ANESTHESIOLOGY

Target-controlled Infusion of Remimazolam in Healthy Volunteers Shows Some Acute Tolerance

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ABSTRACT

Background: Remimazolam exhibits sedative properties by binding to γ-aminobutyric acid type A receptors. Remimazolam is administered as a bolus dose or continuous infusion, but has not been studied using target-controlled infusion (TCI). The study quantified the relationship between the remimazolam concentration, Modified Observer's Assessment of Alertness and Sedation (MOAAS) score, and bispectral index (BIS) using TCI.

Methods: The authors performed a three-period, crossover, dose-ranging clinical trial in 24 healthy volunteers using age and sex stratification. Data collected in the first period, where remimazolam was administered alone using a step-up and step-down TCI protocol, were used for this analysis. Remimazolam concentrations, MOAAS scores, and BIS values were collected at each step at steady state. Data were analyzed using nonlinear mixed-effects modeling methodology.

Results: The relationship between remimazolam, BIS, and MOAAS differed between step-up and step-down infusions at similar remimazolam target concentrations. Tolerance, driven by remimazolam or CNS7054, significantly improved overall model fit ($P < 0.01$) for both BIS and MOAAS models. After 30 min of repeated bolus dosing, mimicking the regimen in the label for procedural sedation, the BIS and probability of MOAAS 2/3 were predicted to be 54 (95% prediction interval, 44 to 67) and 2% (95% prediction interval, 0 to 32%) *versus* 58 (95% prediction interval, 48 to 70) and 8% (95% prediction interval, 0 to 36%) in a model without and with tolerance, respectively. After 60 min of continuous infusion, mimicking the regimen in the label for general anesthesia, the BIS and probability of MOAAS 0 were predicted to be 40 (95% prediction interval, 33 to 50) and 87% (95% prediction interval, 18 to 100%) *versus* 50 (95% prediction interval, 41 to 60) and 59% (95% prediction interval, 6 to 99%) in a model without and with tolerance, respectively.

Conclusions: In this study, it was shown that remimazolam-induced sedation is prone to tolerance development, which is potentially mediated by the CNS7054 concentration. The clinical consequences are, however, limited in situations where remimazolam is titrated to effect.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Remimazolam is a short-acting benzodiazepine that is administered as repeated bolus doses for procedural sedation and as a continuous infusion for general anesthesia in adults
- The pharmacokinetics of remimazolam administered as a bolus dose as well as a continuous infusion have been well-characterized

What This Article Tells Us That Is New

- The pharmacokinetic-pharmacodynamic relationships between remimazolam concentrations and Modified Observer's Assessment of Alertness and Sedation scores and bispectral index were assessed in 24 healthy volunteers using step-up and step-down target-controlled infusions to determine clinically appropriate target concentrations
- Target concentration–dependent sedation was observed with little effect on vital signs
- A difference in the sedative effects of remimazolam at identical target concentrations was observed between the step-up and step-down parts of the titration scheme that could not be explained by a bias in the predicted target concentrations
- Pharmacodynamic models for Modified Observer's Assessment of Alertness and Sedation scores and bispectral index that assumed tolerance development described the observed difference in the sedative effects between the step-up and step-down parts of the titration scheme

This article is featured in "This Month in ANESTHESIOLOGY," page A1. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has a visual abstract available in the online version. Preliminary results were shared at the Annual Meeting of the International Society of Anesthetic Pharmacology in New Orleans, Louisiana, October 21, 2022. R. V. and J. V. K. contributed equally to this article.

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Remimazolam (Byfavo, PAION, Germany) is a short-acting benzodiazepine that is indicated for procedural sedation (European Union, United States, and United Kingdom) and, in the European Union, Japan, and South Korea, for general anesthesia in adults.[1–3] Interestingly, the posology of remimazolam in the procedural sedation setting differs between the European Union and U.S. drug labels. Contrary to the U.S. label, the posology in the European Union label depends on the presence or absence of concomitant administration of opioids.[1,2]

The pharmacokinetics of remimazolam have been extensively characterized after bolus dose administration as well as continuous infusion.[4–7] Remimazolam has a relatively low volume of distribution of approximately 40 l/kg, which indicates that distribution throughout the body is rather limited.[5] Remimazolam has a terminal half-life of approximately 70 min and is therefore rapidly eliminated from the body.[5] Remimazolam is metabolized by carboxylesterase 1A, which is expressed mainly in the liver, into its main metabolite, CNS7054.[8] The pharmacokinetics of CNS7054 are less extensively characterized, but the volume of distribution is reported to be around 8 l, and the terminal half-life is approximately 120 min.[5] This favorable pharmacokinetic profile of remimazolam contributes to the rapid onset and short duration of effect.

Remimazolam exhibits sedative properties by binding to the benzodiazepine binding site at the γ-aminobutyric acid type A ($GABA_A$) receptor with a rapid onset of effect (1 to 3 min).[4,9] The contribution of CNS7054 to the sedative properties of remimazolam is expected to be negligible, because the affinity of remimazolam for the $GABA_A$ receptor is about 320 to 410 times higher compared to CNS7054.[9] Remimazolam does not have analgesic properties.[9] The effect of remimazolam can, however, be enhanced during concomitant administration with an opioid as a synergistic interaction between remimazolam and opioids on the Modified Observer's Assessment of Alertness and Sedation (MOAAS) scores has been reported.[10,11]

The pharmacokinetics and pharmacodynamics under target-controlled infusion (TCI) have not yet been studied. Therefore, we performed a dose-ranging trial under TCI conditions in the absence or presence of concomitant administration of the opioid remifentanil to evaluate appropriate target concentrations for use in clinical practice. In this first analysis, we quantified the pharmacokinetic-pharmacodynamic relationship between remimazolam and the MOAAS score and bispectral index (BIS) in the absence of remifentanil under TCI conditions.

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Materials and Methods

This trial was conducted at the Department of Anesthesiology of the University Medical Center Groningen, Groningen, The Netherlands. The independent Ethics Committee of the Foundation "Evaluation of Ethics in Biomedical Research" in Assen, The Netherlands, has approved the study (clinical trial registry: https://clinicaltrials.gov; NCT04670471; protocol No. CNS7056-025).

Inclusion and Exclusion of Volunteers

Healthy volunteers were stratified across groups by age (18 to 34, 35 to 49, and 50 to 70 yr) and sex. Eligibility criteria were an age between 18 and 70 yr, a body mass index between 18 and 30 kg/m^2, a bilateral patent arteria radialis, and an American Society of Anesthesiologists Physical Status of I. All subjects agreed to abstain from alcohol for 2 days, nicotine for 1 week, and recreational drugs for 2 weeks before the first dose and until the end of the trial. All volunteers agreed to use a medically accepted form of contraception. The exclusion criteria were recent (less than 3 months) use of psychoactive medication, a history of illicit drug or alcohol abuse, known intolerance to any of the medications or ingredients of the drugs that were used during the trial, a positive COVIDd-19 test, a positive pregnancy test, and recent (less than 3 months) blood donation of 500 ml or more. Volunteers with bradycardia (less than 45 beats per minute), hypotension (systolic blood pressure less than 90 mmHg), or hypertension (systolic blood pressure greater than 140 mmHg) were also excluded. Written informed consent was obtained from all volunteers.

Study Design

In this single-center, three-period, crossover, dose-ranging trial, each volunteer received a step-up and step-down remimazolam infusion in the absence or presence of concomitant remifentanil administration. The washout period between periods was at least 1 week. During the first period, remimazolam was administered without remifentanil using TCI with effect-site target concentrations of 150, 300, 400, 800, 1,300, and 2,000 ng/ml during step-up and 1,300, 800, 400, 300, and 150 ng/ml during step-down. The infusion duration was composed of the time to reach pharmacokinetic steady state, which depended on the rate constant for effect-site equilibration and the targeted effect-site concentration and ranged between 2 and 30 min, and an equilibration period of 10 min that was introduced to ensure steady state was reached at each step of the infusion protocol (Supplemental Digital Content 1, figs. 1 and 2, https://links.lww.com/ALN/D354). Subsequently, pharmacokinetic samples and pharmacodynamic outcomes were collected during a period of approximately 10 min. Therefore, each step in this infusion protocol was planned to consist of at least 22 min and up to 50 min of administration of remimazolam. In the second and third periods,

different remifentanil effect-site target concentrations (0.1 to 4.0 ng/ml) were administered, and remimazolam was administered using a similar step-up and step-down infusion protocol as used in session 1. In case of early dropout, additional healthy volunteers were included until a total of 24 healthy volunteers with data available in all three periods of the trial was available. The effect-site target concentrations, the number of targets, the number of volunteers, and the number of blood samples and pharmacodynamic measurements were optimized before the trial using clinical trial simulations based on modeling results of Zhou *et al.*[11] and Schüttler *et al.*[5]

Drug Administration

Drugs were administered by two syringe pumps (AlarisTM GH syringe pump, Becton Dickinson, USA) controlled by a computer running RUGLOOP II software (Demed, Belgium) for Windows (Microsoft, USA). The RUGLOOP II software was programed to deliver remimazolam by effect-site TCI using the model developed by Zhou *et al.* with a rate constant for effect-site equilibration (ke0) of 0.135 min^{-1}.[11]

Study Procedures

During the study, a board-certified anesthesiologist and nurse anesthetist were responsible for the monitoring and safety of the volunteers. Upon arrival, the volunteers were connected to a multichannel electroencephalogram device (BrainAmp DC 23 amplifier, Brain Products, GmbH, Germany) and to a BIS monitor (BIS Vista, Medtronic, USA) using electrodes placed on the forehead. Two 20-gauge IV cannulas were used for fluid and drug administration. For the remimazolam line, a sodium chloride solution (Baxter B.V., The Netherlands) was used. Fluids were administered at a flow rate of 1 ml · kg^{-1} · h^{-1}. After 4 h, fluids were reduced to a flow rate of 0.5 ml · kg^{-1} · h^{-1}. A 20-gauge arterial cannula was placed under local anesthesia for hemodynamic monitoring and blood sampling. Volunteers were connected to a Philips IntelliVue MP50 vital signs monitor (Philips Medical Systems, The Netherlands) using standard monitoring (electrocardiogram, noninvasive blood pressure monitoring, pulse oximetry, and atrial blood pressure). For advanced hemodynamic monitoring, a HemoSphere (Edwards Lifesciences, USA) was used. Ventilation was monitored using a Zeus ventilator (Drager Medical, Germany). The volunteers breathed spontaneously through a tight-fitting mask to measure the frequency, tidal volumes, and the end-tidal carbon dioxide.

Measurement of Drug Effect

The depth of sedation was assessed by the anesthesiologist using the MOAAS score. The MOAAS score contains categories from 5 (fully alert) to 0 (fully sedated).[12] See table 1, Supplemental Digital Content 1 (https://links.lww.com/ALN/D354), for an overview of the individual categories of the MOAAS score. All volunteers were asked to stay in bed with their eyes closed and not to engage in activities or spontaneous conversations except for reacting to the MOAAS assessment. MOAAS scores were collected at baseline, defined as 2 min before drug infusion, and during the last 5 min of each step of the remimazolam infusion protocol, in which it was assumed that the remimazolam concentration was at steady state (see Supplemental Digital Content 1, https://links.lww.com/ALN/D354). An arterial blood sample was collected after each MOAAS assessment. For pharmacokinetic modeling, additional arterial blood samples were collected at defined non–steady state timepoints (see Supplemental Digital Content 2, https://links.lww.com/ALN/D355). The cerebral drug effect was continuously measured using the BIS. The median BIS value from −60 s up to the start of the MOAAS assessment was used for this analysis. See figures 1 and 2 in Supplemental Digital Content 1 (https://links.lww.com/ALN/D354) for an overview of the entire sequence of interventions.

Recovery Period

A recovery period was initiated after completion of all infusion steps or if any of the safety criteria (*i.e.*, heart rate less than 40 beats/min despite the use of atropine, changes in cardiac rhythm resulting in clinically significant hemodynamic instability) were reached. During the recovery period, MOAAS scores were assessed with a 2-min interval for the first 30 min until the volunteer reached two sequential MOAAS scores of 5. A volunteer was discharged from the research unit when the volunteer reached a modified Aldrete score of 10 and met the discharge criteria of the hospital's postanesthesia care unit.

Storage and Analysis of Blood Samples

Remimazolam and CNS7054 concentrations were determined in arterial plasma samples that were collected at baseline (before the start of infusion) and at each step in the infusion protocol at pseudo–steady state (*i.e.*, after a minimum equilibration period of at least 25 min after target adjustment). Blood samples were collected in EDTA tubes and stored on ice. Within 10 min after collection, samples were centrifuged (Labofuge 400R, Heraeus Holding, GmbH, Germany) for 10 min at 2,000*g* at 4°C and transferred to cryovials. Remimazolam samples were stored at −20°C until analysis. Remimazolam and CNS7054 concentrations were extracted from plasma using solid-phase extraction and analyzed using ultra-high-performance liquid chromatography–mass spectrometry at Aptuit (Italy). Deuterium-labeled D4-remimazolam and D4-CNS7054 were used as internal standards. The lower and upper limits of quantification were 2 ng/ml and 2,000 ng/ml (remimazolam) and 20 ng/ml and 20,000 ng/ml (CNS7054), with a within-run precision of 6.6% or less.

Model Development

A population pharmacokinetic model was first developed to characterize the relationship between remimazolam dose, remimazolam plasma concentration, and CNS7054 plasma concentration using a nonlinear mixed-effects modeling approach (full details are provided in Supplemental Digital Content 2, https://links.lww.com/ALN/D355). Subsequently, this model was used to predict the remimazolam and CNS7054 plasma concentration in the population pharmacodynamic models using the sequential population pharmacokinetic parameters and data (PPP&D) approach.[13]

Population pharmacodynamic models were then developed for MOAAS and BIS using all observations collected in session 1. The Laplacian algorithm with interaction as implemented in NONMEM version 7.5 (Icon Development Solutions, USA) was used for parameter estimation in the population pharmacodynamic models. Pre- and postprocessing of data were performed in R version 4.0.3 (R core team, Austria).

For MOAAS, a proportional odds model structure was used in which the ordered categorical nature of the score is preserved by estimating cumulative probabilities.[14] The cumulative probabilities are modeled as follows:

$$Logit\left[P\left(Y \leq i\right)\right] = Baseline\left(i\right) + Drug\ effect. \quad \text{(Eq 1)}$$

In this equation, Y represents the observed MOAAS score, i represents a particular category, and baseline(i) is a parameter that represents the cumulative probability of a particular score in the absence of drug in the logit domain. The baseline parameter is constrained, where baseline(i) < baseline(i + 1), to maintain the order in the cumulative probabilities of the MOAAS scores. The drug effect represents the change in cumulative probability in the presence of drug in the logit domain. Linear, Emax, and sigmoid equations were used to evaluate the influence of the remimazolam concentration on the overall drug effect. The cumulative probabilities in the logit domain can be converted back to the probability domain using the expit transform (equation 2), where × is the logit[P(Y ≤ i)].

$$P(Y \leq i|x) = e^{x} / \left(1 + e^{x}\right). \quad \text{(Eq 2)}$$

Probabilities for each MOAAS category can then be derived using equation 3,

$$P\left(Y = i\right) = P\left(Y \leq i\right) - P\left(Y \leq i - 1\right) \quad \text{(Eq 3)}$$

in which, for the highest category, $P\left(Y \leq i\right) = 1$.

For BIS, similar to the MOAAS model, a logit transform was used to preserve the bounds of the score between 0 and 100 (equation 4).

$$Y = 100 \times \left(e^{(baseline\ -\ drug\ effect\ +\ residual\ error)} / \left(1 + e^{(baseline\ -\ drug\ effect\ +\ residual\ error)}\right)\right). \quad \text{(Eq 4)}$$

In this equation, Y represents the observed BIS value, baseline represents the expected baseline BIS value in the absence of drug, and drug effect represents the change in BIS in the presence of drug. The influence of remimazolam concentration on the overall drug effect was evaluated as described for the MOAAS model. Finally, the residual error describes the discrepancy between the observed BIS and model-predicted BIS and was assumed to follow a normal distribution in the logit domain with a mean of 0 and estimated variance.

Random effect parameters, representing differences between subjects, were evaluated on all structural model parameters assuming either a normal or log-normal distribution. Model comparison was performed by comparing the change in objective function value between competing models. A change in objective function value of –3.84 corresponds to a significant ($P < 0.05$) improvement in model fit. Further, model performance was assessed by visual predictive checks, plausibility and uncertainty of the model parameters, and a condition number less than 1,000. Uncertainty in the model parameters was estimated using likelihood profiling.

Simulations

Simulations were conducted to illustrate the pharmacokinetic-pharmacodynamic relationship between remimazolam dose and MOAAS and BIS. Two scenarios were evaluated. First, repeated bolus dosing was evaluated, as recommended by the current European Union, with an induction dose of 7 mg administered over 1 min.[1] After 2 min, additional bolus doses of 2.5 mg can be administered during a 15-s interval with at least 2 min between dosages. In our simulations, a 3-min interval was used between start and follow-up administrations. Second, a continuous infusion protocol was evaluated, similar to the continuous infusion protocol evaluated in the confirmatory phase III study in the general anesthesia setting, where 6 mg/min remimazolam was administered for 3 min, continued by 2.5 mg/min for 7 min and 1.5 mg/min for 50 min.[1] For the repeated bolus dosing, summary statistics were predicted at 10, 20, and 30 min after treatment initiation. For the continuous infusion protocol, summary statistics were predicted at 10, 20, 30, and 60 min after treatment initiation. The parameters of interest were BIS, the probability of reaching a MOAAS score of 2 or 3, and MOAAS score less than 2 (sedation; repeated bolus dosing scenario) and the probability of reaching a MOAAS score of 0 (anesthesia; continuous infusion scenario). In the simulations (n = 1,000), a reference subject of 70 kg and a rate constant for effect-site equilibration of 0.135 min^{-1} or 0.6 min^{-1} was assumed. All simulations were conducted using the RxODE package (version 1.0.9) in R.

Results

A total of 28 of the 33 screened volunteers were eligible for inclusion. Four volunteers were excluded, two volunteers stopped early (after session 1 and after session 2, respectively) for personal reasons, and two volunteers had a positive drug test upon arrival at the first session. Of the 24 volunteers enrolled in the study, 13 (54.2%) subjects were male. These subjects had a mean (minimum, maximum) age of 43 (19, 70) yr, weight of 74 (51, 106) kg, height of 175 (159, 192) cm, and body mass index of 24.1 (20.2, 29.4) kg/m^2. Cardiovascular and respiratory homeostasis were maintained throughout the study duration (Supplemental Digital Content 3, https://links.lww.com/ALN/D356).

Pharmacokinetics

The measured remimazolam and CNS7054 concentrations per remimazolam target concentration during step-up and step-down are displayed in figure 1. No bias was observed between the observed remimazolam concentrations and the predicted TCI target concentrations (median absolute prediction error, –0.64%; 95% CI, –8.33 to 7.06%), and the precision was acceptable (median absolute prediction error, 18.6%; 95% CI, 14.4 to 22.9%).

A difference in the remimazolam to CNS7054 concentration ratio was observed between step-up and step-down. The largest difference was observed at the 150 ng/ml TCI target concentration (step-up ratio, 0.35 [minimum, maximum: 0.23, 0.60] *vs.* step-down ratio, 0.02 [0.01, 0.05]). A population pharmacokinetic model was fitted to the data to quantify both the remimazolam and CNS7054 concentrations over time. Remimazolam and CNS7054 concentrations were well-captured by the population pharmacokinetic model. No difference between step-up and step-down infusions were observed in bias (step-up: median absolute prediction error, 2.8%; 95% CI, –4.2 to 9.9%; step-down: median absolute prediction error, 0.1%; 95% CI, –11.9 to 12.1%) and precision (step-up: median absolute prediction error, 25.0%; 95% CI, 17.6 to 32.5%; step-down: median absolute prediction error, 18.6%; 95% CI, 14.5 to 22.7%). Population pharmacokinetic model structure, parameter estimates, parameter uncertainty, visual predictive checks, and likelihood profiles can be found in Supplemental Digital Content 2 (https://links.lww.com/ALN/D355).

MOAAS Model Development

A total of 338 MOAAS observations was collected, and they are shown in figure 2 (with the size of the orange circles representing the distribution of observed MOAAS for each TCI target concentration). Model development started by fitting the proportional odds model without a drug effect. A drug effect was then added, which was best described using an Emax equation (change in objective function value, –314.9) *versus* a linear equation (change in objective function value, –255.6). A sigmoid equation (change in objective function value, –315.1) only showed minimal improvement (change in objective function value, –0.2) over the Emax equation, but this improvement was not significant.

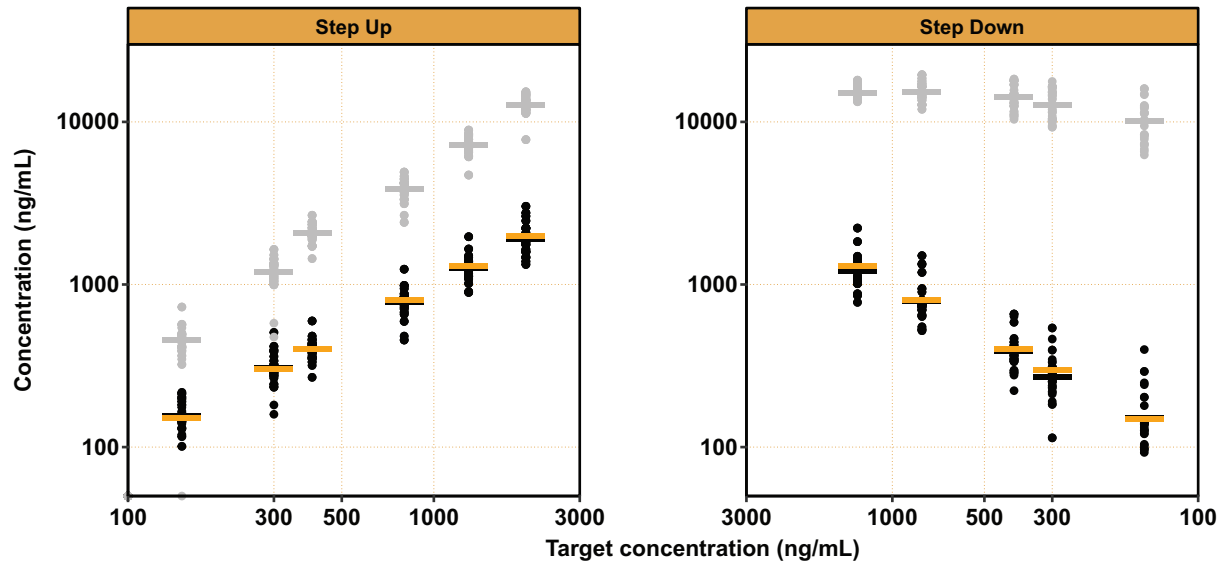

Fig. 1. Plasma remimazolam and CNS7054 concentrations *versus* remimazolam target concentrations in step-up and step-down phases. The *black dots* represent the observed remimazolam concentrations, and the *gray dots* represent the observed CNS7054 concentrations. *Black* and *gray lines* represent the geometric mean of the remimazolam and CNS7054 concentrations. *Orange lines* represent the predicted target concentration.

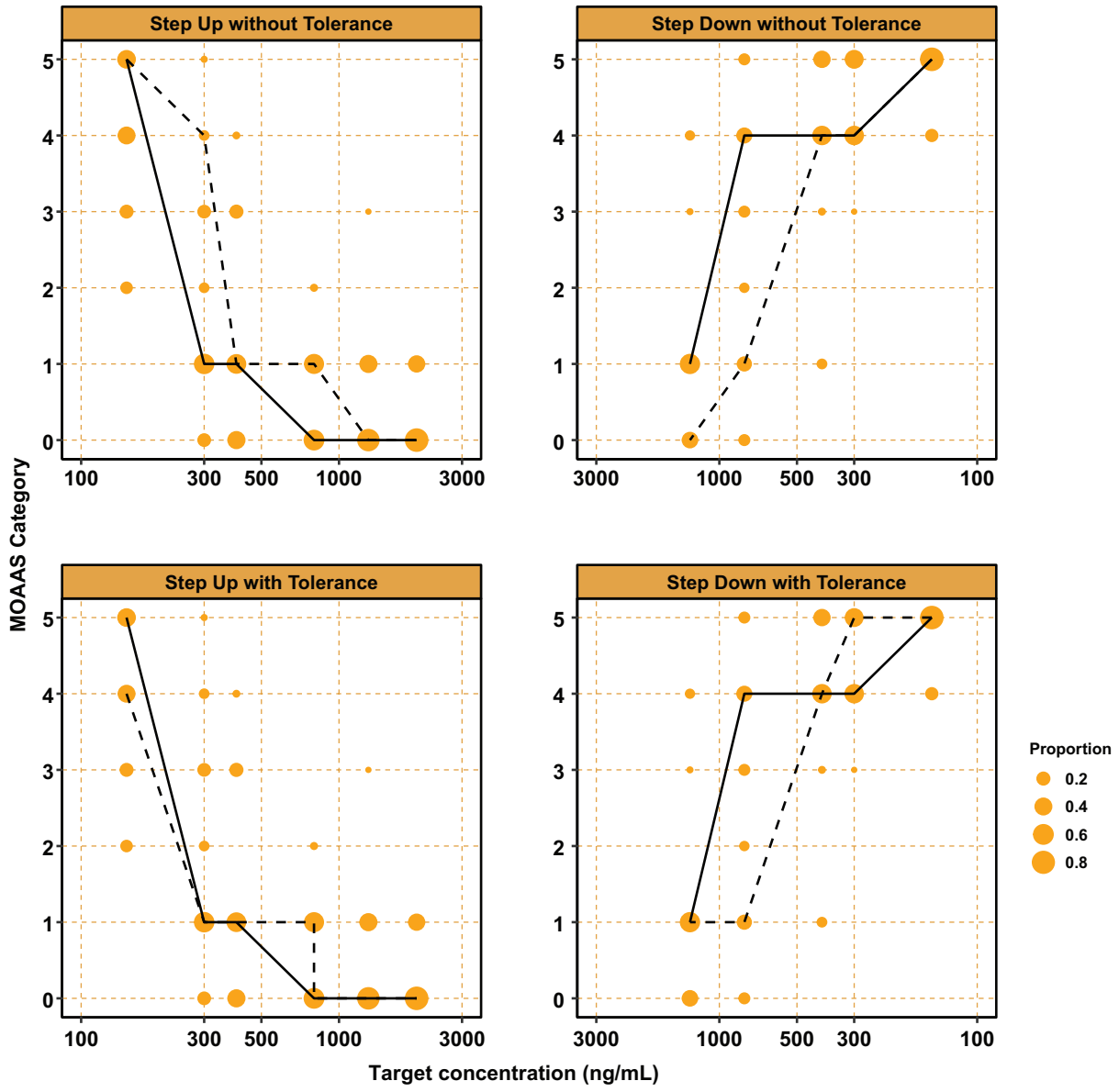

Fig. 2. Modified Observer's Assessment of Alertness and Sedation (MOAAS) scores *versus* remimazolam target concentrations in step-up and step-down phases. *Top*, Model predictions did not account for potential tolerance development. *Bottom*, Model predictions accounted for potential tolerance development. *Orange dots* represent observed MOAAS scores (*size* indicates relative frequency per remimazolam target concentration). *Solid* and *dashed lines* represent the most frequently observed and predicted MOAAS scores, respectively.

An overlay of the predicted MOAAS according to this model and the observed MOAAS (fig. 2, top panels) showed a noticeable difference in the relative distribution of observed MOAAS for each TCI target concentration between step-up and step-down. In an attempt to explain this difference, we explored the addition of a tolerance model to the MOAAS model using two different approaches.

In the first approach, the influence of tolerance was evaluated using a tolerance compartment that drives the influence on the remimazolam drug effect, which was based on the approach described by Ihmsen *et al.*[15] This method will be referred to as "remimazolam-induced tolerance." As a second approach, tolerance development was assumed to be mediated by competitive inhibition by CNS7054, which was based on the approach described by Holford and Sheiner[16] and Tuk *et al.*[17] This method will be referred to as "CNS7054-induced tolerance." Both approaches were evaluated using linear, Emax, or sigmoid-type equations.

Addition of tolerance mechanisms improved the overall model fit with a change in objective function value of −136.7 assuming remimazolam-induced tolerance, and a change in objective function value of −107.4 assuming

CNS7054-induced tolerance. For both CNS7054- and remimazolam-induced tolerance models, the addition of a Hill parameter on the drug effect of remimazolam (remimazolam: change in objective function value, –21.4; CNS7054: change in objective function value, –18.4) and addition of a random effect on Emax (remimazolam: change in objective function value, –20.5; CNS7054: change in objective function value, –39.5) further improved the model. The difference between the two final models was a change in objective function value of –20.5 in favor of the remimazolam-induced tolerance model, but the condition number of remimazolam-induced tolerance model indicated model instability (remimazolam, 8,050; CNS7054, 134). Therefore, the CNS7054 tolerance model was used for the simulations. In the bottom panels of figure 2, the predicted MOAAS with the CNS7054-induced tolerance model is shown. Figure 2 shows that our final model better described the observed difference between the step-up and step-down periods. To ascertain that this observation was not driven by the step-up data only, the model was refitted based on data solely obtained in the down-titration phase. The difference in objective function value between the model with and without tolerance on this subset of data is –59.9 ($P < 0.001$) in favor of the model with tolerance, which also confirms data during the down-titration phase were better described. Model code, visual predictive checks, and likelihood profiles of the CNS7054 tolerance model are shown in Supplemental Digital Content 4 (https://links.lww.com/ALN/D357). Model parameters for the CNS7054-induced tolerance model are provided in table 1.

BIS Model Development

A total of 328 BIS observations were collected and are shown in figure 3. Model development started by fitting a model with a random effect on baseline without a drug effect. A drug effect was then added, which was best described using an Emax equation (change in objective function value, –1,006.4) *versus* a linear equation (change in objective function value, –857.1). A sigmoid equation (change in objective function value, –1,006.8) showed only minimal improvement (change in objective function value, –0.4) over the Emax equation, but this improvement was not significant. Similar to the MOAAS, the observed BIS, shown in the top panels of figure 3, suggests tolerance development. Addition of tolerance mechanisms improved the overall model fit with a change in objective function value of –129.7 assuming CNS7054-induced tolerance, and a change in objective function value of –137.6 assuming remimazolam-induced tolerance.

The difference between the two final models was a change in objective function value of –7.9 points in favor of remimazolam-induced tolerance. The condition number of the remimazolam-induced tolerance model was

Table 1. Parameter Estimates of Bispectral Index and Modified Observer's Assessment of Alertness and Sedation Scores

Parameter Name	Parameter	Estimates	Lower Limit (95% CI)	Upper Limit (95% CI)	Interindividual Variability	Lower Limit (95% CI)	Upper Limit (95% CI)	Shrinkage (%)
Bispectral index								
Baseline (100 – estimate)	BASEB	5.1	4.8	5.5	0.005	0.003	0.01	21.1
Maximum drug effect in logit domain	EMAXB	3.7	3.5	4.0				
EC50 – remimazolam (ng/ml)	EC50B	241	182	311				
EC50 – CNS7054 (ng/ml)	EC50BM	7,838	6,103	10,226				
Additive residual error (SD)		0.41	0.38	0.45				5.5
MOAAS								
Baseline in logit domain MOAAS ≤ 0	BASE0	–10.1	–11.8	–8.9				
Baseline in logit domain MOAAS ≤ 1	BASE1	3.1	2.5	3.9				
Baseline in logit domain MOAAS ≤ 2	BASE2	0.4	0.2	0.8				
Baseline in logit domain MOAAS ≤ 3	BASE3	1.1	0.7	1.7				
Baseline in logit domain MOAAS ≤ 4	BASE4	2.7	2.1	3.4				
Maximum drug effect in logit domain	EMAXM	12.4	10.1	15.3	0.035	0.013	0.078	18.2
EC50 – remimazolam (ng/ml)	EC50M	189	150	245				
EC50 – CNS7054 (ng/ml)	EC50MM	1,351	481	3,108				
Hill parameter	GAMMAM	2.1	1.7	2.6				

The interindividual variability was expressed as SD in the logit domain. The residual variability of the bispectral index model was expressed as SD in the logit domain. The lower limit and upper limit of the 95% CI of the parameters were derived using log-likelihood profiling. Model code, visual predictive checks, and likelihood profiles of the CNS7054 tolerance model are shown in Supplemental Digital Content 4 (https://links.lww.com/ALN/D357).

MOAAS, Modified Observer's Assessment of Alertness and Sedation.

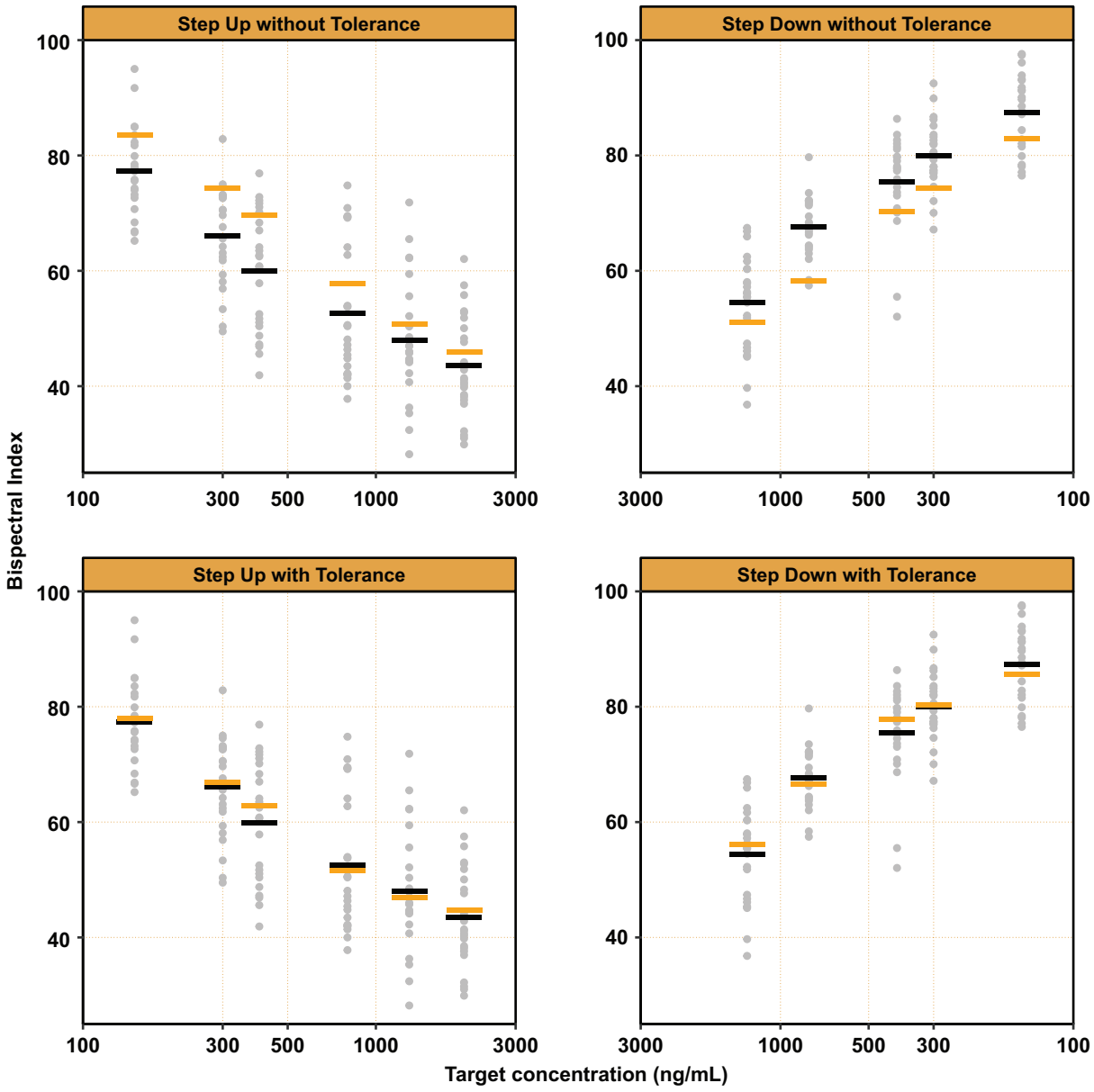

Fig. 3. Bispectral index values *versus* remimazolam target concentrations in step-up and step-down phases. *Top*, Model predictions did not account for potential tolerance development. *Bottom*, Model predictions accounted for potential tolerance development. *Gray dots* represent observed bispectral index values. *Black* and *orange lines* represent the observed and predicted mean bispectral index values, respectively.

higher (remimazolam, 1,051.6; CNS7054, 185.1). No differences between the tolerance models were observed in goodness-of-fit plots. In the bottom panels of figure 3, the predicted BIS according to the CNS7054 tolerance model is shown. This model better described the observed difference between the step-up and step-down periods. The CNS7054-induced tolerance model was used for subsequent simulations. Model code, visual predictive checks, and likelihood profiles of the CNS7054 model are displayed in Supplemental Digital Content 4 (https://links.lww.com/ALN/D357). Model parameters of the CNS7054-induced tolerance model are provided in table 1.

Simulations

Simulations were conducted to visualize the pharmacokinetic-pharmacodynamic relationship between remimazolam and BIS and MOAAS after repeated bolus dosing or continuous infusion (fig. 4; table 2). For the repeated bolus dose simulations, BIS was predicted to be 54 (95% prediction interval, 44 to 67) in the model without tolerance *versus*

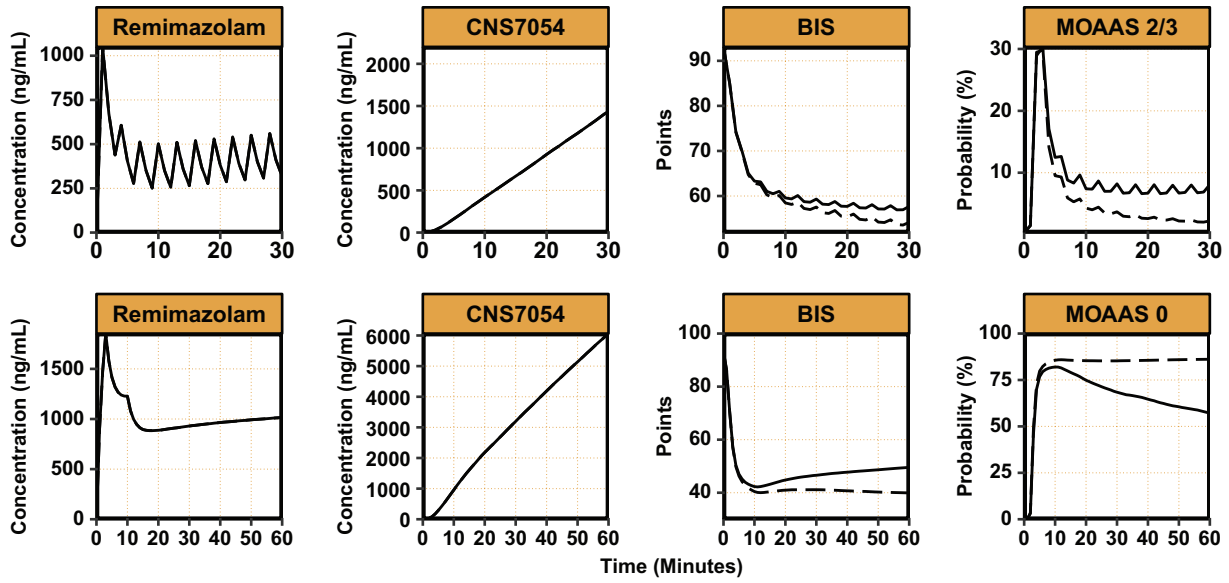

Fig. 4. Simulated bispectral index (BIS) values and probability of Modified Observer's Assessment of Alertness and Sedation (MOAAS) scores 2/3 (sedation) or 0 (anesthesia) after repeated bolus dose administration (*top*) or continuous infusion (*bottom*). The *solid black lines* are simulations that account for potential tolerance development, while *dashed lines* are simulations that do not account for potential tolerance development. The units are nanograms per milliliter for remimazolam and CNS7054 concentration, points for BIS, and percentage for MOAAS 2/3 and MOAAS 0.

Table 2. Simulated Mean (95% Prediction Intervals) BIS Values and Probability of MOAAS 2/3 (Sedation), MOAAS < 2 or 0 (Anesthesia) after Repeated Bolus Dose Administration or Continuous Infusion Assuming a Rate Constant for Effect-site Equilibration of 0.135 min^{-1}

		Timepoint (min)	P(MOAAS 2/3) (%)	P(MOAAS < 2) (%)	P(MOAAS 0) (%)	BIS (Points)
Repeated bolus dose	Without tolerance	10	4 (0–36)	94 (36–100)		59 (49–71)
		20	3 (0–33)	97 (47–100)		55 (45–68)
		30	2 (0–32)	97 (51–100)		54 (44–67)
	With tolerance	10	7 (0–36)	90 (27–100)		60 (50–72)
		20	7 (0–36)	91 (27–100)		58 (48–70)
		30	8 (0–36)	90 (24–100)		58 (48–70)
Continuous infusion	Without tolerance	10			86 (18–100)	41 (33–50)
		20			86 (17–100)	41 (34–51)
		30			86 (18–100)	41 (34–51)
		60			87 (18–100)	40 (33–50)
	With tolerance	10			83 (15–100)	42 (35–52)
		20			76 (11–100)	45 (47–55)
		30			70 (9–99)	47 (38–57)
		60			59 (6–99)	50 (41–60)

BIS, bispectral index; MOAAS, Modified Observer's Assessment of Alertness and Sedation.

58 (95% prediction interval, 48 to 70) in the model with tolerance after 30 min of dosing. The probability of reaching a MOAAS score of 2 or 3 was 2% (95% prediction interval, 0 to 32%) in the model without tolerance *versus* 8% (95% prediction interval, 0 to 36%) in the model with tolerance after 30 min of dosing. The probability for oversedation (MOAAS score less than 2) decreased when tolerance was assumed (table 2).

For the continuous infusion, the predicted BIS was 40 (95% prediction interval, 33 to 50) in the model without tolerance *versus* 50 (95% prediction interval, 41 to 60) in the model with tolerance after 60 min of dosing. The probability of reaching a MOAAS score of 0 was 87% (95% prediction interval, 18 to 100%) in the model without tolerance *versus* 59% (95% prediction interval, 6 to 99%) in the model with tolerance after 60 min of dosing. Simulations

with a rate constant for effect-site equilibration of 0.6 min^{-1} showed similar trends (Supplemental Digital Content 4, https://links.lww.com/ALN/D357).

Discussion

In this dose-ranging trial, a difference in the sedative effects of remimazolam, as measured by MOAAS and BIS, was observed at identical TCI target concentrations between the step-up and step-down parts of the titration scheme. The difference could not be explained by a bias in the predicted remimazolam target concentrations. Pharmacodynamic models for MOAAS and BIS that assumed tolerance development were able to better describe the observed difference in the sedative effects between the step-up and step-down parts of the titration scheme.

The observed difference in sedative effects between step-up and step-down could indicate that the population pharmacokinetic model, used for TCI administration of remimazolam in this trial, displayed a bias in the predicted target concentrations. The observed remimazolam concentrations, however, indicated that the model was able to adequately describe the pharmacokinetics of remimazolam over time. The population pharmacokinetic model developed from these data resulted in very similar pharmacokinetic parameters to those of the model used to design the clinical trial.[11] In addition, model diagnostics, both graphically and numerically, did not show clear signs of a misspecification of remimazolam concentrations between step-up and step-down, which further confirms that the pharmacokinetic profile of remimazolam behaved as expected.

It was therefore hypothesized that the observed difference in sedative effects between step-up and step-down was caused by pharmacodynamic mechanisms, such as development of acute tolerance or tachyphylaxis. Svensson proposed a mechanistic distinction between (acute) tolerance and tachyphylaxis, where tachyphylaxis is reserved for attenuation of drug response by cellular depletion.[18] Cellular depletion is not directly a logical explanation for the observed difference between step-up and step-down as the mechanism of action of remimazolam is based on enhancing the effects of γ-aminobutyric acid, and we are not aware of any depletion downstream of the receptor. Development of acute tolerance was therefore explored.

Acute tolerance has been observed before by Zhou *et al.*,[11] who used the cumulative dose to account for a bias in the relationship between remimazolam concentration and BIS over time, and by Io *et al.*,[19] who demonstrated tolerance development in remimazolam-induced sedation in a miniature pig model. We explored two different, more mechanistic, modeling approaches to describe tolerance in the sedative effects. In the first approach, tolerance was induced by the remimazolam concentration using a tolerance compartment, which is similar to the effect compartment approach, where an additional compartment is used to describe delays between pharmacokinetics and pharmacodynamics. The second approach was inspired by the observed difference in the remimazolam to CNS7054 concentration ratio during step-up and step-down, where the metabolite concentration influenced the affinity of remimazolam assuming competitive antagonism. The data from this trial indicated more plausible model parameters and a more stable model when tolerance was induced by CNS7054.

Tolerance development has also been described previously for another short-acting benzodiazepine, RO48-6791, and other benzodiazepines like midazolam.[15,17] For RO48-6791, tolerance has been successfully described by an additional tolerance compartment that was driven by the concentration of RO48-6791.[15] The drug effect was then modified using a competitive interaction model. For RO48-6791, the hypothetical tolerance compartment followed the pharmacokinetic behavior of the metabolite, and therefore tolerance was also described using a model where the metabolite concentration of RO48-6791 was driving tolerance.[15] This model resulted in a similar fit as the model where tolerance was driven by RO48-6791, suggesting that tolerance of RO48-6791 could be mediated by the metabolite.[15] In our analysis, we also found that both approaches could describe tolerance, which suggests that also for remimazolam, CNS7054 could be driving potential tolerance development in the sedative effects.

The exact tolerance mechanism of remimazolam remains unclear. Tolerance driven by a metabolite has also been described for midazolam and its metabolite α-hydroxymidazolam.[17] The difference, however, between midazolam and RO48-6791 is that the metabolite of RO48-6791 does not have an intrinsic effect for $GABA_A$, whereas α-hydroxymidazolam does have an intrinsic effect for $GABA_A$.[15,17] For RO48-6791, it is expected that the metabolite influences the concentration of parent drug at the receptor site and, consequently, weakens the effect. CNS7054 was also best described when assuming no intrinsic effect for $GABA_A$ (data not shown). Therefore, potential tolerance development of remimazolam resembles tolerance development of RO48-6791 and could also be attributed to a weakening of effect due to influencing the remimazolam concentration at the receptor site. We cannot, however, distinguish remimazolam-induced tolerance development *versus* CNS7054-induced tolerance development. To confirm that CNS7054 induces tolerance, *in vitro* experiments, such as functional assays with recombinantly expressed $GABA_A$ receptors or inducible pluripotent stem cells that are differentiated into GABAergic neurons, should be conducted.

The simulations from our final models for MOAAS and BIS indicate that the clinical impact of tolerance development on the sedative effects of remimazolam is limited for the simulated U.S. dosing regimens for procedural sedation

targeting short procedures (less than 30 min). At the same time, it is noteworthy to appreciate that our results suggest that CNS7054 could be involved in the tolerance development of remimazolam. Hence, tolerance development could be more pronounced in situations where CNS7054 pharmacokinetics are different from those in healthy volunteers. For example, in patients with impaired kidney function, where higher CNS7054 concentrations have been reported, the CNS7054-induced tolerance may have a larger impact on the sedative effects of remimazolam.[20] Unfortunately, the dose in the clinical trial that studied the influence of impaired kidney function was too low to demonstrate an influence on the sedative effects of remimazolam.[20] Tolerance development would also be more pronounced in situations when remimazolam infusion is stopped.

This study aimed to evaluate appropriate target concentrations for use in clinical practice. However, in the presence of tolerance development, the effects of remimazolam on MOAAS and BIS change over time. One consequence of this finding is that TCI targets aimed at attaining a fixed sedative effect (*e.g.*, targeting a BIS of 50) will change with time, which complicates remimazolam administration using TCI. In addition, administration of remimazolam using TCI is especially beneficial for appropriate sedation in long-term procedures. The impact of the quantified tolerance development for long-term procedures is difficult to determine as titration steps in this study had a relatively short duration (approximately 25 min) and did not take into account the titration to effect that is part of common clinical practice. Future studies will therefore need to determine appropriate ways of managing tolerance development in the sedative effects of remimazolam at the bedside.

A limitation of the trial is that relatively few observations of MOAAS 2 and 3 were collected, which increases uncertainty in the predictions for these MOAAS scores, which are most relevant for sedation. Additionally, limited CNS7054 pharmacokinetic samples were collected after stop of infusion. Therefore, we could not quantify a second distribution compartment of CNS7054, which has been described previously. This is, however, not expected to influence the described tolerance development of remimazolam. The tolerance phenomenon was unexpected, and therefore, the clinical trial was not *a priori* designed to characterize the observed tolerance development. It is unclear to what extent the tolerance phenomenon observed in this study depends on our study design. A study with prolonged target-controlled infusion at a constant effect-site concentration would be highly informative for studying the tolerance phenomenon and could be used in a future study to confirm our findings. An effect-site elimination rate constant of 0.135 min^{-1} was assumed in the simulations as the rate constant for effect-site equilibration could not be estimated in this clinical trial due to the collection of MOAAS and BIS at steady state. An alternative effect-site elimination rate constant did not alter conclusions that were based on the simulations as additional simulations using an effect-site elimination rate of 0.6 min^{-1} showed similar trends to those displayed in figure 4. Finally, this trial included healthy volunteers, and it is therefore unknown whether the observed tolerance development is also apparent in the patient setting. Therefore, future studies are needed to confirm or negate our findings (*e.g.*, in patients with impaired kidney function using a higher remimazolam dose) and, if relevant, establish appropriate ways of handling tolerance in a broad patient population (*e.g.*, by studying different dosing regimens).

In conclusion, we showed that there is target concentration–dependent sedation for remimazolam with little impact on vital signs. However, remimazolam-induced sedation is prone to tolerance development, which is potentially mediated by the CNS7054 concentration. The clinical consequences for short-term procedures for the general population are limited, but a more pronounced tolerance effect can be expected in long-term procedures and in patients with impaired kidney function. This does not have clinical consequences when titrating to effect.

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Competing Interests

Dr. Struys reports that his research group or department received (during the last 3 yr) research grants and consultancy fees from The Medicines Company (Parsippany, New Jersey), Masimo (Irvine, California), Becton Dickinson (Eysins, Switzerland), Fresenius (Bad Homburg, Germany), Dräger (Lübeck, Germany), Paion (Aachen, Germany), Medtronic (Dublin, Ireland), Medcaptain Europe (Andelst, The Netherlands), Baxter (Chicago, Illinois), and HanaPharm (Seoul, Republic of Korea). He receives royalties on intellectual property from Demed Medical (Sinaai, Belgium) and Ghent University (Ghent, Belgium). He is an editorial board member and director for the *British Journal of Anesthesia*. Dr. Eleveld received travel from Becton Dickenson and is an editorial board member of Anesthesiology. Dr. Colin reports that during the last 3 yr, his research group has been involved in contract research for PAION UK Ltd. (London, United Kingdom) and Acacia Pharma Ltd. (Cambridge, United Kingdom). Drs. Stöhr and Pesic are/were employees of Paion at the time of study conduct. The other authors declare no competing interests.

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Supplemental Digital Content

Supplemental Digital Content 1. Sequence of interventions, definition MOAAS categories, https://links.lww.com/ALN/D354

Supplemental Digital Content 2. Development population pharmacokinetic model, https://links.lww.com/ALN/D355

Supplemental Digital Content 3. Hemodynamic effects, https://links.lww.com/ALN/D356

Supplemental Digital Content 4. Diagnostics population pharmacodynamic models, https://links.lww.com/ALN/D357

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