First Human Administration of MR04A3

A Novel Water-soluble Nonbenzodiazepine Sedative


ABSTRACT

Background: JM-1232(–), (–)-3-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-2-phenyl-3,5,6,7-tetrahydrocyclopenta[f]isoindol-1(2H)-one, molecular formula, C_{24}H_{27}N_{3}O_{2}; molecular weight, 389.49, is a novel isoindoline water-soluble benzodiazepine receptor agonist with favorable anesthetic/sedative properties in animals. MR04A3 is a 1% aqueous presentation of JM-1232(–).

Methods: In Step 1, healthy male volunteers received 10-min infusions of MR04A3, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/kg, with three MR04A3 subjects and one placebo subject per dose concentration. In Step 2, doses were 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, and 0.4 mg/kg over 1 min with six MR04A3 subjects and one placebo subject per dose concentration.

Results: Hypnotic effects of MR04A3 were seen at all dose concentrations in Step 1 and at doses of 0.075 mg/kg or more in Step 2. Central nervous system effect was seen at all dose concentrations with larger doses of MR04A3 producing a deeper and longer reduction in bispectral index. Ramsay sedation scores were increased with higher doses causing sedation and then unresponsiveness. The adverse event profile of subjects receiving MR04A3 was similar to that of subjects given placebo except that some subjects receiving MR04A3 developed upper airway obstruction while sedated. This responded to simple maneuvers (i.e., chin lift). Changes in systolic arterial blood pressure and heart rate were minimal.

Conclusions: MR04A3 is hypnotic in man with a satisfactory hemodynamic and safety profile.

What This Article Tells Us That Is New

• Water-soluble benzodiazepines produce dose-dependent sedation, but have active metabolites which can result in prolonged effect with prolonged administration
• MR04A3 is a nonbenzodiazepine, but targets the same molecular site as benzodiazepines to produce sedation in animals

ENZODIAZEPINES are used extensively for human sedation with indications ranging from brief procedures to prolonged periods of critical care. Diazepam is presented in a lipid emulsion and has an active metabolite desmethyl-diazepam. Diazepam was used widely for intravenous sedation until the introduction of the water-soluble midazolam. Although the solubility of midazolam avoids the use of a lipid or Cremofo vehicle, its onset is slower than diazepam.1 Midazolam has an active metabolite α-hydroxy-midazolam and is prone to accumulation when infused over prolonged periods.

What We Already Know about This Topic

• In a small number of healthy subjects, MR04A3 produced dose-dependent sedation with rapid onset and resolution, accompanied by upper airway obstruction at deep levels of sedation.
• These results support continued investigation of this novel compound for sedation.

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longed periods with subsequent slow recovery. Blood-brain equilibration of midazolam is slower than that of diazepam with $t_{1/2\text{keo}}$ for midazolam being 2.5–3 times greater for midazolam than for diazepam. However, comparative clinical studies suggest that differences between the two agents are modest and inconsistent. Attempts to develop new water-soluble benzodiazepines with properties superior to midazolam have been unsuccessful. Propofol is widely used as an anesthetic and sedative; however, it causes pain on injection and is typically presented in lipid formulations which may support bacterial growth. Reformulation of propofol in nonlipid vehicles remains the subject of intense development activity.

In 1997, Maruishi started a screening program to search for water-soluble compounds with nonbenzodiazepine structures and favorable anesthetic/sedative properties. Among the compounds synthesized, a water-soluble benzodiazepine agonist JM-1232(−) showed the best efficacy and safety profile. MR04A3 is an aqueous presentation of JM-1232(−). JM-1232(−) is a novel isoindoline hypnotic [(−)-3-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-2-phenyl-3,5,6,7-tetrahydrocyclopenta[f]isoindol-1(2H)-one; molecular formula, C$_{24}$H$_{27}$N$_3$O$_2$; molecular weight, 389.49] (fig. 1).

Although JM-1232(−) has a nonbenzodiazepine structure, it binds to the central benzodiazepine binding site of the γ-aminobutyric Acid receptor and acts as an agonist. The effects of JM-1232(−) are inhibited by flumazenil. In mice, the 50% hypnotic dose (HD$_{50}$) was estimated at 3.12 mg/kg whereas the therapeutic index (LD$_{50}$/HD$_{50}$) was more than 38.5. Intrathecal and intraperitoneal administration of JM-1232(−) is noiceptive in rat thermal tail withdrawal response and tail pressure models. This nociceptive effect was reversed by flumazenil but not by naloxone. Standard preclinical safety and toxicology studies were conducted, and their outcomes were compatible with progression to human studies.

We aimed to assess the safety, efficacy, and tolerability of MR04A3 (a 1% solution of JM-1232(−)) and evaluate its pharmacokinetics and pharmacodynamics when administered to healthy male subjects.

**Materials and Methods**

**Ethical Approval and Research Environment**

The protocol for this investigation was approved by the Independent Ethics Committee, Plymouth, England, United Kingdom (Non-National Health Service Phase 1) and registered with EudraCT Number 2007-003791-18. All subjects gave written informed consent. The study was conducted in a specialist clinical trials unit with standard equipment for induction and maintenance of anesthesia and subsequent supervised recovery. A medically qualified anesthetist was present for every administration of sedative or placebo.

**Inclusion and Exclusion Criteria**

Subjects were recruited by local advertisement and screened before enrollment. We studied males aged 18–35 yr, 50–90 kg, who were in good health based upon medical history, physical examination, respiratory function test, hematology and clinical chemistry, 12-lead echocardiography and exercise test performed at the screening visit.

Subjects avoided medications, vitamins, or herbal remedies for 21 days before drug administration, abstained from strenuous exercise, alcohol, or caffeine for 48 h before admission, and fasted for 6 h before drug administration.

**Study Drug Administration**

This was a dose escalation study in which single intravenous doses of MR04A3 solution or saline placebo were administered over 10 min, Step 1 or 1 min, Step 2. The initial dose was chosen to allow a substantial safety margin based on results from the nonclinical toxicity studies. Step 1 doses were 0.05, 0.1, 0.2, 0.4, and 0.8 mg/kg, with three MR04A3 subjects and one placebo subject per dose concentration; Step 2 doses were 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, and 0.4 mg/kg with six MR04A3 subjects and one placebo subject per dose concentration. An Efficacy and Safety Judgment Committee evaluated data from each dose concentration before progression to the next dose or step. Within each dose group, allocation to MR04A3 or placebo was determined by a random code. Subjects were blinded to treatment allocation and dose.

MR04A3 intravenous injection was reconstituted from a lyophilized powder with physiologic saline to form a clear and colorless solution. The dose to be administered was contained in a volume of 20 ml.
**Study Procedure**

Arterial and venous cannulae for blood sampling were placed in one forearm and investigational drug or placebo infused through a venous cannula in the other forearm. Skin electrodes for recording of bispectral index (BIS) (BIS XP® version 2.1 high pass filter 2 Hz, low pass filter 50 Hz, notch filter 50 Hz) and a bioimpedance cardiac output monitor EBI100C (Biopac systems Incorporated, Goleta, CA) were placed in accordance with manufacturer’s instructions. The subject was instructed to hold a 20-ml water-filled syringe. All administrations were video recorded. Heart rate, SpO2, arterial blood pressure, BIS, and other monitor outputs were logged using a GE Medical Systems Information Technologies Unity Network and BedMaster software (Excel Medical Electronics, Jupiter, FL, version 3.7).

**Biologic Samples and Electrocardiographs**

Arterial blood samples (4 ml) were collected as follows: Step 1; before infusion; 0.5, 1, 2, 3, 5, and 8 min after start of infusion; 0, 0.5, 1, 2, 3, 5, 8, 15, 30, 60, 120, and 240 min after completion of infusion. Step 2: before infusion; 0, 0.5, 1, 2, 4, 8, 15, 30, 60, 120, and 240 min after completion of infusion.

Venous blood samples (4 ml) were collected at the same time as arterial samples and at 8, 16, 24, and 48 h after completion of infusion. Blood samples were processed to plasma and frozen until analysis.

Urine was collected over the periods 0–2, 2–4, 4–8, 8–12, and 12–24 h.

Electrocardiograph and venous blood samples for hematology and biochemistry were collected before treatment and up to 7 days postdose.

**Assay Procedures**

JM-1232(–) and the main metabolite of JM-1232(–), JM-Metabo-3, concentrations in plasma were quantified by LC-MS/MS using Atmospheric Pressure Chemical Ionization in the positive ionization mode, after a solid phase extraction procedure. Assays were performed by Huntingdon Life Sciences Limited, Huntingdon, Cambridgeshire, United Kingdom.

Full validation of the liquid chromatography-mass spectrometry assay consisted of analysis of six calibration curves, QC samples prepared at 0.4, 1.2, 7.5, and 75 ng/ml, and analyzed on three separate occasions, specificity checks in blank plasma, matrix effects (n = 6 different subjects), recovery, dilution of over-range samples and stability evaluation of the analyte in plasma, following sample preparation and in standard solution.

Recovery of the analyte after extraction from plasma ranged from 70% to 77% for JM-1232(–) and from 60% to 70% for JM-Metabo-3 over the assay calibration range. The assay demonstrated linearity of response in the range 0.4–100 ng/ml. Dilution factors of 1–10 and 1–50 were validated, allowing samples to be measured up to a concentration of 5,000 ng/ml. The accuracy (expressed in terms of the relative error) of the back-calculated concentrations of all acceptable standards (parent and metabolite) was between −6.6% and 3.0%. The intrabatch coefficient of variation ranged from 1.9% to 7.7% for QC samples analyzed at 1.2, 7.5, and 75 ng/ml on three separate occasions. Intrabatch relative error ranged from −10.8% to 5.3% for QC samples analyzed at 1.2, 7.5, and 75 ng/ml on three separate occasions. The precision at the lower limit of quantification (0.4 ng/ml) ranged from 3.5% to 9.5%. Relative error at this concentration ranged from −2.8 to 14.3% following the analysis of the QC sample on three separate occasions. JM-1232(–) and JM-Metabo-3 were shown to be stable in human plasma for up to 3 months at −20°C, after three freeze/thaw cycles.

**Efficacy Endpoints**

Sedation was assessed at 1-min intervals using the Ramsay score16 by the attending anesthetist. The onset of hypnotic effects was defined as the time when any of the following occurred: loss of verbal contact with the subject, loss of the eyelash reflex, or the subject dropping a water-filled 20-ml syringe from his hand. Dipping the syringe was discounted in obviously awake subjects. Time to eyes open on command was recorded as minutes from the end of the infusion. Effective sedation was defined as a Ramsay score16 of 3 or 4, effective hypnosis was defined as a score of 5 or 6.

**Statistical Analysis**

All efficacy data were evaluated by means of descriptive statistics. Kaplan–Meier analysis was used to estimate median time to any hypnotic effect. Logistic regression models were used to estimate the 25%, 50%, and 75% percentiles for the effective sedative dose (ED) and the effective hypnotic dose (HD).

**Pharmacokinetics and Pharmacodynamics**

**Noncompartmental Analysis.** The maximum plasma concentrations (Cmax) of JM-1232(–) and its metabolite JM-Metabo-3 were the observed values during a 48-h sampling period. The areas under the plasma concentration–time curves to the last quantifiable sample point (AUC0–t) and up to 48 h postdose (AUC0–48) were estimated by the linear trap-ezoidal rule, and the areas under the plasma concentration–time curves to infinite time (AUC0–∞) were calculated as AUC0–t + Clast/λz. Where appropriate, terminal rate constants (λz) were estimated by fitting a linear regression of log mean concentration against time using data points randomly distributed approximately a single straight line. Pharmacokinetic analysis of urinary data of JM-1232(–) and its metabolite JM-Metabo-3 included the following parameters; Ae, the amount excreted; fe the fraction of the administered dose excreted unchanged in urine, CLr (renal clearance). Renal clearance was calculated as Ae/AUC0–48. Assessment of dose proportionality (Power Test) was performed using AUC0–t.
because the parameter AUC_{0,\infty} could not be calculated adequately for every subject.

**Population Analysis.** JM-1232 plasma concentration versus time data from Step 1 (10-min infusion group) and Step 2 (1-min infusion group) were pooled and the data used to construct a population pharmacokinetic-pharmacodynamic model using NONMEM software (version 7.1.0., ICON Development Solutions, Ellicott City, MD). An Intel Visual Fortran compiler was used (Professional edition, version 11.1.048) with a Dual Xeon Quad Core E5620 2.4GHZ CPU (Intel, Santa Clara, CA) under Windows 7 Professional 64-bit. A sequential approach was taken to pharmacokinetic-pharmacodynamic modeling: that is, the final population pharmacokinetic model was used to derive individualized pharmacokinetic parameter estimates (clearances and central compartment volume) for each volunteer which were then used as inputs, along with the drug dose and effect measure data (BIS), for the estimation of pharmacodynamic parameters.

**Pharmacokinetics.** Arterial plasma JM-1232(−) concentrations were available and used for modeling, up to and including the 250-min postdose sample time in the 10-min infusion group, and up to and including the 241-min postdose sample time in the 1-min infusion group. After those times, the arterial line was removed, and subsequent samples were venous. Models were fitted using the first order conditional estimation method with interaction between the interindividual error terms and the random residual error term allowed.

The drug concentration versus time data were applied to one-, two-, and three-compartment mammillary models. Allometric scaling was applied to all structural model parameters, standardized to a 70-kg person. Intraindividual variability was described using a log error model. The appropriateness of the base model and the requirement for interindividual variability parameters (ETAs) were assessed using the likelihood ratio test (where appropriate, i.e., for nested models) and by consideration of the Akaike Information Criterion (nonnested models) and the precision of the final parameter estimates (all models). For nested models, the justification for each additional effect (additional parameter) was for it to improve the goodness-of-fit statistic (−2 log likelihood) by more than 3.84 (evaluated against the chi-square distribution, this is equivalent to significance at the 0.05 concentration). The improvement (or lack of) in model goodness-of-fit was also assessed visually by the examination of diagnostic plots.

Classic mammillary models assume that drug enters the arterial circulation instantly. However, it has been demonstrated that there is a lag time between drug administration and its appearance in the central circulation. After establishing the number of model compartments required to describe drug distribution and elimination, we then used transit compartments, rather than empirical lag time parameters, to describe this initial delay in drug reaching the central compartment. The appearance of drug in the central compartment is delayed, in part, because of unavoidable dead space in the intravenous infusion system, specifically the internal volume of the three-way tap and the internal volume of the length of the cannula. Hence, we allowed the transit rate constant, $k_{tr}$, to vary with infusion group, i.e., 10-min group and 1-min group, reflecting the differences in infusion pump speed. The number of transit compartments required to characterize the delay adequately was established by stepwise addition and subsequent examination of the diagnostic plots and the NONMEM objective function value.

**Pharmacodynamics.** The pharmacodynamic effect (BIS) was described using a sigmoid $E_{\text{max}}$ model of MR04A3 concentration ($C_e$) in a hypothetical effect site compartment, according to the following equation:

$$\text{Effect} = E_0 + \left( E_{\text{max}} - E_0 \right) \frac{C_e}{EC_{50} + C_e}$$

where $E_0$ is the baseline BIS value, $E_{\text{max}}$ is the maximum BIS response, $EC_{50}$ is the effect site concentration corresponding to half of the maximum response, and $\gamma$ describes the slope of the drug concentration-effect relationship. The incorporation of a hypothetical effect compartment accounted for the hysteresis (temporal delay) between the plasma MR04A3 concentration and the onset of drug effect. This delay was characterized by the estimation of $K_{e0}$, the blood-brain equilibration rate constant. The requirement for the inclusion of model parameters for $\gamma$ (slope factor) and $K_{e0}$, the blood-effect site equilibration rate constant was assessed by consideration of the NONMEM objective function value and the Akaike Information Criterion. The improvement (or lack of) in model goodness-of-fit was also assessed visually by the examination of diagnostic plots.

**Evaluation of Final Models**

The median prediction error and median absolute prediction error were calculated for the final population pharmacokinetic model as described by Varvel et al. Likelihood profiling and bootstrap simulations were used to generate 95% confidence intervals for the final pharmacokinetic and pharmacodynamic model parameters. Jackknife analyses were performed to evaluate whether any one particular subject’s data unduly influenced the final parameter estimates. A licensed version of the software PLT Tools (version 4.1.1, D. Fisher, PLessThan, San Francisco, CA) was used to facilitate the jackknife, bootstrap, and likelihood profile analyses, which were performed as follows.

**Jackknife.** Multiple datasets ($n = 56$) were produced, each of which excluded one volunteer from the analysis, a different volunteer being excluded from each dataset. The final population models were applied to each dataset and the parameter estimates compared with the estimates resulting from the analysis of the entire dataset to identify any individuals who may have exerted a large influence on the parameter values.

**Log-likelihood Profiling.** Log-likelihood profiling is a method of estimating parameter confidence intervals that
makes no assumptions regarding the symmetry of the resulted intervals. The relationship between the model parameter estimates and the NONMEM objection function value was explored by individually fixing each parameter estimate to values close to the final estimate, and then refitting the model, allowing all other parameter values to vary. The 95% confidence interval was estimated from the log-likelihood profile at 3.84 units from the minimum objective function value. When a single parameter of the full model is fixed, a decrease of 3.84 in the minimum value of the objective function is significant at $P < 0.05$.

**Bootstrap.** One thousand bootstrap datasets were created by sampling the data, with replacement, from the original dataset. The final pharmacokinetic and pharmacodynamic models were then fitted to each of the resulting datasets. The mean parameter values and the 2.5 and 97.5 percentiles for all successful runs (where the model minimized successfully and final estimates were produced to at least 3 significant digits) were determined, and 95% confidence intervals for the parameter estimates were obtained.

**Results**

One hundred thirty-five subjects were screened and 69 included in the study (table 1).

**Hypnotic Effects**

Hypnotic effects of MR04A3 were seen at all dose concentrations in Step 1 and at doses of 0.075 mg/kg or more in Step 2 (table 2). Ramsay scores were increased with higher doses causing sedation and then unresponsiveness (fig. 2). Larger doses of MR04A3 produced longer periods of unresponsiveness.

### Table 1. Demographic Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Step 1, 10-min Infusions n = 20 Males</th>
<th>Step 2, 1-min Infusions n = 49 Males</th>
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<tbody>
<tr>
<td>MR04A3/placebo</td>
<td>15/5</td>
<td>42/7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26.8 ± 4.6</td>
<td>25.6 ± 4.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179 ± 6.9</td>
<td>178 ± 6.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.1 ± 8.1</td>
<td>74 ± 7.9</td>
</tr>
<tr>
<td>BMI, kg m²</td>
<td>23.1 ± 2.1</td>
<td>23.2 ± 2.2</td>
</tr>
</tbody>
</table>

Values for age, height, weight, and BMI (body mass index) are means ± SD.

### Table 2. Hypnotic Effects

<table>
<thead>
<tr>
<th>Steps</th>
<th>Group Size (n)</th>
<th>Hypnotic Effect (n)</th>
<th>Onset (min), Median (min), Mean (SD)</th>
<th>Eyes Open to Command (min), Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1, 10-min infusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>5</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>0.05 mg/kg</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>n/a</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>13 (1.4)</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>17.7 (2.5)</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>32 (19.9)</td>
</tr>
<tr>
<td>0.8 mg/kg</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>67.3 (17.8)</td>
</tr>
<tr>
<td>Step 2, 1-min infusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>7</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>0.025 mg/kg</td>
<td>6</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>0.05 mg/kg</td>
<td>6</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>0.075 mg/kg</td>
<td>6</td>
<td>4</td>
<td>4.5</td>
<td>5.7 (2.3)</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>6</td>
<td>2</td>
<td>2.5</td>
<td>22.5 (19.1)</td>
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<tr>
<td>0.2 mg/kg</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>15 (11.9)</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>6</td>
<td>6</td>
<td>1.5</td>
<td>28 (13.9)</td>
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<tr>
<td>0.4 mg/kg</td>
<td>6</td>
<td>6</td>
<td>1.5</td>
<td>38.2 (24.5)</td>
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</table>

The absence or presence of hypnotic effect was assessed, and the time of its onset was defined as the time from the start of infusion to the occurrence of any of the three following events: loss of verbal contact, loss of eyelash reflex, or syringe drop. Time to eyes open on command was recorded as min from end of the infusion. n/a = not applicable.
Hemodynamic Effects
Both 1- and 10-min infusions of MR04A3 were well tolerated with modest decreases in systolic arterial blood pressure (fig. 3).

Electroencephalogram Effects
Bispectral index was decreased by all doses of MR04A3 with larger doses causing a greater reduction is BIS and a longer duration of effect (fig. 4).

Adverse Events
The adverse event profile of subjects receiving MR04A3 was similar to that of subjects given placebo except that some subjects receiving MR04A3 developed upper airway obstruction while sedated. This responded to simple maneuvers (i.e., chin lift).

Noncompartmental Pharmacokinetics
Arterial concentrations of JM-1232(−) and the metabolite JM-Metabo-3 increased with increasing dose (fig. 5). Administration of single intravenous infusion doses of 0.05–0.8 mg/kg MR04A3 over 10 min resulted in increasing values for the parameters $C_{max}$ and $AUC_{0-t}$ of JM-1232(−). Increases were proportional to increase in dose, and there was no statistically significant evidence for nonproportionality for either parameter. The metabolite, JM-Metabo-3, increased with increasing dose of MR04A3, and there was no statistically significant evidence of nonproportionality for this metabolite. A full description of the results of the noncompartmental analysis and associated tables of pharmacokinetic parameter values are provided in Supplemental Digital Content 1, http://links.lww.com/ALN/A808.

Population Pharmacokinetics and Pharmacodynamics
Pharmacokinetics. MR04A3 pharmacokinetics were best described using a three-compartment mammillary model, with transit compartments preceding the central compartment. The transit compartment approach was found to be superior (in terms of the objective function value and diagnostic plots) to a simple lag time model. Models including both transit compartments and lag time parameters showed no advantage over models containing transit compartments only. The optimal number of presystemic transit compartments was nine. Reductions in the NONMEM objective function value were observed for models with further transit compartments (up to 12), but no further improvements in the diagnostic plots were observed be-
yond nine transit compartments. Interindividual variance was modeled in all structural parameters with the exception of V1 and V3. The typical parameter values for the final model are given in table 3. Goodness-of-fit plots for the final pharmacokinetic model are provided in Supplemental Digital Content 2, http://links.lww.com/ALN/A809.

**Pharmacodynamics.** A full sigmoid $E_{\text{max}}$ model with an effect compartment best described the relationship between BIS and MR04A3 concentration. Random residual error was described using an additive error model. A proportional variance model was used to describe the interindividual variability in $E_{\text{max}}$ and $E_0$. Goodness-of-fit plots are shown as Supplemental Digital Content 3, http://links.lww.com/ALN/A810. The typical value for EC$_{50}$ was 200 ng/ml. The typical $K_e0$ value was 0.137 min$^{-1}$, resulting in a typical value for $K_e0$ half-life of 5.1 min.

Table 4 lists all the final pharmacodynamic parameter values.

**Model Evaluation**

The population model predicted MR04A3 plasma concentrations, based on the typical pharmacokinetic parameter values, demonstrated a median prediction error (reflecting model bias) of $-0.7\%$ and a median absolute prediction error (a measure of model precision) of 20.0%. A bias of up to 20% and an imprecision of 20–30% are considered acceptable for clinical use. Figure 6 demonstrates population and individualized model fits for a typical individual, and the most extreme over- and underpredictions.

**Jackknife Analyses**

**Pharmacokinetic Model.** Subject 18 was relatively influential in terms of the impact that removal of the data collected from this subject had on the resultant parameter estimates. The concentration versus time profile for this subject (fig. 6C) demonstrates that the measured JM-1232(−) concentration (and the individualized model prediction) describe a significantly faster decrease in concentration than that predicted using the population model. Hence, removing subject 18 from the dataset decreased the typical value estimate for V2 by 15%. Estimates of interindividual variability associated with Q2 and V2 were reduced by 61% and 80%, respectively. However, there was no clinical reason for excluding this volunteer from the analysis, i.e., there was no significant protocol deviation, so final results presented are based on data from all subjects. Structural and random effect parameter estimates were minimally affected by the removal of any other subject from the dataset. The mean jackknife estimates for each parameter differed by less than 1% from the NONMEM typical parameter values based on the full dataset (table 3).

**Pharmacodynamic Model.** Structural parameter estimates were minimally affected by the removal of any subject from the dataset. The mean jackknife estimates for each parameter differed by less than 1% from the NONMEM typical parameter values based on the full dataset (table 4).

**Log-likelihood Profiling.** The resulting changes in the objective function values were plotted against the fixed values for each structural model parameter. Confidence intervals resulting from the likelihood profiling exercise are given in tables 3 and 4 for the pharmacokinetic and pharmacodynamic models, respectively. The likelihood profile generated confidence intervals were very similar to those produced from the bootstrap procedure for the pharmacokinetic model. For the pharmacodynamic model, likelihood generated confidence intervals tended to be closer to the NONMEM generated typical value than those produce from the bootstrap process.

**Bootstrap**

The mean parameter values resulting from the bootstrap procedure ($n = 922$ successful runs for the pharmacokinetic model, $n = 852$ for the pharmacodynamic model) were comparable with the NONMEM estimates from the original dataset. The mean bootstrap values for the structural pharmacokinetic model parameters differed from the final NONMEM model values by less than 3%. The mean
bootstrap values for the pharmacodynamic parameters differed by less than 6% from the final NONMEM model estimates. The 95% confidence intervals resulting from the bootstrap procedures are provided in tables 3 and 4 for the pharmacokinetic and pharmacodynamic models, respectively.

Discussion

MR04A3 is hypnotic in man with a satisfactory safety profile. Onset of hypnotic effect was free from excitation, and recovery from sedation was uncomplicated. The hemodynamic effects of MR04A3 in our subjects were minimal.

We studied healthy male volunteers. Females, older males, and patients with comorbidities may demonstrate different effects. We compared MR04A3 with placebo and did not include an active control. Subsequent investigation of the compound should compare MR04A3 with clinically relevant comparators, notably midazolam and propofol.

Study Design

The study drug was infused over two periods: Step 1 comprised 10-min infusions to properly populate the hysteresis loop for arterial drug concentration and electroencephalogram effect.23 In Step 2, 1-min infusions were given to more closely replicate potential clinical use for brief sedation or induction of anesthesia.

The study design of ascending doses administered to consecutive groups was pragmatic and allowed a formal safety evaluation after each dose group. The sample size (three active treatments per dose group in Step 1 and six active treatments per group in Step 2) allowed timely progressive dose escalation and exposed subjects to the whole of the dose–response curve (from no effect to maximum effect). The larger numbers at each dose concentration in Step 2 reflect increasing experience with the drug and provide a more substantial dataset.

Choice of BIS as an Electroencephalogram Measure

Multiple candidates are available as electroencephalogram–derived measures of drug effect. We selected BIS because it is widely used by clinicians to monitor anesthesia and sedation.

Fig. 5. Arterial concentrations of JM-1232(−) (A and C) after 10- and 1-min infusions of JM 1232(−), respectively. Arterial concentrations of the JM-1232(−) metabolite, JM-Metabo-3, are shown in B and D after 10- and 1-min infusions of JM-1232(−), respectively. Data are means, times are minutes from the start of the infusion. n = 3 per MR04A3 group for 10-min infusions and n = 6 per MR04A3 group for 1-min infusions.
and has previously been shown to be effective for computation of $k_{e0}$ of propofol, alfentanil, and midazolam.\textsuperscript{24}

**Pharmacokinetics and Pharmacodynamics of MR04A3**

We estimated EC\textsubscript{50} as 200 ng/ml and $t_{1/2}K_{e0}$ as 5.1 min. This $t_{1/2}K_{e0}$ value compares with published estimates of 1.3–4.8 min for midazolam (\textsuperscript{1.3,25,26} and 0.6–3.7 min for propofol\textsuperscript{27–30}). Comparisons of $t_{1/2}K_{e0}$ across different studies are subject to methodological concerns and have limited validity. However, this initial analysis suggests that the onset time of MR04A3 may be similar to onset times achieved with propofol and midazolam. Direct, within-subject comparisons between MR04A3 and other sedative agents are necessary to evaluate this finding.

**Limitations of This Study**

This was a preliminary investigation and did not include any active control. Comparisons with other sedative agents are therefore speculative.

MR04A3 appears to be safe, efficacious, and well tolerated in man. However, the limited numbers of subjects studied and the nonclinical nature of the study mandate caution before extrapolation to clinical practice. Nevertheless, the quick onset of sedation, minimal hemodynamic disturbance, and uneventful recovery are attractive characteristics for clinical use.

The JM-1232(–) metabolite JM-Metabo-3 requires further investigation. If it has hypnotic effects, then these could delay recovery after large single doses of MR04A3 or after infusions.

**Table 3. Parameter Estimates for the Final Pharmacokinetic Model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NONMEM Typical Value</th>
<th>CV%</th>
<th>Log-likelihood Profile (95% Confidence Intervals)</th>
<th>Bootstrap (95% Confidence Intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL ml min</td>
<td>654 × (WT/70)\textsuperscript{0.75}</td>
<td>17.3</td>
<td>622 – 687</td>
<td>655 – 623 – 688</td>
</tr>
<tr>
<td>Q2 ml min</td>
<td>213 × (WT/70)\textsuperscript{0.75}</td>
<td>75.9</td>
<td>167 – 244</td>
<td>210 – 155 – 269</td>
</tr>
<tr>
<td>Q3 ml min</td>
<td>2,530 × (WT/70)\textsuperscript{0.75}</td>
<td>33.2</td>
<td>2,294 – 2,805</td>
<td>2,527 – 2,300 – 2,790</td>
</tr>
<tr>
<td>V1 ml</td>
<td>5,050 × (WT/70)\textsuperscript{1}</td>
<td>—</td>
<td>4,679 – 5,428</td>
<td>5,058 – 4,700 – 5,430</td>
</tr>
<tr>
<td>V2 ml</td>
<td>50200 × (WT/70)\textsuperscript{1}</td>
<td>72.6</td>
<td>44,912 – 64,907</td>
<td>48,802 – 36,700 – 66,900</td>
</tr>
<tr>
<td>V3 ml</td>
<td>36300 × (WT/70)\textsuperscript{1}</td>
<td>—</td>
<td>34,007 – 38,094</td>
<td>36,461 – 33,400 – 39,800</td>
</tr>
<tr>
<td>$K_r$ min\textsuperscript{-1} 10-min cohort</td>
<td>11.5</td>
<td>66.0</td>
<td>8.8 – 15.8</td>
<td>11.7 – 8.7 – 15.3</td>
</tr>
<tr>
<td>$K_r$ min\textsuperscript{-1} 1-min cohort</td>
<td>64.4</td>
<td>—</td>
<td>43.0 – 129</td>
<td>79 – 42.0 – 135</td>
</tr>
<tr>
<td>Residual error (SD) ng/ml</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The coefficient of variation (CV%) was determined, where possible, as the typical magnitude of the ETA parameter associated with the pharmacokinetic parameter.

CL = irreversible systemic clearance from the central compartment; $K_r$ = transit rate constant; Q2 = intercompartmental clearance between the central and the first peripheral compartment; Q3 = intercompartmental clearance between the central and the second peripheral compartment; V1 = volume of the central compartment; V2 = volume of the first peripheral compartment; V3 = volume of the second peripheral compartment.

and has previously been shown to be effective for computation of $k_{e0}$ of propofol, alfentanil, and midazolam.\textsuperscript{24}

**Table 4. Pharmacodynamic Parameter Estimates**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NONMEM Typical Value</th>
<th>CV%</th>
<th>Log-likelihood Profile (95% Confidence Intervals)</th>
<th>Bootstrap (95% Confidence Intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{max}$</td>
<td>58.4</td>
<td>23.5</td>
<td>54.6 – 65.0</td>
<td>58.0 – 45.8 – 66.1</td>
</tr>
<tr>
<td>$E_0$</td>
<td>94.9</td>
<td>3.78</td>
<td>94.0 – 96.05</td>
<td>94.8 – 90.1 – 96.0</td>
</tr>
<tr>
<td>$E_{50}$ ng/ml</td>
<td>200</td>
<td>—</td>
<td>193 – 208</td>
<td>204 – 166 – 270</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>2.52</td>
<td>—</td>
<td>2.33 – 2.71</td>
<td>2.68 – 1.64 – 4.20</td>
</tr>
<tr>
<td>$K_{e0}$ min\textsuperscript{-1}</td>
<td>0.137</td>
<td>—</td>
<td>0.128 – 0.147</td>
<td>0.140 – 0.112 – 0.173</td>
</tr>
<tr>
<td>Residual error (SD)</td>
<td>6.58</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The coefficient of variation (CV%) was calculated, where possible, as the typical magnitude of the $\gamma$ variable associated with that parameter.

$E_0$ = baseline BIS (bispectral index) value; $E_{50}$ = effect site concentration corresponding to half of the maximum response; $E_{max}$ = maximum BIS response; $\gamma$ = the slope of the drug concentration–effect relationship; $K_{e0}$ = the blood-brain equilibration rate constant.

**Limitations of This Study**

This was a preliminary investigation and did not include any active control. Comparisons with other sedative agents are therefore speculative.

MR04A3 appears to be safe, efficacious, and well tolerated in man. However, the limited numbers of subjects studied and the nonclinical nature of the study mandate caution before extrapolation to clinical practice. Nevertheless, the quick onset of sedation, minimal hemodynamic disturbance, and uneventful recovery are attractive characteristics for clinical use.

The JM-1232(–) metabolite JM-Metabo-3 requires further investigation. If it has hypnotic effects, then these could delay recovery after large single doses of MR04A3 or after infusions.
Comparison with Other Studies

How might we improve on midazolam and propofol? In the case of propofol, water solubility and freedom from pain on injection would be material improvements. For midazolam, faster onset and a steeper dose–response relationship would be advantageous. JM-1232(−) is not a benzodiazepine, and its development aims to address the deficiencies of both propofol and midazolam. In the absence of an active control, the current study offers only a limited opportunity to make the necessary comparisons.

Certainly, JM-1232(−) is water-soluble, and the formulation studied, MR04A3, is a particular water-based presentation. Our subjects did not report pain during injection of MR04A3; however, the use of large forearm veins and particularly the antecubital fossa, is known to attenuate pain during propofol injection, so caution needs to be extended to this experience with MR04A3.

In the 1990s, Roche examined novel benzodiazepines in the search for compounds superior to midazolam. Ro48-6791 was four to six times as potent as midazolam with a similar onset and duration of effect. Ro48-8684 was of potency similar to midazolam with a steeper dose–response curve and a shorter duration of effect. Development of these compounds was discontinued, presumably because the differences from the licensed compound, midazolam, were insufficient to justify further investment. For a new nonbenzodiazepine compound with benzodiazepine-like effects to justify clinical development, it must show characteristics significantly superior to midazolam. This would imply that in addition to water solubility, already possessed by midazolam, there be faster onset (i.e., shorter \( t_{1/2} k_e0 \)) and faster recovery. How might “faster recovery” be characterized? Derived electroencephalogram measures of effect have been used extensively in drug discovery and characterization of existing compounds. However, these are surrogates, and real clinical advantage cannot be described in these terms. Recovery from sedation and readiness for discharge after same-day procedures are limited by the psychomotor effects of sedative agents. Accordingly, meaningful comparisons between such compounds must include psychometric testing.

The possibility of an analgesic effect of JM-1232(−) is of real interest; however, the current observations can only be regarded as preliminary and require confirmation in other models and particularly in man.

Further development of MR04A3 requires direct comparison with the contemporary agents propofol and midazolam using psychometric testing as well as electroencephalogram-derived measures of effect.

The authors are grateful to Kenichi Masui, M.D., Ph.D., Department of Anaesthesiology, National Defense Medical College, Japan, for advice on the implementation of transit compartment models.

References


Fig. 6. Example of pharmacokinetic model fits to three individuals’ JM-1232(−) plasma concentration data. The blue line represents the prediction of the typical value or population model. The red line is the individualized model. Markers show the observed values. A, typical fit; B, most extreme under-prediction; C, most extreme over-prediction.