Pharmacotherapy and ventilatory control in health and disease

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Chapter 3

S-ketamine oral thin film – Part II: population pharmacodynamics of S-ketamine, S-norketamine and S-hydroxynorketamine

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Introduction

The N-methyl-D-aspartate receptor antagonist ketamine experiences a clinical renaissance due to the introduction of various new indications.[1] While initially developed as an anesthetic and a substitute for phencyclidine, it later gained popularity as an analgesic and currently is available as a rapid-acting antidepressant.[1] Ketamine has multiple administration routes that may be divided into those that require a sometimes painful injection or venipuncture (intravenous and subcutaneous delivery) and those that circumvent the disadvantages of delivery by injection and allow an easy and painless treatment. The latter route includes delivery by oral, intranasal, transcutaneous, or rectal routes. Here, we study the pharmacodynamics [and in part 1 of this study, the pharmacokinetics, see chapter 2][2] of sublingual and buccal fast-dissolving oral-thin-films (OTFs) that contain 50 mg of S-ketamine, one of the isomers of ketamine. The OTF is a rectangular 4.5 cm^2 thin film that is loaded with an active substance that immediately dissolves in the mouth and is rapidly absorbed through the mucosa. Ketamine is a complex drug for various reasons; it is a racemic mixture of S- and R-isomers and is metabolized into active compounds such as norketamine and hydroxynorketamine.[3,4] All these differ in pharmacokinetics and dynamics, and consequently may influence the ultimate effect of the drug and consequently the S-ketamine OTF.[3,4]

In the current study, we present the results of a pharmacodynamic analysis of the effect of one and two OTFs, containing, respectively, 50 and 100 mg S-ketamine, administered sublingually or buccally. In the accompanying report, we showed that the S-ketamine OTF undergoes a large first-pass effect causing relatively high concentrations of S-norketamine and S-hydroxynorketamine.[3] We studied the OTF on two end-points, nociception, by testing three distinct pain assays (pressure pain, electrical pain, and thermal pain), and drug high, one of the psychotomimetic effects of S-ketamine. Our main interest is the description of the pharmacodynamic effects of S-ketamine in the S-ketamine OTF. Additionally, we quantified the contribution of the S-ketamine metabolites in the production of antinociception and drug high. Earlier studies demonstrated that S-norketamine has a little analgesic effect in humans and is possibly even pro-algesic,[5] while animal data indicate that S-hydroxynorketamine has potent analgesic and antidepressant properties.[6,7]

We performed a population pharmacodynamic analysis of the S-ketamine OTF in a group of healthy volunteers and built a pharmacodynamic model that incorporates the contribution of S-norketamine and S-hydroxynorketamine. Pharmacokinetic–pharmacodynamic modeling is an important tool in the development of new therapies (including new administration modes of existing therapies) to quantify the therapeutic index or utility in terms of wanted and unwanted effects and determine the contribution of metabolites.

Methods

This report is accompanied by a report on the population pharmacokinetics of the *S*-ketamine OTF, in chapter 2.[8] Here we describe the pharmacodynamic endpoints that were collected simultaneously with the pharmacokinetic data.

Ethics and Subjects

The protocol was approved by the Central Committee on Research Involving Human Subjects (Competent authority: Centrale Commissie Mensgebonden Onderzoek (CCMO), The Hague, the Netherlands; registration number NL75727.058.20) and the Medical Research Ethics Committee of Leiden University Medical Center (Medisch Ethische Toetsingscommissie Leiden-Den Haag-Delft, The Netherlands; identifier P20.111) and was registered at the trial register of the Dutch Cochrane Center (www.onderzoekmetmensen.nl) under identifier NL9267. Healthy male and female volunteers (aged 18-45 years, body mass index 19 and 30 kg.m^{-2}) were recruited. All recruited subjects gave written and oral informed consent, after which they were screened. Inclusion and exclusion criteria are given in chapter 2. Eating, drinking, brushing teeth or gum chewing was not allowed in the morning of the OTF application to avoid changes/variabilities in saliva pH, which could potentially affect the mucosal permeability and *S*-ketamine plasma concentration variability.

Study Design

This phase 1 study had an open-label randomized crossover design. The subjects were randomized to receive one OTF on one occasion (50 mg *S*-ketamine) and two on another (100 mg *S*-ketamine) with at least 7 days between visits. The thin film is a rectangular 4.5 cm^2 orodispersible film containing 57.7 mg *S*-ketamine hydrochloride (*S*-ketamine HCL). The *S*-ketamine HCL is dispersed within a matrix to produce a film corresponding to 50 mg *S*-ketamine free base. The film(s) was/were placed either under the tongue or buccally on the mucosa. After placement of the films, the subject was not allowed to swallow for 10 min. The randomization sequence was determined by the randomization option in the Electronic Data Capture system CASTOR (www.castoredc.com). The OTFs were obtained from LTS Lohmann Therapie-Systeme AG (Andernach, Germany) and were dispensed by the pharmacy on the morning of dosing. Measurement of pharmacodynamic endpoints lasted for 6 h. For blood sampling and measurement of *S*-ketamine, *S*-norketamine and *S*-hydroxynorketamine, see the accompanying report.[2]

In all subjects, on both occasions, an intravenous *S*-ketamine infusion followed the 6 h OTF test phase and was included in the pharmacokinetic

analysis to determine the S-ketamine bioavailability. Here we only present the pharmacodynamic data obtained during the OTF test phase.

Noxious assays

Three independent pain assays, namely thermal noxious pain, electrical noxious pain and pressure pain, were randomly applied around predefined time intervals: t = 0, 10, 20, 30, 40, 60, 80, 100, 120, 150, 180, 240, 300 and 360 min after placement of the OTF(s) with 3-5 min in between tests.

Electrical pain was induced by an in-house manufactured transcutaneous electrical current stimulator.[9] A constant current electrical stimulus train (stimulation at 20 Hz, pulse duration 0.2 ms) was applied on the skin over the tibial bone on the non-dominant side of the body through two surface electrodes. The location of the electrodes was such that muscle contractions did not occur. The current that it induced a numerical pain rating score (NRS) of 8 on a pain scale from 0 (no pain) to 10 (worst pain imaginable) at baseline was used in the remainder of the study. The search for the correct current was performed three times before any drug administration at 5-10 min intervals in steps of 0.5 mA.

Thermal noxious stimulation was applied on the volar side of the non-dominant forearm using a 3 cm^2 Peltier element or thermal probe of the Pathway device (Medoc Ltd., Israel) that allows computer-controlled changes in contact heat changes in steps of $\pm$ 0.5 °C.[9] In the current study a heat level was chosen that at baseline caused an NRS of 8 on the above-mentioned 11-point pain scale. The correct heat level was derived from three tests at 5-10 min intervals.

Pressure pain was induced using an Algometer (FDN 200 series, Wagner Instruments Inc., Greenwich, CT).[10] Pressure pain was delivered on a 1 cm^2 skin area between the thumb and index finger of the non-dominant hand. The device has a force capacity ($\pm$ accuracy) of 200 $\pm$ 2 N (= 20 $\pm$ 0.2 kgf) and graduation of 1 N (100 gf), respectively. A gradually increasing pressure was applied and the subjects indicated when the pressure became painful (pressure pain threshold). Three tests were applied at baseline; the obtained pressure values were averaged and served as the baseline value. A researcher well trained in this assay performed the pressure pain tests throughout the study visit days.

Questionnaire

The Bowdle questionnaire was taken at regular intervals to determine the effect of treatment on mental and psychotomimetic side effects.[11] The timing of the questionnaires was at baseline (prior to any drug administration) and at 30 min intervals until 6 h after thin film application. In case the querying coincided with pain testing, the questionnaires were taken prior to pain testing. The Bowdle questionnaire allows the derivation of three factors of psychedelic ketamine effects: drug high and changes in internal and external perception. All

three were measured on a visual analogueue score from 0 (no effect) to 10 cm (maximum effect). In the pharmacokinetic-pharmacodynamic data analysis, we included the effect of the OTF on drug high derived from the Bowdle questionnaire. The description of the effect of the *S*-ketamine OTF on the other endpoints, internal and external perception, is given in the in Supplementary Figure 1 online.

Population pharmacodynamic analysis

Data were analyzed in a stepwise fashion. First, the pharmacokinetic data were analyzed using a population-based approach (see chapter 2). Next, the pharmacodynamic data were analyzed with individual concentration profiles of *S*-ketamine and its metabolites (based on the empirical Bayesian estimates of the pharmacokinetic parameters) as input of the sigmoid E_{MAX} pharmacodynamic models. The metabolites were assumed to be agonists or antagonists, with the total effect, EFF, modeled as:

$$EFF = EEF(K) + EFF(NK) + EFF(HNK)$$

With

$$EFF(K) = C_{E,K}/C_{50,K}$$

$$EFF(NK) = C_{E,NK}/C_{50,NK}$$

$$EFF(HNK) = C_{E,HNK}/C_{50,HNK}$$

or

$$EFF = EFF(K) = C_{E,K}/C_{50}$$

with

$$C_{50} = C_{50,K} \times [1 + EFF(NK) + EFF(HNK)]$$

and

$$EFF(NK) = C_{E,NK}/C_{100,NK}$$

$$EFF(HNK) = C_{E,HNK}/C_{100,HNK}$$

under the agonistic and antagonistic assumptions, respectively. $C_{E,K}$, $C_{E,NK}$ and $C_{E,HNK}$ are the effect-site concentrations of *S*-ketamine, *S*-norketamine and

S-hydroxynorketamine, respectively; $C_{50,K}$, $C_{50,NK}$ and $C_{50,HNK}$ are the steady-state or effect-site concentrations causing 50% of the pharmacodynamic effect; and $C_{100,NK}$ and $C_{100,HNK}$ are the S-norketamine and S-hydroxynorketamine concentrations causing a 100% increase of S-ketamine C_{50}.

An effect compartment was postulated to account for the hysteresis between the S-ketamine plasma concentrations (and possibly its metabolites) and its effect. This effect compartment equilibrates with the plasma compartment with plasma-effect-site equilibration half-life ($t_{1/2}k_{e0}$).

The results of the electrical and thermal noxious assays were analyzed using the following inhibitory sigmoid E_{MAX} model:

$$NRS(t) = NRS_0 \ \times \ [1 + (C_E(t)/C_{50})^\gamma]^{-1}$$

where NRS(t) is the NRS in response to the noxious stimulation at time t and NRS_0 is the NRS at baseline (pre-drug condition), and γ is a dimensionless shape parameter.

For pressure pain, we assume that S-ketamine (and possibly its metabolites) attenuates the response to the applied noxious pressure stimulus by the inhibition of signal propagation and central nociceptive processing. As a consequence, stronger stimuli are needed before the subjects indicate that he or she experiences pain. The attenuation (A) is described by an inhibitory sigmoid E_{MAX} model:[12]

$$A = [1 + \ C_E(t)/C_{50})^\gamma]^{-1}$$

Since a response of the subjects occurs just above the response threshold, we use the following equation for the pressure pain threshold at time t:

$$P(t) = \ P_0 \ \times \ 1/A = \ P_0 \times \ [1 + \ C_E(t)/C_{50})^\gamma]$$

where P_0 is the baseline or pre-drug pressure that elicited a pain threshold response.

Drug high was modeled using a sigmoid E_{MAX} model:

$$VAS \ drug \ high \ (t) = [E_{max} \ \times \ C_E(t)^\gamma] / \ [C_{50}{}^\gamma + \ C_E(t)^\gamma]$$

where C_{50} is the S-ketamine concentration that causes a drug high of 50% of E_{MAX}, and E_{MAX} the maximum possible effect on drug high (10 cm).

Data analysis was performed using NONMEM version 7.5.0 (ICON Development Solutions, Hanover, MD, USA). Inter-occasion variability (ν^2) was determined for baseline values only, as we assumed that other parameter values would not differ between the two occasions and were drug-dose independent. Determining whether the metabolites contributed to the measured effect and the level of significance of model parameters were based on the log-likelihood criterion (-2LL; a decrease of more than 6.6 is significant at the $p < 0.01$ level for one additional parameter). The Goodness-of-Fit was based on the visual inspection of the model fits and Goodness-of-Fit plots (individual predicted *versus* measured, individual weighted residuals *vs.* time and normalized prediction discrepancy error *vs.* time). Additionally, visual predictive checks (PVCs) were generated to ensure that the models were able to reproduce the data used for model building. Although no pharmacokinetic differences were observed in the sublingual and mucosal applications, we compared the location of the application on the pharmacodynamic parameter estimates.

Results

Twenty subjects participated in the study to receive, in random order, a single OTF (50 mg) or, on a second occasion, two OTFs applied simultaneously (100 mg) sublingually ($n = 15$) or on the buccal mucosa ($n = 5$). One subject participated only once (receiving 100 mg S-ketamine), due to side effects experienced from the intravenous S-ketamine infusion. Since no differences were observed in the sublingual and mucosal application regarding pharmacokinetics and pharmacodynamics of S-ketamine and its metabolites, we combined the two in the pharmacokinetic and pharmacodynamic model analyses. The mean age of the volunteers was 24 years (with range 19-32 years), mean weight 73 kg (53-93 kg), and mean body mass index 23 kg/m^2 (19-27 g/m^2) with an equal number of men and women participating (10/10).

To summarize the pharmacokinetic data that stands at the basis of the pharmacodynamic analyses, we give the average plasma concentrations for S-ketamine, S-norketamine and S-hydroxynorketamine following 50 and 100 mg S-ketamine OTF in Figure 3.1 on page 49. It shows the large first-pass effect, with relatively high concentrations of the S-ketamine metabolites. No serious adverse events occurred during the study (see chapter 2 for a description of adverse events). The effects of the 50 and 100 mg S-ketamine OTF on pain responses are given in Figure 3.2 on page 49 for the three assays: electrical pain, heat pain and pain pressure. The data indicate that the OTF produces antinociception in all three assays, but irrespective of the pain assay a clear dose-response relationship was absent. Among subjects, pain relief was most variable in the pain pressure test compared to the other pain test with pain relief lasting two hours (heat pain test) or longer (electrical pain test). Comparing Figures 3.1 and 3.2, gives rise to the suggestion that the S-ketamine effect is pharmacokinetically-driven, *i.e.* that the pain responses closely follow the S-ketamine concentration profile, without any effect of the metabolites. In Figure 3.4, panels A and B, the individual median drug high visual analogueue scores are given in gray and red, respectively. The peak median effect is higher for the 100 mg S-ketamine OTF compared to the lower dose OTF.
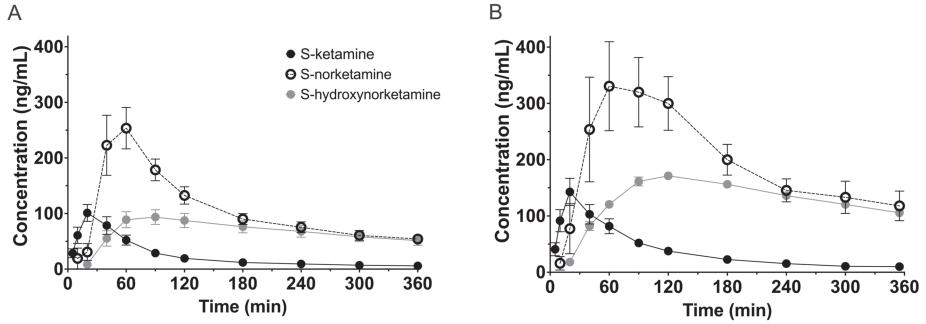
Figure 3.1: Pharmacokinetic data. Average plasma concentrations $\pm$ 95% confidence intervals for *S*-ketamine, S-norketamine and *S*-hydroxynorketamine following administration of 50 mg (**A**) and 100 mg (**B**) *S*-ketamine oral thin film.
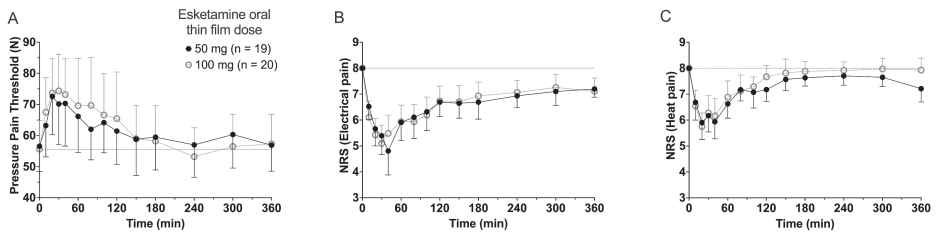

Figure 3.2: Antinociceptive data. The effects of the 50 mg and 100 mg *S*-ketamine oral thin film on pain responses for the three pain assays, pressure pain threshold (**A**), electrical pain numerical rating scale (**B**), and heat pain numerical rating scale (**C**). Data are mean $\pm$ 95% confidence interval.

Population pharmacodynamic analysis

Population parameters estimates are given in Table 3.1 on page 51, the best median and worst fits (based on R^2) for all 4 endpoints are given in Supplementary Figure 1 online. The Goodness-of-Fit plots are given in Figure 3.3 and Figure 3.4 on page 52 and 53, panels C-E. Visual Predictive Checks (VPCs), comparing observations with model predictions, are given in Figure 3.5 on page 54. Inspection of the fits, Goodness-of-Fit plots and VPCs indicate that the PKPD models adequately describe the data with no differences between the 50 and 100 mg OTF-related data. No contribution of either *S*-norketamine or *S*-hydroxynorketamine could be detected, *i.e.* EFF(NK) and EFF(HNK) approached zero. Consequently, the antinociceptive and drug high effects are attributed solely to *S*-ketamine. *S*-ketamine potency was about 3- to 5-fold lower for antinociception than for drug high: C_{50} 1.2-1.7 nmol/mL *vs.* 0.3 nmol/mL for the nociceptive tests and for drug high, respectively. The onset/offset of the S-ketamine effect was similarly fast for all tests, nociceptive and drug high, and ranged from a value not different from zero (Table 3.1), indicative of an instantaneous effect to 5 min. In Figure 3.6 on page 55, the steady-state or effect-site concentration-effect relationships are given for the four pharmacodynamic endpoints. The dots in the figure depict the C_{50} values. No effect of the location of the OTF on parameter estimates was observed ($p > 0.05$).

Table 3.1: Pharmacodynamic parameter estimates

	Estimate $\pm$ SEE	$\omega^2 \pm$ SEE	$\nu^2 \pm$ SEE
Electrical pain NRS (0-10)			
Baseline NRS	7.2 ± 0.2		0.025 ± 0.010
$t_{1/2}k_{e0}$ (min)	3.4 ± 0.4	1.13 ± 0.81	
C_{50} (nmol/mL)	1.3 ± 0.2	1.01 ± 0.32	
γ	1 (FIX)		
σ	0.75 ± 0.07		
Heat pain NRS (0 -10)			
Baseline NRS	7.9 ± 0.2		0.011 ± 0.006
$t_{1/2}k_{e0}$ (min)	-		
C_{50} (nmol/mL)	1.2 ± 0.32	0.57 ± 0.27	
γ	1 (FIX)	0.57 ± 0.31	
σ	0.66 ± 0.08		
Pressure pain			
Baseline (N)	56 ± 5	0.09 ± 0.03	0.03 ± 0.01
$t_{1/2}k_{e0}$ (min)	-		
C_{50} (nmol/mL)	1.7 ± 0.29	0.27 ± 0.11	
γ	1 (FIX)		
σ (N)	9.26 ± 1.18		
Drug high VAS (0-10 cm)			
$t_{1/2}k_{e0}$ (min)	5.4 ± 0.2	1.1 ± 0.69	
C_{50} (nmol/mL)	0.31 ± 0.03	0.16 ± 0.08	
γ	2.8 ± 0.3	0.15 ± 0.05	
σ	0.89 ± 0.10		

NRS = numerical rating scale; $t_{1/2}k_{e0}$ is blood-effect-site equilibration half-life; C_{50} is the concentration at steady-state causing 50% effect; γ is a shape parameter and σ a measure of residual noise.
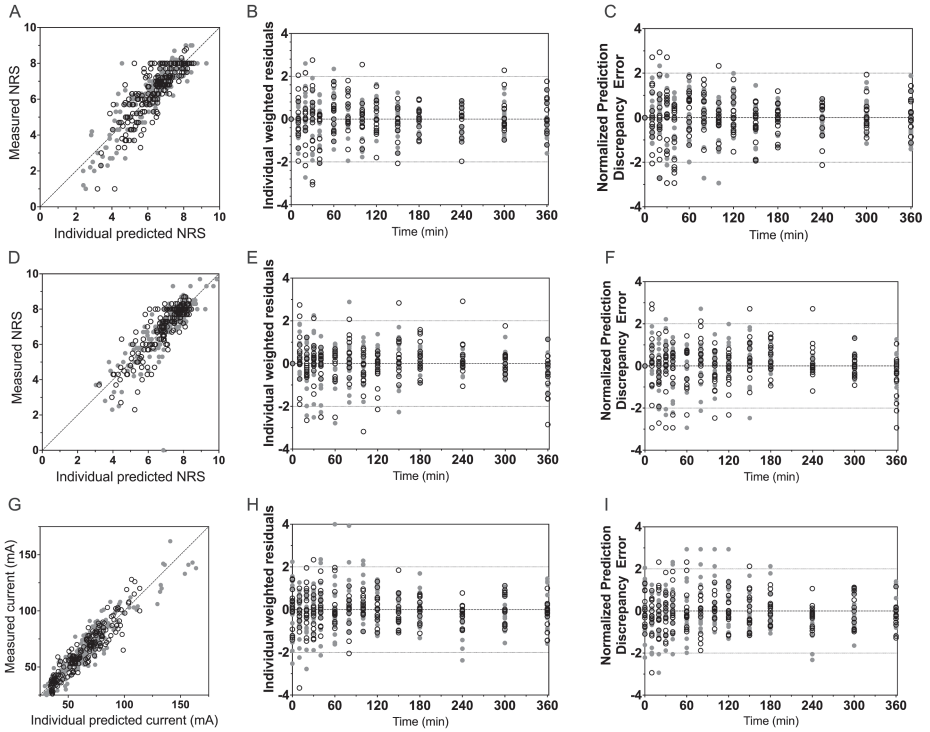
Figure 3.3: Goodness-of-Fit plots. A-C electrical pain numerical rating scale; **D-F** heat pain numerical rating scale; **G-I** pressure pain threshold. **A**, **D**, and **G**: measured versus individual predicted; **B**, **E**, and **H**: individual weighted residuals versus time. **C**, **F** and **I**: normalized prediction discrepancy errors versus time. Dashed lines are the 95% confidence intervals. Open circles denote data from the 50 mg *S*-ketamine oral thin film, closed circles data from the 100 mg *S*-ketamine oral thin film.
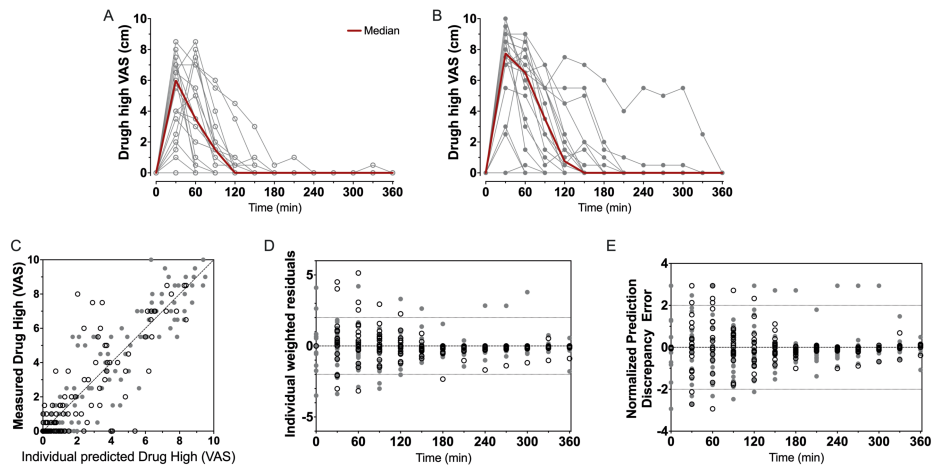
Figure 3.4: drug high data. Individual and median drug high visual analogueue scores following the 50 mg (**A**) and 100 mg (**B**) *S*-ketamine oral thin film. **C-E**: Goodness-of-Fit plots, **C**. measured versus individual; **D**. individual weighted residuals versus time; **E**. normalized prediction discrepancy errors versus time. Dashed lines are the 95% confidence intervals. Open circles denote data from the 50 mg *S*-ketamine oral thin film, closed circles data from the 100 mg *S*-ketamine oral thin film.
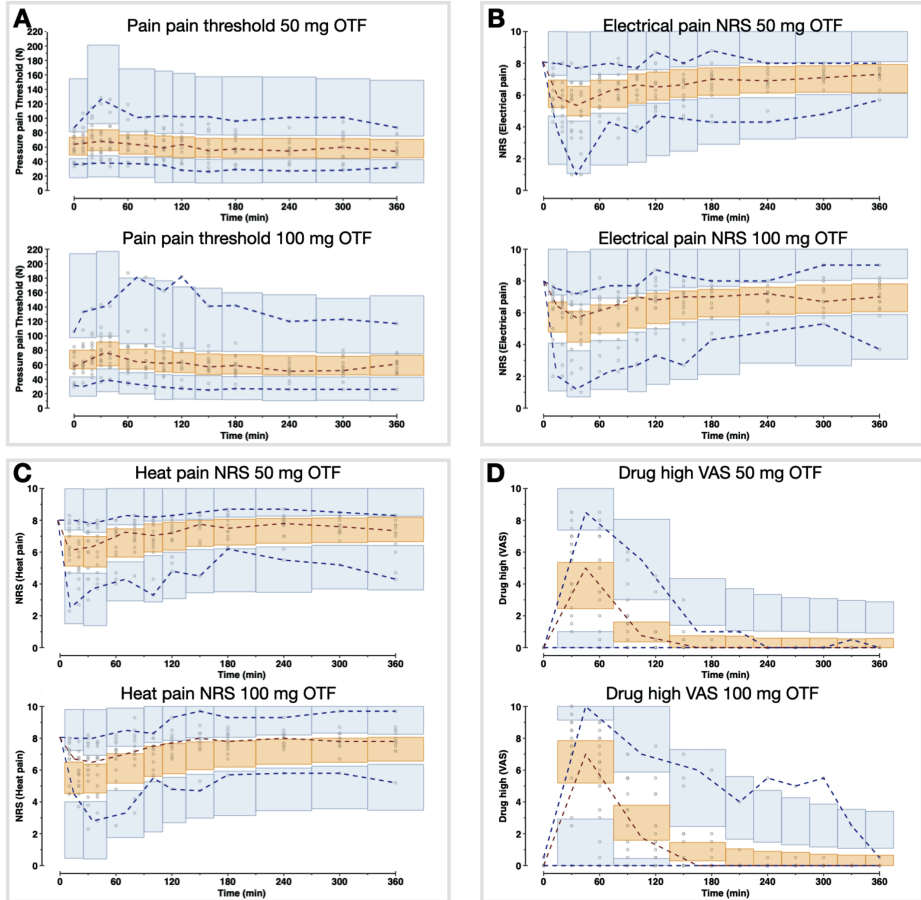
Figure 3.5: Visual predictive checks of the pharmacodynamic model in for the two *S*-ketamine OTF doses. The circles are the measured data points; the broken lines are the observed percentiles (dark red: median, dark blue: 2.5th and 97.5th percentiles); the bins are the 95% confidence intervals of simulated percentiles (orange bins: median, blue bins: 2.5th and 97.5th percentiles). **A**. Pain threshold; **B**. Electrical pain numerical rating scale (NRS); **C**. Heat pain NRS; **D**. Drug high visual analog scale (VAS).
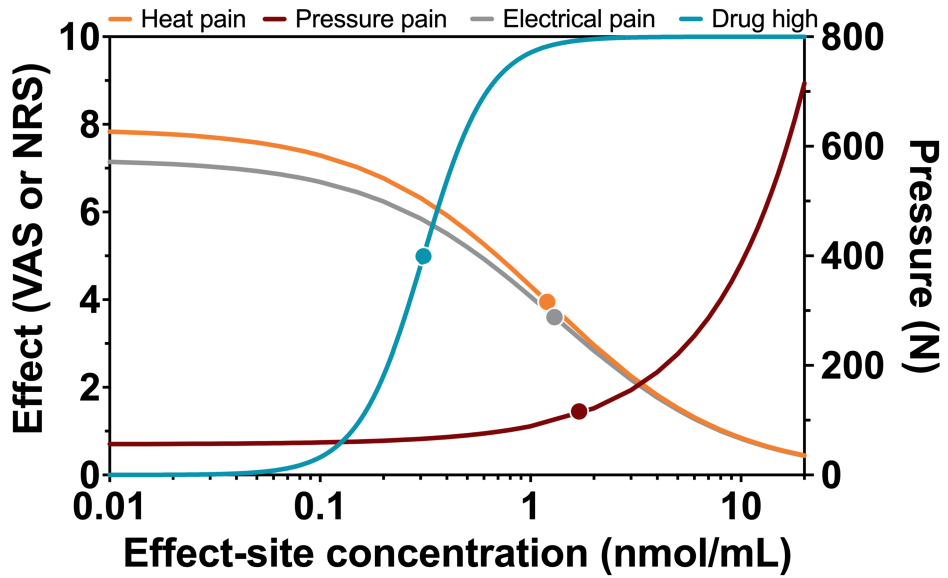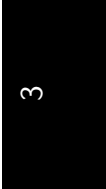
Figure 3.6: Steady-state concentration-effect relationships for heat pain, pressure pain, electrical pain, and drug high. Relationship between steady-state or effect-site *S*-ketamine plasma concentrations and effect with dots representing $_{50}$ values or half-effect concentration values.

Discussion

The main findings from our population pharmacodynamic modeling study of an
S-ketamine OTF are summarized as follows: (i) the sublingual and buccal 50
and 100 mg *S*-ketamine OTFs are antinociceptive with effects lasting at least
2 h; (ii) the onset of effect is rapid with peak effects within 30 to 45 min; (iii)
drug high had a peak effect at 30 min and lasted at least 2 h; (iv) *S*-ketamine
potency was lower for antinociception than for drug high by a factor of 3 to 5;
(v) there were no contributions of *S*-norketamine and *S*-hydroxynorketamine
to the antinociceptive or drug high *S*-ketamine effects detected in our model.

A clear dose-response relationship was not observed in the nociceptive data.
For all three pain assays, the effect of doubling the dose of the OTF did not
produce a significant increase in antinociception. Several mechanisms may be
involved: (i) this may be related to a 20% lower S-ketamine bioavailability for
100 mg OTF compared to 50 mg OTF (bioavailability 50 mg = 29% *vs.* 100 mg
23%);[2] (ii) as observed in Figure 3.6, for electrical and heat pain, the effect-site
concentration-effect curve becomes ultimately flat at high concentrations; for
pressure pain, the slope of the response is initially not particularly steep and
some overlap in the response data may be expected; (iii) The high concentra-
tions of the measured and non-measured metabolites may have an antagonistic
effect on the antinociceptive response.[5] High concentrations of *S*-norketamine
and *S*-hydroxynorketamine were observed after the 50 and 100 mg *S*-ketamine
OTFs due to the high first-pass effect. We earlier showed that *S*-norketamine
counteracts the effects of *S*-ketamine,[5] but see below; (iv) possibly some noise
in the data and variability in the day-to-day analgesic drug efficacy may have
caused the overlap of antinociceptive response; (v) since we applied several nox-
ious stimuli in a relatively short period of time to the volunteers, this may have
altered the discriminatory ability of the nociception signaling pathways. Such
an effect was earlier observed for volunteers treated with an opioid;[13] (vi) and fi-
nally, theoretically, there may be a ceiling in the ability of the nociceptive assays
at higher drug doses. In a *post hoc* analysis, we observed that the separately es-
timated C_{50s} following the two *S*-ketamine OTFs did not differ, indicative that
the absence of a dose-dependency of antinociceptive effect is probably related to
the following items (i) reduced bioavailability for the higher dose OTF, (ii) flat
concentration-effect relationship, and (iii) lesser discriminatory ability of the
pain signaling pathways when multiple stimuli are administered. An effect of
metabolites on the antinociceptive and drug high responses is further discussed
below.

Norketamine and hydroxynorketamine

Due to the large first-pass effect, plasma concentrations of *S*-norketamine and *S*-hydroxynorketamine were relatively high compared to intravenous ketamine administration (chapter 2 and Figure 3.1). Our modeling approach detected no contribution of these two *S*-ketamine metabolites to the antinociceptive and drug high effects of the *S*-ketamine OTF. Animal studies do show an antinociceptive effect from both ketamine and norketamine,[14,15] while we earlier observed a small negative contribution of *S*-norketamine to the antinociceptive and hemodynamic effects of *S*-ketamine in healthy volunteers.[5] We argued that this is one of the main reasons for the observation of pain facilitation after ketamine treatment, when norketamine concentrations exceed ketamine concentrations in plasma. Such observations are sometimes observed both clinically and in experimental studies.[16,17,18] The absence of a negative *S*-norketamine contribution to the antinociception from the OTF, suggests that *S*-norketamine has either no antinociceptive effect in humans, or the effect is small and was not detected from the noise in the data in our current study. Still, the absence of treatment arms that received *S*-norketamine precludes a definite conclusion regarding the effect of either metabolite on the pharmacodynamic responses in our study. For now, we cautiously infer from our modeling approach that *S*-norketamine has no or just little effect on either analgesia or drug high in human volunteers. The excitatory phenomena observed after ketamine infusion in other studies may then be related to the rebound activation of *N*-methyl-D-aspartate receptor and non-*N*-methyl-D-aspartate receptor glutamate receptors from accumulated excitatory amino acids in the synaptic cleft.[18]

In an animal study, Kroin et al.[7] showed earlier that (*2R,6R*)-hydroxynorketamine is an efficacious analgesic in mice. In three pain models, nerve-injury neuropathic pain, tibia fracture complex regional pain syndrome, and plantar incision postoperative pain, (2R,6R)-hydroxynorketamine was effective and superior to ketamine in terms of efficacy and side effect profile. Hence, we anticipated a long-term analgesic effect from *S*-hydroxynorketamine in our model with sustained and relatively high *S*-hydroxynorketamine concentrations in plasma. While after intravenous *S*-ketamine the ratio peak concentration *S*-hydroxynorketamine to *S*-ketamine equals 0.3,[3] this ratio ≈ 1 after application of the *S*-ketamine OTF, irrespective of dose (Figure 3.1). The absence of an *S*-hydroxynorketamine contribution may be dose-related (*i.e.* at higher concentration an effect may become visible), related to the stereoselectivity in the effect of hydroxynorketamine (*S*-hydroxynorketamine in our study *vs.* *R*-hydroxynorketamine in the study of Kroin et al.,[7] and finally it may be due to the cancellation of an antinociceptive effect from *S*-hydroxynorketamine by a pronociceptive rebound effect from the accumulated excitatory amino acids following the decline in *S*-ketamine plasma concentration

and reduced blockade of NMDA glutamatergic receptors.[19] This later mechanism would then suggest that S-ketamine and S-hydroxynorketamine act at different receptor systems to induce analgesia. Zanos et al.[6] showed indeed that (*2R,6R*)-hydroxynorketamine, but not ketamine, acts at the non-NMDA glutamate -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. Moreover, a recent study from Bonaventura et al.,[19] showed that (*2R,6R*)-hydroxynorketamine displays minimal brain uptake and rapid clearance from the brain, without any affinity for opioid receptors or any other known ketamine targets. Evidently, we need to consider the stereoselective effects of ketamine's metabolites. We argue therefore that, similar to S-norketamine, S-hydroxynorketamine needs to be administered to humans, in future studies to quantify its analgesic effect.

Drug high *vs.* analgesia

For drug high, a dose dependent S-ketamine effect was observed (Figure 3.4A,B), without any contribution from its metabolites. Relative to the S-ketamine antinociception, S-ketamine was about 3-5 times more potent in producing its drug high effects with a C_{50} (S-ketamine concentration causing a drug high of 5 on an 11-point scale from 0 to 10) of 0.31 nmol/ml. Interestingly, earlier studies showed that the racemic ketamine C_{50} for drug high is at least a factor 2 greater than that of S-ketamine, indicative of a greater S-ketamine potency compared to the R-isomer and the racemic mixture in producing drug high effects with some studies finding that R-ketamine does not produce any psychotypical effects.[19,20]

Drug high effects were predominantly present during the analgesic period, suggestive of a connection between drug high and pain relief. A connection or association between the various ketamine endpoints such as analgesia and its psychotomimetic effects has been a matter of debate and has recently been refuted.[20,21,22] However, the current data set and earlier studies from our laboratory support an intricate association between analgesia and psychotomimetic side effects.[20] What this means is still unclear. It may relate to a similar site of action within the brain, or more probably, a connection between distinct brain areas that fire together upon exposure to ketamine. The latter would cause similar dynamics of the response (*i.e.* with similar onset/offset times), although potency between the two endpoints may differ. We plan further studies to increase our insights into this matter.

Conclusions

In this pharmacokinetic-pharmacodynamic modeling study, we tested the antinociceptive and drug high effects of an *S*-ketamine OTF. The OTF was safe and side effects were related to ketamine itself and not to the thin film. Despite low bioavailability (on average 26%), the *S*-ketamine OTF produced potent antinociceptive responses in all three assays lasting 2-6 h, effects that were related to *S*-ketamine and not to its two metabolites, *S*-norketamine and *S*-hydroxynorketamine. The clinical indication of the OTF is primarily treatment of acute pain, for example in the emergency room, in the ambulance following acute trauma or for wound dressing. Additionally, we see a place for the *S*-ketamine OTF in the treatment of severe (cancer and non-cancer) breakthrough pain. However, further clinical studies are needed to address this issue.

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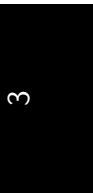
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