ro 48–8684. This is close to the normal liver blood flow, and more than three-fold higher than for midazolam [2, 5]. Formation of the metabolite Ro 61–2466 was in accordance with linear pharmacokinetics. Ro 61–2466 concentrations remained at a plateau for a relatively long time after termination of the infusion. The elimination half-life could not be determined due to lack of sufficient data points, but it appeared to be markedly longer than of the parent compound.

The doses of Ro 48–8684 and midazolam that were administered until loss of voluntary saccadic eye movements were similar. At first sight, this would suggest that the potencies of the two benzodiazepines are also comparable. The corresponding predicted effect compartment concentrations, however, were on average 2.6 times higher for midazolam than for Ro 48–8684. Similar differences were found after comparison of the concentration–effect profiles: slopes of these consistently linear profiles were on average 2.2 times as steep with Ro 48–8684 as with midazolam. This similarity suggests that predicted effect compartment concentrations at comparable clinical effects can be used to compare the potencies of compounds, even if their concentration–effect profiles are heterogeneous. This has the advantage over a comparison of potencies based on equipotent doses that pharmacokinetic differences are accounted for: in the present study equipotent doses of midazolam and Ro 48–8684 were similar, but equipotent predicted effect compartment concentrations differed considerably. Although potencies could be compared on the basis of plasma instead of predicted effect compartment concentrations, this does not take account of hysteresis.

A comparison of the consistently linear concentration–effect-relationships of ascending doses of Ro 48–8684 showed that the slopes for saccadic peak velocity were on average 4.4 times as steep with 1 mg as with 10 mg (*c.f.* Figure 5 and Table 4). Thus, sensitivity to Ro 48–8684 seemed to decrease with repeated administration of rising doses. For safety reasons, the doses of the new benzodiazepine in this entry-into-man study were administered in ascending order. This resulted in potentially confounding factors such as a time-bias, increasing velocity and quantity of drug administration and repetitive dosing. However, these factors would also apply to the study with Ro 48–6791, in which no significant change in slope was observed [1]. Hence, it is more likely that factors specific to Ro 48–8684, such as the development of tolerance or metabolite formation, have contributed to the reduction of sensitivity to increasing doses. In various animal species, the identified monopropyl metabolite Ro 61–2466 appears to be about tenfold less active than the parent compound Ro 48–8684 [6], but its antagonistic properties or the formation of other active metabolites in man have not been studied. The pharmacokinetic and pharmacodynamic properties of Ro 61–2466 and possible other metabolites require further investigations, in the light of a prolonged or repeated application of Ro 48–8684, as anticipated in intensive care practice. The possibilities of competing metabolites, and a stronger than expected sedation when Ro 48–8684 is administered to naive subjects, should be taken into account in future studies.

The present study was the counterpart of a study with another new benzodiazepine, Ro 48–6791, described elsewhere in this issue of the journal [1]. Although the two studies were not designed for a direct comparison of Ro 48–6791 and Ro 48–8684, a general comparison is allowed because of the very similar study designs, and the lack of obvious differences in midazolam results between the two study groups. The safety and effect profiles of both drugs were similar to midazolam. Both Ro 48–6791 and Ro 48–8684 had a several-fold larger volume of distribution and a much higher clearance, compared with midazolam. Ro 48–6791 appeared to be several-fold more potent than Ro 48–8684, judged from the potencies relative to midazolam. The main differences between the two compounds appeared to be pharmacodynamic. Equipotent doses of Ro 48–6791 had a duration of action similar to midazolam, whereas recovery from Ro 48–8684 was almost half an hour faster. The potency of Ro 48–6791 did not change with administration of rising doses, whereas the sensitivity to ascending doses of Ro 48–8684 appeared to decrease. These results should facilitate the design of further studies in the clinical development of one or both of these new benzodiazepines.

This work was sponsored by F. Hoffmann-La Roche Ltd, Basel, Switzerland.

References

1 Dingemanse J, Van Gerven JMA, Schoemaker RC, *et al*. Integrated pharmacokinetics and pharmacodynamics of Ro 48–6791, a new benzodiazepine, in comparison to midazolam during first administration to healthy male subjects. *Br J Clin Pharmacol* 1997; **44**: 477–486.

2 Heizmann P, Eckert M, Ziegler WH. Pharmacokinetics and bioavailability of midazolam in man. *Br J Clin Pharmacol* 1983; **16**: 43S–49S.

3 Heinzel G, Woloszczak W, Thomann P. *TOPFIT version 2.0: Pharmacokinetic and pharmacodynamic data analysis system*. Stuttgart: Gustav Fischer Verlag, 1993.

4 Bates DM, Watts DG. *Nonlinear regression analysis and its applications*. Wiley & Sons, New York, 1988, pp 103–104.

5 Allonen H, Ziegler G, Klotz U. Midazolam kinetics. Clin Pharmacol Ther 1981; **30**: 653–661.

6 Investigational drug brochure. F.Hoffmann-La Roche, Basel, 1994.

(*Received 22 July 1996, accepted 3 July 1997*)