Safety - efficacy balance of S-ketamine and S-norketamine in acute and chronic pain

Ingeborg M Noppers

The studies described in this thesis were performed at the Department of Anesthesiology of the Leiden University Medical Center, Leiden, The Netherlands.

This PhD project was performed within TREND (Trauma RElated Neuronal Dysfunction), a Dutch Consortium that integrates research on epidemiology, assessment technology, pharmacotherapeutics, biomarkers and genetics on Complex Regional Pain Syndrome type 1. The consortium aims to develop concepts on disease mechanisms that occur in response to tissue injury, its assessment and treatment. TREND is supported by a government grant (BSIK03016).

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	Dr. J Vuyk

So long as men can breathe or eyes can see,
So long lives this and this gives life to thee.

William Shakespeare, from Sonnet 18, 1609

Voor mijn ouders

Contents

Chapter 1

Introduction

Ketamine - *the tiger still roars*[1]

Ketamine, 2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone, was first developed in 1962 as an alternative to phencyclidine, and first used as an anesthetic in humans in 1964. Phencyclidine produced an anesthetic state coupled to a prolonged emergence delirium (a "centrally-mediated sensory deprivation syndrome" which resembles some of the symptoms of schizophrenia).[1] Ketamine produces a so-called dissociative anesthetic state in which the patient is dissociated from their surroundings, and although it also causes an emergence reaction, the symptoms are less severe than those produced by phencyclidine. Both drugs have effects at multiple receptor systems, but their main effect is blockade of the *N*-methyl-D-aspartate receptor (NMDAR), an excitatory ionotropic glutamate receptor present in the spinal cord and brain. Ketamine is considered a 'safe' anesthetic, as it is not associated with profound respiratory depression or hypotension; however, when anesthetics that caused fewer or no emergence reactions became available, the use of ketamine as an anesthetic declined and became restricted to specific indications, e.g. patients with severe hypotension or trauma.

Numerous studies in volunteers and patients have shown that apart from its anesthetic action, ketamine produces potent analgesia at subanesthetic plasma concentrations (Chapter 2 of this thesis). Anesthesiologists and pain physicians make use of this by combining opioids and ketamine to reduce opioid consumption and improve the quality of pain relief in patients after surgery. These observations led to a significant expansion of ketamine's use as an analgesic in chronic (neuropathic) pain patients and ketamine began a second life as an analgesic. Because the evidence that ketamine is efficacious in these patients is limited (Chapter 2 of this thesis), studies are still conducted to establish efficacy and improve administration strategies in a variety of chronic pain conditions (Chapters 3 and 4 of this thesis). For ketamine it is obvious that it produces pain relief during intravenous infusion, but its effect following infusion is dependent on the duration of infusion and long-term infusions are probably required to cause long-term analgesic effects (Chapter 2 of this thesis). The use of drugs outside of their initial indication (so-called off-label use), in this case the use of ketamine for analgesia, raises important questions, not only regarding efficacy, but also regarding short-term and long-term safety. This is especially relevant when the mode of administration changes from single or short-term infusions for induction of anesthesia to long-term and/or repeated administration for treatment of chronic pain.

Ketamine - *side-effects and safety*

Ketamine-induced side-effects may be subdivided in:

(i) transient cardiovascular effects;
(ii) psychotomimetic or schizophrenia-like effects;
(iii) cognitive impairment;
(iv) long-term neurotoxic effects; and
(v) other somatic effects (including liver injury, renal injury and bladder dysfunction).

(i) Ketamine has a biphasic action on the cardiovascular system[2]: a direct cardiac depressive effect (i.e., a direct negative inotropic effect) and an indirect stimulatory effect (due to activation of the sympathetic system). Cardiac depression precedes stimulation after high-dose ketamine administration or after repeated infusions when presynaptic catecholamine stores become depleted. Cardiovascular stimulation occurs with low dose-ketamine infusion and is characterized by tachycardia, systemic and pulmonary hypertension, and an increase in cardiac output and myocardial oxygen consumption. These properties restrict the use of ketamine in the cardiac compromised patient, even when used at low-dose. Sympathetic stimulation may also cause other symptoms including nausea and vomiting.

(ii) Psychotomimetic effects mimic symptoms observed in schizophrenia.[3] Symptoms include feelings of euphoria or dysphoria, depersonalization, out of body experiences, hallucinations, anxiety, fear and panic attacks. The incidence of these side effects is dose related and there is a wide variety in occurrence and severity between patients. During prolonged continuous administration side effects will often decline even though the infusion rate is not changed. Side effects usually disappear rapidly upon termination of the low dose ketamine administration. In some patients side effects will persist for some time, and may even recur after initially disappearing. Simultaneous treatment with a benzodiazepine or clonidine reduces the severity of side effects.

(iii) Cognitive impairment, including memory and learning deficits can occur during and following ketamine treatment (frequent abuse of ketamine has been shown to cause long-lasting memory impairment and so-called flash-backs).[4] See also Chapter 6.

(iv) Animal studies associate ketamine with neurotoxicity (Chapter 2 of this thesis). Neuronal injury (vacuolization in neurons and apoptotic neuro-degeneration) is caused by loss of inhibitory pathways leading to an increase of excitatory neuronal activity. Studies on this topic have not been performed in

humans. Data from one case report on the epidural use of ketamine indicated that neurotoxicity occurred. This was based on histological findings, clinical signs were absent. This patient received long-term high-dose preservative free S-ketamine, suggesting a role for the NMDA receptor in causing neurotoxicity.

(v) The effects of ketamine on non-neuronal or non-cardiovascular tissues have not been widely studied. 'Older' studies (1979-1980)[5] indicate increases in liver enzymes from anesthetic doses of the racemic mixture (at higher incidences than observed during halothane anesthesia) (Chapter 3 of this thesis). This topic has been relatively untouched until recently other publications became available. Recreational ketamine abusers and ketamine addicts often present themselves at the emergency department with kidney injury, elevated liver enzymes and hemorrhagic cystitis.[6]

There is a thin line between short-term transient ketamine side effects and long-lasting ketamine-induced tissue injury. Despite the above-mentioned adverse effects, ketamine has been used with success as an anesthetic agent for the last 50 years.[1] This indicates its safety when used by anesthesia specialists for short-term administration. Knowledge on the safety of ketamine in chronic clinical use is limited and deserves further study (see also Chapter 3).

Ketamine - *is it the parent or the metabolite?*

Norketamine is the main metabolite of ketamine. It is an active NMDAR antagonist, albeit with lesser affinity for the receptor. Few animal studies have addressed the issue of norketamine potency with respect to the spectrum of effects elicited by ketamine. They show that norketamine produces analgesia in acute and chronic pain models, but that its potency is only about one-third of that of the parent compound.[7] Similarly, side effects were present after norketamine administration that were indistinguishable from those observed after equi-analgesic doses of ketamine, although there are some indications that the potency of norketamine for causing side effects is less than that for analgesia. No human data exist on the potency of norketamine, as norketamine is not available for use in humans. Previous modeling studies, assuming an additive affinity of ketamine and norketamine for the same receptor, suggest that norketamine does not contribute significantly to the effects of ketamine because its potency in humans is probably lower than that suggested from animal studies.[8]

Outline of this thesis

This theses has three major topics:

1. S-ketamine efficacy (Chapters 2, 3 and 4);
2. S-ketamine safety focusing on liver enzymes (Chapter 3), cognition (Chapter 6), and other side effects (Chapter 4); and
3. S-ketamine metabolism and contribution of norketamine to effect (Chapters 5 and 6).

Experiments were performed in chronic pain patients with complex regional pain syndrome type 1 (Chapter 3) and fibromyalgia (Chapter 4) and in healthy volunteers (Chapters 5 and 6).

In **Chapter 2** an overview is given of the efficacy and safety of ketamine in the treatment of chronic non-cancer pain. The available randomized controlled trials (RCTs) on ketamine in chronic non-cancer pain patients were evaluated and a semi-quantitative analysis of the data was performed.

The efficacy and safety of a repeated S-ketamine infusion on pain relief in chronic pain patients was studied in **Chapter 3**. A 100-h infusion of S-ketamine was repeated three weeks after the start of an initial infusion period of 100 h in chronic pain patients with complex regional pain syndrome type 1 (CRPS-1). The emphasis of this report will be on the effects of ketamine on the liver function of these patients.

Chapter 4 consists of a study on the efficacy and side-effect profile of a short-term infusion of relatively high-dose S-ketamine (0.5 mg/kg) in patients with fibromyalgia. The emphasis of this study was on the long-term effects of ketamine (i.e., did pain relief sustain following the infusion period?).

In **Chapter 5** the metabolism of S-ketamine and S-norketamine was manipulated by induction of the cytochrome P450 system. This provides information on the specifics of the metabolism of S-ketamine and its active metabolite S-norketamine. A simulation study was performed to predict the contribution of norketamine to ketamine's analgesic effects in the context of acute and chronic pain relief.

In healthy volunteers the contribution of S-norketamine to S-ketamine-induced pain relief, psychotomimetic side-effects and cognitive effects was measured in **Chapter 6**. To that end the plasma concentrations of S-ketamine and its metabolite were manipulated by cytochrome P450 induction.

Chapter 7 consists of a summary of the topics discussed in this thesis, followed by the conclusions and future perspectives.

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Chapter 2

Ketamine for the treatment of chronic non-cancer pain

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Introduction

Worldwide the number of patients affected by chronic pain is growing. Presently, in the US alone, chronic pain affects over 70 million people costing the economy more than 100 billion US$ per year.[1] Management of chronic pain syndromes is characterized by a trial-and-error approach, with interventions including psychotherapy, physiotherapy, drug treatment (including opioids, anti-depressants, anti-epileptics, NSAIDs, and their combinations) and spinal cord stimulation, often with limited success. Recently, the importance of the *N*-methyl-D-aspartate receptor (NMDAR) in the etiology and perseverance of chronic pain was established.[2] In chronic pain the NMDAR is activated and upregulated in the dorsal horn of the spinal cord (i.e., sensitization) which causes enhanced signal transmission in the pain circuitry and leads to chronic pain that is often coupled to allodynia and hyperalgesia.[2,3] Consequently, drugs that block the NMDAR may be able to relief chronic pain and possibly modulate the underlying disease process. The most studied NMDAR antagonist currently available is ketamine.[4]

Here we will discuss the use of low-dose ketamine in the treatment of chronic non-cancer pain, reviewing the complete ketamine database and highlighting recent clinical and preclinical studies (published after 2008). While ketamine was initially marketed as anesthetic agent it recently began its second life in the treatment of chronic and acute (perioperative) pain.[5-9] In chronic pain, ketamine is used in the treatment of cancer and non-cancer pain. A major problem with the use of ketamine is the development of psychotropic side effects, especially when used at high dose, while animal data suggests that high-dose and long-term ketamine infusion may be associated with neurotoxicity.[7]

Recent studies (published after 2008), focusing on long-term infusion of low-dose ketamine in the treatment of chronic non-cancer pain, demonstrated efficacy and safety of the ketamine infusion, although the patients were not followed for > 3 months.[10-12] Previous reviews have addressed the efficacy of ketamine in acute pain, cancer pain and chronic non-cancer pain (covering studies until 2008), predominantly of studies employing short-term administration paradigms.[5-8]

Chemistry

Ketamine (2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone) is an arylcycloalkylamine molecule, a phenylpiperidine derivative, structurally related to phencyclidine (PCP). It was first synthesized in the early 1960s and initially introduced as safer alternative to PCP. In 1965 the anesthetic properties of ketamine became apparent. At anesthetic doses it causes a dissociative anesthetic

state (i.e., dissociation between the thalamus and limbic system) while at subanesthetic doses it is a potent analgesic. Ketamine exists in two stereo-isomeric forms, S(+) and R(-), due to the presence of a chiral center located on C-atom 2 of the cyclohexane ring (Figure 1).[5] The S(+)-variant has about two and four times greater analgesic potency compared to the racemic mixture and the R(-)-enantiomer, respectively.[5] There are two different commercial forms of ketamine available: the racemic mixture (Ketalar™, Pfizer Inc.) and in some countries (e.g., The Netherlands, Germany, Austria) the S(+)-enantiomer (S-ketamine or Ketanest-S™, Pfizer Inc.).

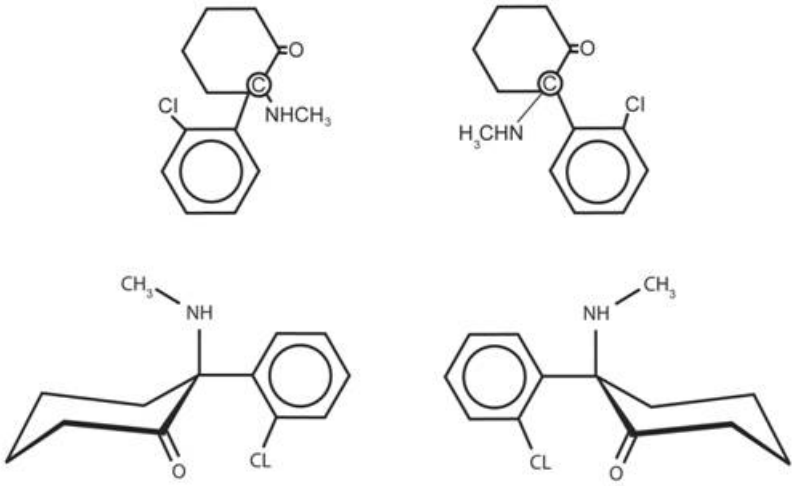

Figure 1 left S(+)-ketamine; **right** R(-)-ketamine. The chiral center © is at C-atom 2 of the cyclohexane ring.

Pharmacokinetics and metabolism

Few studies addressed the pharmacokinetics of ketamine in chronic pain patients using an extended administration paradigm.[10] Studies on the acute (i.e. short-term) administration show limited bioavailability after oral, sublingual and rectal administration (20-30%), partly because of the large first-pass effect, while bioavailability is somewhat higher after intranasal application (45%), with no differences between the two enantiomers of the racemic mixture.[13] Peak concentration after oral ketamine ingestion is reached after 20-30 min. The pharmacokinetics after a single or short-term infusion have well been described; volume of distribution, distribution and elimination half-life are 0.3 L/kg, 15 min and 2-3 h, respectively.[14,15] After intravenous infusion of the racemic mixture, the R(-)-enantiomer inhibits the elimination of the S(+)-variant (magnitude of effect about 30%).[16] Furthermore, for the S(+)-enantiomer a sex difference in pharmacokinetics has been observed with a 20% greater clearance in women.[15]

Ketamine rapidly passes the blood-brain barrier due its high lipophilicity (blood-effect-site equilibration half-life 1-3 min), ensuring a rapid onset of analgesic effect.[15,17]

Ketamine is extensively metabolized by the hepatic cytochrome P450 enzyme system (by CYP3A4, CYP2B6 and CYP2C9 enzymes).[18,19] The main pathway is *N*-demethylation to norketamine with subsequent metabolism of norketamine into 6-hydroxynorketamine. Norketamine and the hydroxy-product are glucuronidated and eliminated via the kidney and bile.[20,21] Induction of the CYP system will have limited effect on the ketamine concentration as its hepatic clearance before induction is high and approaches liver blood flow (1 L/min).[15] Drugs that inhibit CYP enzymes involved in ketamine's metabolism (such as clarithromycin), will increase ketamine plasma concentrations, in particular after oral administration.[22] Norketamine appears within minutes in plasma after the intravenous administration of ketamine, and, particularly after long-term infusions, reaches values similar or even greater than that of ketamine.[10,15,17] Upon the termination of ketamine infusion, the plasma concentrations drop rapidly and norketamine concentrations exceed ketamine concentrations.[10,15,17]

Mechanism of action

While ketamine acts at multiple receptor systems (such as the μ-opioid receptor and the HCN1 pacemaker cell), the analgesic effect of ketamine in chronic pain is attributed to its effects at the NMDAR.[2,10,23,24] The NMDAR is an excitatory ionotropic glutamate receptor present in the spinal cord and the brain. In the resting state the receptor is blocked by Mg^{2+} ions. The block is lost upon strong and sustained nociceptive activation of the receptor by presynaptic release of glutamate (in the presence of co-agonist glycine). This results in a neuronal influx of positive ions (Na^{+}, Ca^{2+}) and an increase in discharge of dorsal horn nociceptive neurons (causing enhanced pain perception). Prolonged activation of the NMDAR results in plastic changes in the spinal cord with upregulation of NMDAR and central sensitization leading to the chronification of pain.[2,3,25] Ketamine is a non-competitive antagonist of the NMDAR, reverting the NMDAR to its resting state and consequently causing the impairment of nociceptive signal propagation to the brain and, especially after long-term administration, restoration of the physiological balance between pain inhibition and facilitation.[10] Of further interest is that ketamine's metabolite norketamine is a non-competitive antagonist of the NMDA receptor.[26] Animal data indicate that norketamine passes the blood-brain barrier, has about one-third the potency of ketamine, and is thought to be involved in ketamine's analgesic effect as well as (though to a lesser extent) the development of psychotropic side effects.[26] No data are presently available to substantiate this in humans.

Clinical efficacy

Randomized clinical trials: 1992-2010

Most studies published on the effect of ketamine on chronic pain are open-label studies, case series or case reports. We searched seven electronic databases (PubMED, EMBASE, Web of Science, the Cochrane Library, CINAHL, PsychINFO and Academic Search Premier) in June 2010 for papers assaying ketamine's analgesic effect in chronic pain patients using a prospective, randomized, controlled design (Key words included pain, chronic pain, chronic disease, neuralgia, neuropathic pain, complex regional pain syndrome, fibromyalgia, neuropathic pain, neuropathy, low back pain, diabetic neuropathy, migraine, multiple sclerosis, postherpetic neuralgia, trigeminus neuralgia, phantom limb, ketamine, S-ketamine, ketanest, ketalar, ketaset, calipsol, kalipsol, calypsol, 2-(2-Chlorophenyl)-2-(methylamino)-cyclohexanone, CI 581. Limits included human, English, French, German and Dutch).

We retrieved 36 RCTs (first publication date 1992, 6 published after 2008) of which the majority (21) were on iv ketamine (20 using the racemic mixture, 1 S-ketamine).[10-12,27-61] See tables 1 and 2 for the study characteristics.

The infusion duration of ketamine in studies on iv administration varied from single injections to multiple day infusions (max. infusion duration 2 weeks) with large variations in doses (Tables 1 and 2). We refrained from performing a meta-analysis as the heterogeneity between studies was large and the quality of the majority of studies poor to moderate (most studies did not present the method of randomization, refrained from stating how dropouts and withdrawals were taken into account, did not present information on allocation concealment). Furthermore, various studies did not give quantitative data on the ketamine analgesic effect and some studies were ended prematurely.

The number of studies that we graded as good[10,11] were insufficient to perform a meta-analysis. Hence we decided to perform a semi-quantitative analysis on the effect of intravenous infusion duration on treatment effect (magnitude and duration). We included studies that tested the effect of at least 0.15 mg/kg ketamine on chronic pain intensity (Figure 2).

Table 1 Randomized controlled trials on the effect of intravenous ketamine on chronic pain (in chronological order).

Ref	Year	N*	Chronic pain disease	Cross over	Design and treatment	Results
11	2010	20	Neuropathic pain from SCI		20 patients received 80 mg KET infusion in 5 h for 1 week + 3 times/day gabapentin vs 20 patients received a PLCB infusion + 3 times/day gabapentin	Ketamine caused effective analgesia in weeks 1 and 2 following treatment
12	2009	9	Complex regional pain syndrome		KET 4-h infusion (n = 9) vs PLCB (n = 10) infusions for 10 days. Max. infusion = 0.35 mg/kg/h	KET NRS from 7.66 to 6.13 ($P < 0.05$ at week 3-4) vs PLCB NRS from 7.7 to 7.5. Other pain indices improved for at least 12 weeks
10	2009	30	Complex regional pain syndrome		4.2 day S-KET infusion (increasing dose, max. 20 mg/h, n = 30) vs PLCB (n = 30)	KET caused analgesic effects lasting up to 11 weeks. Maximum effect during treatment week
49	2007	20	Whiplash	+	4 treatment combinations: PLCB/PLCB, PLCB/remifentanil, KET/PLCB, KET/remifentanil. iv TCI system with target KET concentration of 100 ng/ml. Infusion duration 65 min	KET/PLCB and KET/remifentanil reduced VAS scores from 3.9 to 1.8 and 3.5 to 1.0 cm ($P < 0.001$) during infusion
40	2006	20	Nerve injury pain	+	0.24 mg/kg KET over 30 min vs lidocaine (5 mg/kg) vs PLCB	Spontaneous pain reduction by KET only; evoked pain reduced by both drugs
48	2005	30	Whiplash	+	0.3 mg/kg KET infusion vs 0.3 mg/kg morphine vs 5 mg/kg lidocaine vs PLCB. Infusion duration 30 min	KET = 14 responders; duration of effect no longer than 1-1.5 h
46	2004	10	SCI and neuropathic pain below the level of injury	+	KET 0.4 mg/kg injection vs lidocaine 2.5 mg/kg vs PLCB	KET responders 5/10 vs 1/10 after lidocaine and 0/10 after PLCB. KET effect = 38% VAS reduction (lidocaine = 10% and PLCB = 3%, $P = 0.01$)
44	2003	12	Chronic neuropathic pain	+	KET iv 60 μg/kg bolus + 6 μg/kg/min for 20 min vs alfentanil vs PLCB	KET (and alfentanil) produced significant reductions of pain and hyperalgesia but not cold pain detection threshold
45	2003	12	Peripheral neuropathic pain of traumatic origin	+	Singe KET 0.4 mg/kg injection vs lidocaine 2.5 mg/kg vs PLCB	KET response in 7/12 patients: KET caused a 55% reduction in VAS vs 34% and 22% for lidocaine and PLCB ($P = 0.009$)

54	2002	18	Painful limb ischemia		KET infusion + opioid vs PLCB infusion + opioid. KET dose 0.6 mg/kg infused over 4 h	Improved pain relief by KET of 65% 1 day post-treatment and 69% 5 days post-treatment. Also significant effects on general activity and quality of life
50	2001	12	Post-nerve injury	+	KET TCI concentration 50, 100 and 150 ng/ml vs alfentanil (TCI 25, 50 and 75 ng/ml) vs PLCB	KET reduced hyperalgesia
41	2000	29	Fibromyalgia	+	0.3 mg/kg KET over 30 min vs PLCB in 29 patients	17/29 patients showed pain relief > 50%
58	1998	8	Pain from arteriosclerosis of the lower extremities	+	KET bolus injection 0.15, 0.30 or 0.45 mg/kg vs morphine 10 mg bolus injection	Dose dependent analgesic effect from KET with greater effect than morphine at 0.3 and 0.45 mg/kg
60	1997	18	Fibromyalgia	+	KET 0.3 mg/kg infusion over 30 min vs PLCB vs 0.3 mg/kg morphine vs 5 mg/kg lidocaine	KET responders = 8, non responders = 8. Effect in responders 1-5 days
56	1997	81	Chronic migraine with a temporal pattern	+	KET infusion 0.15-1 mg/kg per 24 h for 2 weeks vs PLCB	Chronic migraine became episodic in 76/81 patients with a reduction of intake of co-analgesics
57	1996	11	Phantom limb pain	+	KET 0.1 mg/kg bolus injection followed by 7 μg/kg/min for max. 45 min. vs PLCB	KET reduced stump and phantom pain by 100%
36	1995	10	Peripheral neuropathic pain	+	KET (0.2 mg/kg bolus + 0.3 mg/kg over 1 h) vs magnesium (bolus + cont. infusion)	KET produced a 57% reduction of pain and 33% reduction of area of allodynia
53	1995	8	Chronic posttraumatic pain and widespread mechanical allodynia	+	KET infusion for 2 h (mean dose 58 mg) vs alfentanil (11 mg) vs PLCB	Pain relief: KET 65%, alfentanil 46%, PLCB 22% ($P < 0.01$). Similar observations for relief of allodynia. Pain relief disappeared upon end of infusion
29	1994	6	Chronic neuropathic pain (central pain, peripheral neuropathy)	+	Single or series of 0.25 mg/kg KET injections vs PLCB	5/6 patients had pain relief lasting 2-3 h, 1 had 2 weeks effect; 1 patient showed no effect
34	1994	8	Post herpetic neuralgia	+	Single 0.15 mg/kg KET injection vs morphine (0.075 mg/kg) or PLCB	Pain relief by KET (but not morphine or placebo)
35	1994	9	Spinal cord injury	+	KET (0.06 mg/kg bolus + 6 μg/kg/h for 20 min) vs alfentanil vs PLCB	Pain relief by KET of 40% (and alfentanil of 20%)

N* = number of patients receiving ketamine, **KET** = ketamine, **NRS** = numerical rating scale, **PLCB** = placebo, **SCI** = spinal cord injury, **S-KET** = S-ketamine, **TCI** = target controlled intravenous infusion, **VAS** = visual analogue score.

Table 2 Randomized controlled trials on the effect of non-intravenous ketamine on chronic pain (in chronological order).

Ref	Year	N*	Chronic pain disease	Cross over	Design and treatment	Results
30	2010	101	Chemotherapy-induced neuropathy		**Topical.** KET-amitriptyline-baclofen cocktail (n = 101) vs PLCB (n =104)	No effect on pain relief greater than placebo
43	2010	16	Chronic neuropathic pain		**Intranasal.** low dose 0.2 mg/kg, n = 8 and high dose 0.4 mg/kg, n = 8	Pain scores decreased with max. effect at t = 60 min. No effect on QST
37	2009	20	Complex regional pain syndrome	+	**Topical.** KET (10% in organogel) vs PLCB	KET caused reduction of allodynia and hyperalgesia
27	2008	18	Temporomandibular joint arthralgia	+	**Intraarticular**. 18 patients received one injection with KET or saline	No effect on pain or somatosensory end-points
32	2008	14	Chronic myofascial pain in temporomandibular disorder	+	**IM.** injection of KET or PLCB in m. masseter	No relief of spontaneous pain
52	2005	45	Neuropathic pain patients with allodynia, hyperalgesia or pinprick hypesthesia		**Topical.** 4 Groups: PLCB (n = 25), 2% amitriptyline (n = 22), 1% KET (n =22), 1% KET + 2% amitriptyline (n = 23)	Effects no larger than PLCB
61	2005	22	Central neuropathic pain		**Iontophoresis.** KET 50 mg (n = 11) vs 75 mg (n = 11) per day for 1 week vs PLCB (n = 11)	No effect on pain scores from KET but 75 mg improved quality of life and health status
31	2004	20	Breakthrough pain in chronic pain patients (n = 16) and cancer patients (n = 4)	+	**Intranasal.** KET 10-50 mg (1-5 sprays) vs PLCB (1-5 sprays)	KET produced analgesia within 10 min lasting at least 1 h. Max effect occurred after 40 min (NRS change = 3.13. vs PLCB = 0.8, P = 0.0001)
51	2003	20	Chronic neuropathic pain	+	**Topical.** KET for 2-days: 0.5% KET vs 1% amitriptyline vs 0.5% KET + 1% amitriptyline vs PLCB	Effects no larger than PLCB

47	2002	10	Chronic neuropathic pain		**Epidural**. KET (0.3 mg/kg/day) + lidocaine (n = 10) vs epidural clonidine (90 μg/day) + lidocaine (n = 13) using PCEA device. Treatment duration 3 weeks	Significant reductions in pain intensity (VAS) in both groups from 9 to 2 cm. Effect persisted for 2-5 weeks following epidural catheter removal
38	2002	8	Chronic neuropathic pain	+	**Oral.** KET syrup 0.5 mg/kg vs PLCB every 6 h for 1 week	KET caused pain relief: VAS from 78 to 49 mm
59	1999	26	Trigeminal neuropathic pain	+	**IM.** Study A: KET 0.4 mg/kg vs pethidine 1 mg/kg **Oral.** Study B: KET 4 mg/kg vs PLCB for 3 days	Study A. KET: 9/26 no effect; 9/26 effect 1 h; 8/26 effect > 12 h. Study B. 5/26 had an analgesic effect
42	1999	21	Chronic neuropathic pain		**Oral.** N = 1 trial in 9 patients: KET 10 mg once/day for 6 weeks vs PLCB	3/21 patients showed a consistent analgesic effect
55	1995	34	Acute migraine (n = 17), Chronic episodic migraine (n = 17)	+	**SC.** 80 μg/kg in acute sufferers and 80 μg/kg 3 times daily in chronic sufferers for 3 weeks	KET produced significant pain relief. Acute sufferers: 3/17 100% relief, 6/17 90-70% relief and 8 45-50% relief (duration of effect 4 h). Similar observations for chronic sufferers. Variable effects following infusion
39	1992	20	Whiplash, postdiscoidectomy, chronic back-ache		**Intraligamentous.** injection with 0.25 mg/kg ketamine vs 2% lidocaine	VAS reduced from 8 to 2 after KET with increased functionality (no effect of lidocaine)

N* = number of patients receiving ketamine, **IM** = intramuscular, **KET** = ketamine, **NRS** = numerical rating scale, **PCEA** = patient controlled epidural anesthesia, **PLCB** = placebo, **QST** = quantitative sensory testing, **SC** = subcutaneous, **VAS** = visual analogue score.

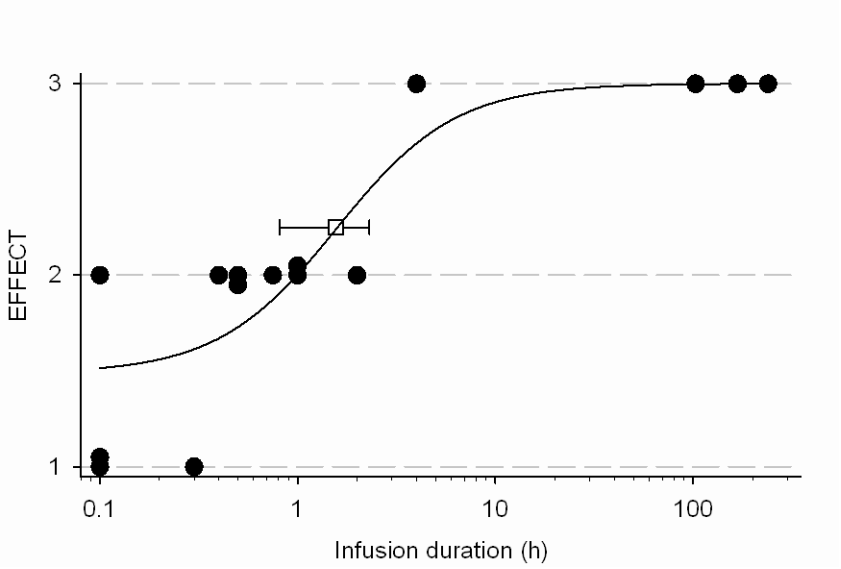

Figure 2 Semi-quantitative analysis on the effect of infusion duration on intravenous ketamine analgesic efficacy. Randomized controlled trials that infused at least 0.15 mg/kg were included in the analysis. On the x-axis infusion duration in hours, on the y-axis effect defined as follows: no analgesic effect (effect = 0), a reduction in pain intensity no greater than 50% of pre-treatment pain during infusion (effect = 1), a reduction in pain intensity greater than 50% of pre-treatment pain during infusion (effect = 2) and a significant reduction in pain intensity with pain relief persisting for at least 48 h following the termination of infusion (effect = 3). Each filled circle is one study. The continuous line is the result of a logistic regression analysis. The open square is the ID50 or infusion duration causing a median analgesic effect (in the current set of studies a median effect = 2.25 was estimated). ID50 = 1.7 ± 0.8 h (median ± SEM) in a dose range of 0.15-0.5 mg/kg. At infusion duration > 10 h the probability of an effect lasting > 48 h approaches 95%, while at durations > 30 h the probability approaches 99%.

The results show that the majority of studies demonstrate a more than 50% reduction of pain intensity, but the effect did not persist beyond the 48 h following infusion. The infusion duration of these studies ranged from 30 min to 2 h. Only four studies reported an analgesic effect persisting beyond the 48 h following infusion, three of which (all published after 2008) employed long-term infusion schemes (4.2 days continuous to daily 4-h infusions for 10 days, with a dose range of 16 to 25 mg/h).[10-12] Our analysis indicates that at an infusion duration > 10 h the probability of an effect lasting > 48 h approaches 95%, while at durations > 30 h the probability approaches 99% (Figure 2). Note, however, that following infusion analgesia slowly dissipates over time (see, for example, Figure 3).[10,11] This indicates that these infusion paradigms were insufficient to cause a permanent reduction of pain. Possibly other infusion regimes (e.g. regular 10-h infusions, daily 1- to 2-h infusions) may have a more permanent effect. None of the published RCTs addressed this issue.

Randomized clinical trials on long-term intravenous infusion: 2009-2010

Three RCTs on iv ketamine published after 2008 were identified[10-12]; all used a multiple day infusion scheme. The most recent study by Amr is on the effect of ketamine in spinal cord injury related chronic pain.[11] Group 1 (n = 20) received 80 mg intravenous ketamine over 5 h daily for one week, plus 300 mg gabapentin three times daily; Group 2 (n = 20) received a 5-h placebo infusion once daily, plus 300 mg gabapentin three times daily. Pain relief was significantly greater in Group 1 relative to Group 2 during infusion and during the first 2 weeks following infusion. Thereafter, there were no more differences between treatment groups in pain scores, although pain scores remained decreased versus baseline for at least 4 weeks following the end of treatment in both groups. Ketamine-related side effects occurred in 3/20 patients (short-lasting delusions). None of the side effects required intervention. We consider this a qualitatively good study that may lead the way to outpatient treatment of chronic pain patients with intravenous ketamine.

Schwartzman et al. assessed the effect of daily 4-h iv ketamine infusions (max. dose = 0.35 mg/kg/h) for 10 days in Complex Regional Pain Syndrome (CRPS) chronic pain patients.[12] Subjects in both arms of the study received clonidine and midazolam. The subjects receiving ketamine had consistent decreases for all pain-related parameters that lasted for the 12-week post-treatment evaluation period (total McGill pain score pre-treatment = 23.1, post-treatment weeks 1-2 = 16.2, post-treatment weeks 3-4 = 13.4 and post-treatment weeks 9-12 = 15.4). Ketamine-related side effects included nausea, headache, tiredness and dysphoria. This study was criticized for reasons of early termination, small sample size, and relatively limited analgesic effect.[62] While powered to study 20 patients per arm, the study was stopped after 9 patients were enrolled in the ketamine arm and 10 in the placebo arm. The reasons given for the premature end of the trial were that the authors had seen greater pain relief with a higher dose of ketamine than allowed in the protocol (max. dose 25 mg/h) and the absence of a placebo effect. Note, however, that these greater analgesic responses were based on an open-label study.[62]

Sigtermans et al. studied the effect of iv ketamine in CRPS patients with moderate to severe chronic pain.[10] Thirty patients (disease duration: mean 7.4 years, range 0.1-32 years; mean baseline pain score 7.2 on a scale of 0-10) were treated with a 4.2 day continuous infusion of ketamine's S(+)-enantiomer with a mean dose of 20 mg/h. Significant analgesic effects were observed in the 4.2 day-treatment phase of the study (pain score 2.7 versus 5.5). Over the 12-week duration of the study ketamine modulated the course of chronic pain more favorably than placebo (Figure 3).

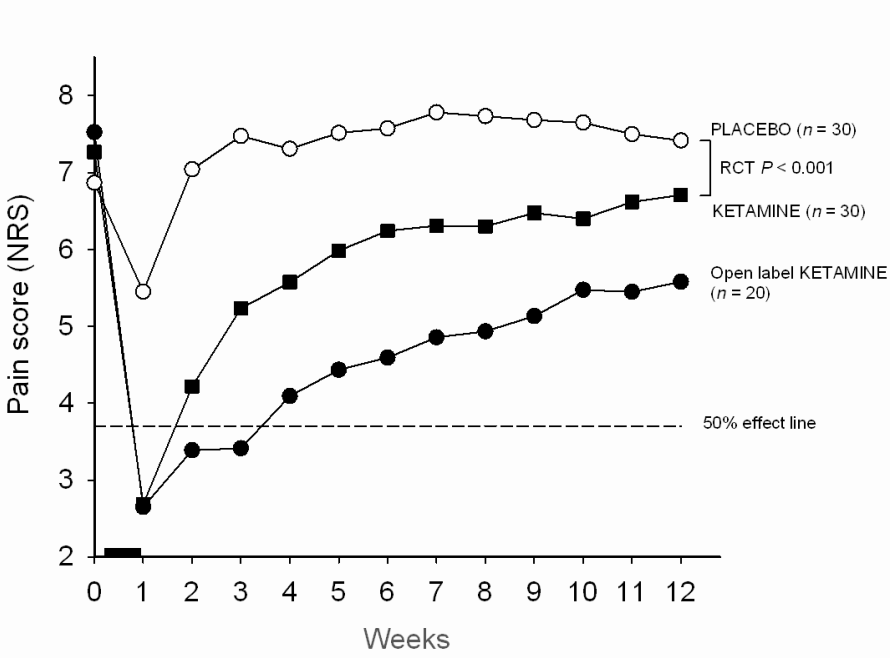

Figure 3 Results of the Sigtermans et al. trial on a 4.2-day continuous intravenous ketamine infusion in 60 patients with complex regional pain syndrome chronic pain. The results of the randomized trial are given (squares: ketamine data, open circles: placebo data) showing a 12-week significant effect on pain intensity scores (P < 0.001). Twenty subjects initially receiving placebo returned to receive open-label ketamine (closed circles). The black bar indicates the infusion period. NRS = numerical rating scale. Adapted from reference 10.

Ketamine-related side effects included nausea/vomiting and psychotropic effects. Although this study is considered qualitatively "good", points of critique include the absence of improvement of function and the cost of the intensive and long-term in-house treatment.[62,63] Twenty patients that initially had received placebo were allowed to receive the identical ketamine treatment, but now in an open-label fashion. As expected, their analgesic responses were larger by at least 1 NRS point at 2 weeks and pain relief > 50% lasted for more than 3 weeks (Figure 3). This suggests that a large part of the responses seen in this patient group is expectancy-related.

Safety and tolerability

Ketamine causes a variety of dose-dependent side effects ranging from nausea/vomiting, sedation, vertigo, tachycardia, hypertension to increased cardiac output.[7,10,15,64] Recent discussions indicate that three important issues regarding safety and tolerability of ketamine are of concern[62]: the occurrence of psychotropic side effects; possible neurotoxicity; and the abuse potential of repeated or long-term ketamine use.

Cardiovascular side effects

The effects of ketamine on the cardiovascular system are related to activation of the sympathetic system (ketamine causes the systemic release of catecholamines and inhibition of norepinephrine re-uptake at peripheral nerves and non-neuronal tissues such as the myocardium).[64] At the doses used in the treatment of chronic pain the effects on cardiovascular system seem moderate and well tolerated. However, care is always required in patients with cardiovascular disease (e.g., patients with ischemic heart disease and hypertension).

Psychotropic side effects and abuse potential

After intravenous dosing psychotropic side effects are common ranging from 'drug high' to derealization/depersonalization, hallucinations and fear/panic attacks. These symptoms resemble psychotic episodes in schizophrenia, but disappear rapidly upon the termination of the infusion.[10,65] These effects are dose dependent.[66] Patient compliance with psychotropic symptoms is variable. When unacceptable psychotropic effects occur, the ketamine infusion rate may be lowered or a benzodiazepine or α2-adrenergic receptor agonist (clonidine, dexmedetomidine, neuroleptics, antiepileptics) may be added.[67] During prolonged infusions the occurrence and severity of psychotropic effects seems to decline over time. However, the psychotropic effects may cause the patient to abandon further treatment.[10] In our experience there are no differences in occurrence of psychotropic side effects during treatment with the racemic mixture or the S(+)-enantiomer.

Ketamine is a drug of abuse and increasingly used for recreational use (mostly by sniffing ketamine powder known as Special K or Vitamin K).[68] Side effects related to the chronic use of ketamine are cognitive dysfunction (memory deficits) and mild psychotic attacks (e.g., flashbacks) occurring up to weeks after the intake.[69] In animals regular ketamine use has been associated with typical addictive behaviour.[70]

Neurotoxicity

Animal studies show that under specific circumstances and in specific areas of the brain NMDAR antagonists, including (high-dose) ketamine, have neuroprotective effects, while under other circumstances these same agents are neurotoxic.[67,71,72] Neuronal injury (vacuolization in neurons and apoptotic neurodegeneration) is caused by loss of inhibition of inhibitory pathways leading to the enhancement of excitatory neuronal activity. Several drugs are able to prevent neuronal injury from NMDAR antagonists, including benzodiazepines and α2-agonists.[73] Despite the observation in animals, ketamine is still widely used in humans, partly because no data in humans are available that cause concern about neurotoxicity

("no hint of a suggested association between ketamine and brain damage or learning delay, the presumed sequelae of neuroapoptosis, has been reported")[74] and the fact that most infusions are combined with either a benzodiazepine or an α2-agonist.

Summary and conclusions

Worldwide the number of patients affected by chronic pain is growing and conventional treatment is often insufficient.[1] Recently the importance of the NMDAR in the etiology and maintenance of chronic pain has been established. Of all available NMDAR antagonists ketamine is the most potent and consequently has been applied in the treatment of various chronic pain syndromes. Most frequently studied is the intravenous route of administration followed by topical, oral and intranasal routes.

The majority of RCTs on intravenous ketamine published since 1992 indicate that when applying short-term infusions (< 4 h) chronic pain is reduced by about 50% irrespective of the dose given, but the effect dissipates rapidly upon the termination of infusion. Three recent studies[10-12] (published in 2009 and 2010) on long-term (days to weeks) ketamine administration (mean infusion rate 20 mg/h) show that the duration of effect lasts multiple weeks, although a slow return to pretreatment pain scores is observed in all studies. In none of the studies a full effect of ketamine on pain or any effect on function or quality of life has been detected, possibly due to the fact that none of the studies was properly powered to study these latter end-points. Evidently, further RCTs of good quality are required before any conclusion may be drawn on the efficacy of ketamine, specifically of long-term ketamine infusions, on long-term pain relief in chronic pain patients.

In most RCTs, various side effects were noted. Most worrisome side effects to the patients are psychotropic in nature (ranging from 'drug-high' to panic/fear and hallucinations) that may be prevented/treated by co-administration of benzodiazepines and/or α2-adrenergic receptor agonists. Other side effects include nausea/vomiting, sedation and hypertension/tachycardia. The latter symptoms are related to ketamine's activation of the sympathetic system and seem of minor importance at low-infusion rates. Finally, there is concern for the possibility of neurotoxicity and potential for abuse from long-term or repetitive ketamine use. Although indications for these concerns were derived from animal studies at relatively high ketamine dose, these issues remain understudied in humans.

The expert's opinion

Ketamine's use as an anesthetic and analgesic in the treatment of acute pain is driven by its pharmacokinetics (i.e., upon the termination of infusion anesthesia and analgesia dissipate rapidly).[12,14] In contrast, long-term (days to weeks) continuous or repetitive infusion of low-dose ketamine results in pain relief persisting for weeks following treatment.[9,24,58] This suggests that ketamine has a disease modulatory role and initiates a cascade of events of which the first step is probably desensitization in instances where NMDAR sensitization is an important factor in the process of the development and chronification of pain. This effect of ketamine in the chronic pain process is exceptional and different from most other analgesic agents that do not have the ability to interfere with the process of central sensitization. Despite these promising results ketamine is not (yet) viable as routine treatment for chronic pain.

Several important issues have to be resolved. First, safety/toxicity studies in humans aimed at cognitive (e.g. memory deficits) and long-term effects of ketamine, as well as studies on ketamine's abuse liability, are needed. Second, administration modes allowing outpatient treatment have to be developed and tested. This will not only be less costly compared to multiple-day in-patient treatment, but will allow the patient to be treated in his/her own surroundings. Finally, safe dosing schemes need to be developed that ensure persistent relief of pain. When these issues have been resolved satisfactorily, ketamine may gain a prominent place in the treatment of chronic pain.

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Chapter 3

Drug-induced liver injury following a repeated course of ketamine treatment for chronic pain in CRPS type 1 patients:
A report of 3 cases

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Pain, in press

Introduction

It is well established that the *N*-methyl-D-aspartate receptor (NMDAR) plays an important role in the etiology and duration of chronic pain.[1,2,3,4] Chronic pain activates and upregulates the NMDAR in the dorsal horn of the spinal cord, which causes enhanced signal transmission in the pain circuitry and leads to chronic pain, often coupled with allodynia and hyperalgesia.[1,2,3] Consequently, drugs that block the NMDAR are able to relieve chronic pain and possibly modulate the underlying disease process.[1,2,3,4] The most potent NMDAR antagonist currently available is ketamine, and the number of studies on the efficacy of ketamine increases rapidly. Since 1992 there are 38 published randomized controlled trials on ketamine use in chronic (non-cancer) pain patients and even more open-label and case studies.[4] Most of the published randomized controlled trials (36/38) are of poor-to-moderate quality. Despite the absence of good quality studies, ketamine treatment seems to get a definite place in the treatment of chronic pain in clinical practice.[4]

The use of ketamine has raised the concern for toxicity.[5] Animal studies indicate that ketamine use is associated with neurotoxicity and learning disabilities, while human studies indicate abuse potential and a high frequency of psychotropic side effects. Case reports on the side effects and toxicity of the recreational abuse of ketamine indicate a pattern of renal and liver toxicity.[6,7,8] During the course of a study on repeated administrations of ketamine for treatment of chronic pain in patients with complex regional pain syndrome type 1 (CRPS-1), we encountered hepatotoxicity in a subset of patients that received 2 100-hour infusions of S-ketamine at a 16-day interval. Six subjects were enrolled in that study arm and liver damage was observed in 3 of them. This prompted us to end the trial prematurely. Liver damage is considered a rare side effect of ketamine use, but since repeated dosing is often necessary, we believe that awareness of this side effect is needed. We therefore present the course of events of the 6 subjects enrolled in the 16-day interval study arm.

Methods

The patients presented were involved in a study registered in the Netherlands Trial Register (www.trialregister.nl) under number NTR1550. This pilot study was aimed at generating exploratory data on the effect of two 5-day (i.e., 100 h) ketamine treatments (treatment 1 in week 1, treatment 2 in week 4) on pain relief in CRPS-1 patients.

Patients

Patients eligible for the study were those referred to our outpatient pain clinic and who were diagnosed with CRPS-1, as based on the International Association for the Study of Pain CRPS-1 criteria[9], and who had pain scores of 5 or higher (on a numerical rating scale (NRS) from 0 to 10, where 0 = no pain and 10 = worst pain). Exclusion criteria included age < 18 years, inability to give informed consent, serious medical disease (e.g., cardiovascular, renal, or liver disease), use of strong opioids or baclofen, pregnancy/lactation, and history of psychosis. Patients were asked not to change their pain medication from the start of the study until completion of follow-up.

Trial design

The study design was single blind. Patients were admitted twice for 5 days and randomly allocated to 1 of 3 groups: Group 1 was admitted in weeks 1 and 4 and received ketamine on both occasions (i.e., they had a 16-day ketamine-free interlude); Groups 2 and 3 were admitted in weeks 1 and 13 and received ketamine on both occasions (Group 2) or midazolam on the first occasion and ketamine on the second (Group 3). Follow-up was performed during the 12 weeks after the second admission. Since the focus of this report is on the side effects observed in the Group 1 subset of patients, we restrict our presentation to these patients.

Treatment

S-ketamine (Ketanest S, Pfizer BV, Capelle aan de IJssel, The Netherlands) was administered continuously by intravenous route for 5 days according to an infusion scheme of Sigtermans et al.[10] On day 1, infusion started at 8 am at 1.2 μg/kg/min. Three times a day (at 8 am, noon, and 4 pm), the infusion rate could be increased in steps of 0.6 μg/kg/min until a maximum infusion rate of 7.2 μg/kg/min was reached. When the patient reached a pain rating of zero, the infusion rate was not further changed. In case of severe side effects, the infusion rate was lowered in steps of 0.6 μg/kg/min and later increased again if possible. On day 5, at noon, the infusion ended. In case of nausea, 10 mg oral domperidone could be given, with a maximum of 40 mg per day.

Measurements

The primary outcome measure of the study was pain relief as measured by the 10-point NRS ranging from 0 (no pain) to 10 (worst pain), measured 3 times daily (8 am, 12 pm, and 4 pm) during treatment, and weekly in between treatments and during follow-up. Secondary outcome parameters were psychotropic side effects, nausea, and headache, all scored on a range from 0 (not present) to 10 (maximal presence). Liver enzymes (alkaline phosphatase (ALP; reference values

40-120 U/L), alanine transaminase (ALT; reference values 5-34 U/L), aspartate transaminase (AST; reference values 5-30 U/L), total bilirubin (TBIL; reference values 0-17 U/L), γ-glutamyltransferase (γGT; reference values 5-40 U/L)) were measured during ketamine treatment (in weeks 1 and 4) on days 1 (before the start of drug infusion), 3, and 5. In case of liver enzyme elevation, the frequency of testing increased to twice daily. Heart rate, blood pressure, and tympanic temperature were obtained 3 times per day.

Statistics

The total number of subjects in this pilot study was arbitrarily set at 30 (10 per group). No comparative analysis was planned, and the data are presented in a descriptive manner only.

Results

Patient admissions took place between December 2008 and February 2010. The inclusion of patients in the study was ended prematurely after 13 subjects had been admitted. Five of the 6 subjects randomized to Group 1 developed side effects: one developed severe hypertension and another psychotropic side effects during their first exposure to S-ketamine, and 3 developed elevated liver enzymes prior to or during their second exposure to S-ketamine. The development of hepatotoxicity was such that we decided that continuation of the trial was unjustifiable. See Figure 1 for a flow chart of the study. None of the subjects randomized to Groups 2 (n = 2) and 3 (n = 5) developed severe side effects. In Table 1, the characteristics of patients randomized to Group 1 are given.

Patient A: a 65-year-old woman with CRPS in her left foot

During treatment in week 1, pain score reduced from 7 to 2 on day 2 of the ketamine infusion. Due to the development of severe psychotropic side effects, nausea, and dizziness, the infusion was reduced to 2.1 μg/kg/min on day 2. At this infusion rate, side effects were bearable. An increase in infusion rate was not possible, and the pain score increased to 5 at the end of the infusion. No increase in liver enzymes was detected in treatment week 1 (Figure 2). Upon admission in week 4, pain had returned to prestudy baseline level and on day 2 of the treatment, similar side effects occurred as had been seen in week 1 (ketamine infusion rate 2.7 μg/kg/min, pain score 2). After 72 h of ketamine infusion, the patient developed an itching rash on legs, abdomen, back, and upper arms, combined with an increase in tympanic temperature to 38.3 °C. The blood tests performed on day 3 revealed elevated liver enzymes (see Figure 2; ALP, ALT, AST, TBIL, and γGT exceeded the upper reference values). A diagnosis of drug-

induced liver injury (DILI) was made and the ketamine infusion was terminated (total amount of ketamine infused at that time was 1.3 g). Also, all other medication was stopped. Jaundice and tenderness or enlargement of the liver were absent on physical examination. No further abnormalities were observed in blood hematology or chemistry, apart from a small increase in eosinophilic and neutrophilic leukocytes (to 8% and 77%, respectively). The patient received topical corticosteroid and oral clemastine to treat the pruritus. Liver enzymes decreased upon termination of the ketamine infusion, except for ALP and γGT, which increased for another day. The patient was discharged on day 5 with an NRS of 3. After discharge the rash slowly improved, disappearing completely within 2 weeks. Liver enzymes returned to normal within 1 month, except for γGT, which normalized within 2 months. The patient continued to experience a lack of energy following discharge for 6 months. The pain score returned to baseline 5 weeks after discharge.

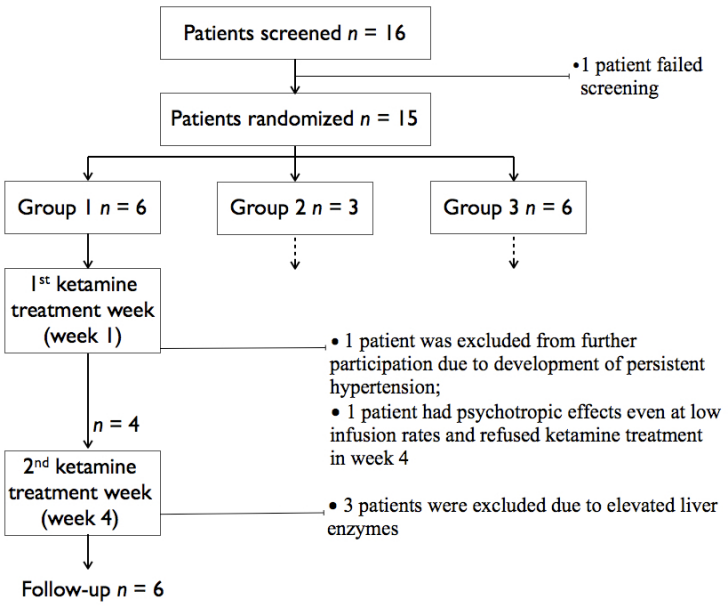

Figure 1 Flow chart of Group I of the study at the time of study termination.

Patient B: a 53-year-old woman with CRPS in her right arm

Ketamine produced a reduction in CRPS pain score from 8 to 2 on treatment day 2. On day 3 the patient was pain free. On day 4 she experienced severe psychotropic side effects: fearsome hallucinations and a panic attack. She refused further treatment and was discharged with a pain score of 4. Pain relief lasted for another 13 weeks. No increase in liver enzymes was detected during ketamine treatment.

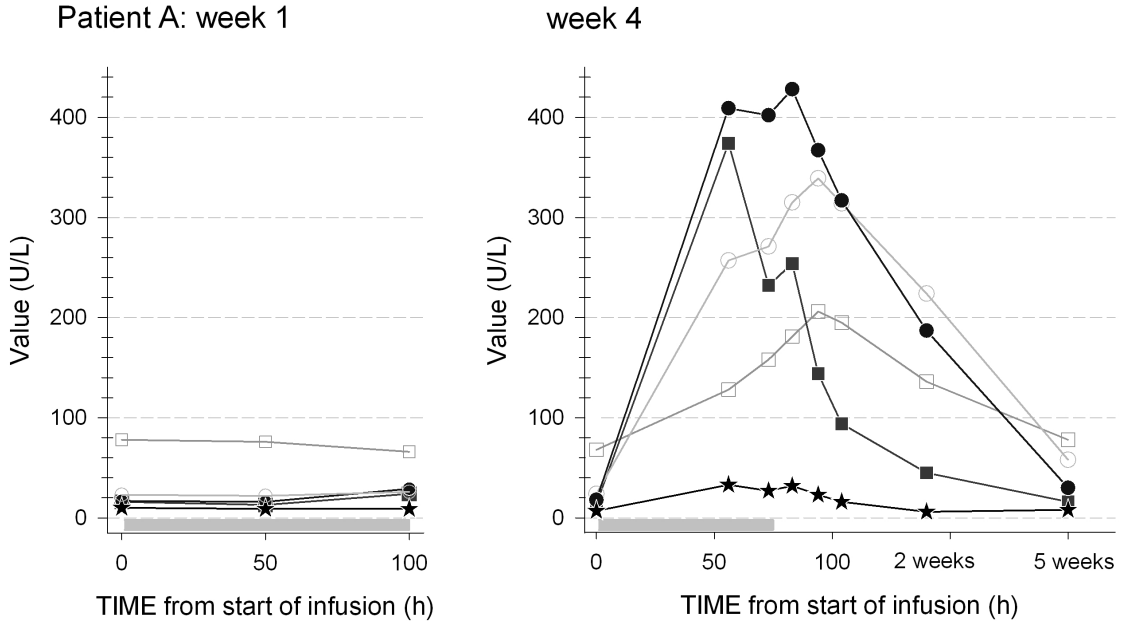

Figure 2 Serum liver enzymes in the first (**left**) and second (**right**) S-ketamine treatment week (= study week 4) of patient A. Alkaline phosphatase, alanine transaminase, aspartate transaminase, total bilirubin, and γ-glutamyltransferase exceeded the upper reference values. Reference values: alkaline phosphatase (open square) 40-120 U/L, alanine transaminase (closed circle) 5-34 U/L, aspartate transaminase (closed square) 5-30 U/L, total bilirubin (black star) 0-17 U/L, and γ-glutamyltransferase (open circle) 5-40 U/L. The gray bar indicates the ketamine infusion.

Patient C: a 39-year-old woman with CRPS in both arms

In treatment week 1 there was a slow and modest reduction in CRPS pain score from 9 to 6 on day 3. She developed a gradual increase in mean arterial blood pressure from 93 to 135 mmHg on day 3. Decreasing the infusion rate did not lower the blood pressure and the treatment was terminated on day 4 (pain score 6, mean arterial pressure 130 mmHg). The decision was made by the investigators and patient to not participate in the second ketamine session. The high blood pressure was successfully treated with antihypertensive medication that is continued to date. No increase in liver enzymes was detected during ketamine treatment.

Patient D: a 20-year-old woman with CRPS of the left leg

Upon admission in week 1, CRPS pain score was 9. The ketamine infusion rate was increased to the maximum dose without any effect on the pain score. The patient experienced no side effects. On day 5 the patient was discharged with pain score 9. A similar course was observed during treatment in week 4. The pain score was 9 upon discharge and remained between 8 and 9 during the follow-up period. No increase in any of the liver enzymes was detected during ketamine treatment in weeks 1 and 4.

Table 1 Patient characteristics, NRS and ketamine treatment.

Patient	Age (y); Sex	BMI (kg/m²)	Cause of CRPS	Duration of CRPS (months)	Affected limbs	Pain medication at admission	NRS at baseline	Ketamine amount in week 1 (mg)	Ketamine amount in week 4 (mg)
A	65; f	38	Surgery	74	Left leg	Tramadol 3 dd 50 mg Paracetamol/codeine od	7	1575	1301
B	53; f	34	Surgery	30	Both arms	Ibuprofen 600 mg od	8	1220	–
C	39; f	32	i.v. line placement	144	Right arm	Tramadol 3 dd 100 mg Gabapentin 4 dd 600 mg Ibuprofen 2 dd 800 mg Oral contraceptive	9	1288	–
D	20; f	20	Contusion	78	Left leg	Tramadol 1 dd 150 mg Tramadol/paracetamol 4 dd 37.5/325 mg Amitriptyline 1 dd 20 mg	9	1412	1416
E	48; f	29	Fracture	105	Right leg	Naproxen 2 dd 500 mg Cannabis tea 1–2/wk	6	2084	794
F	46; m	30	Fracture	11	Left arm	-	8	3297	88

BMI = body mass index; **CRPS** = complex regional pain syndrome; **NRS** = numerical rating scale; **i.v.** = intravenous; **od** = on demand; **dd** = times daily.

Patient E: a 48-year-old woman with CRPS of the right foot

Ketamine induced gradual pain relief, with CRPS pain NRS 6 to 0 on day 4. During treatment the patient experienced various side effects, including psychotropic effects, sedation, dizziness, and nausea. No increase in liver enzymes was detected during the first ketamine treatment week. Upon the start of treatment in week 4, CRPS pain score was 3. Within 1 day of ketamine treatment, the pain score was reduced to 1. Side effects were again present, but they seemed of lesser intensity compared to the first admission. For nausea, domperidone was given. Routine blood screening on day 3 revealed elevated liver enzymes (Figure 3; ALP, ALT, AST, TBIL, and γGT exceeded the upper reference values). The ketamine infusion was ended on that same day (total dose given, 800 mg). The patient had no fever, jaundice, abdominal tenderness, or enlargement of the liver.

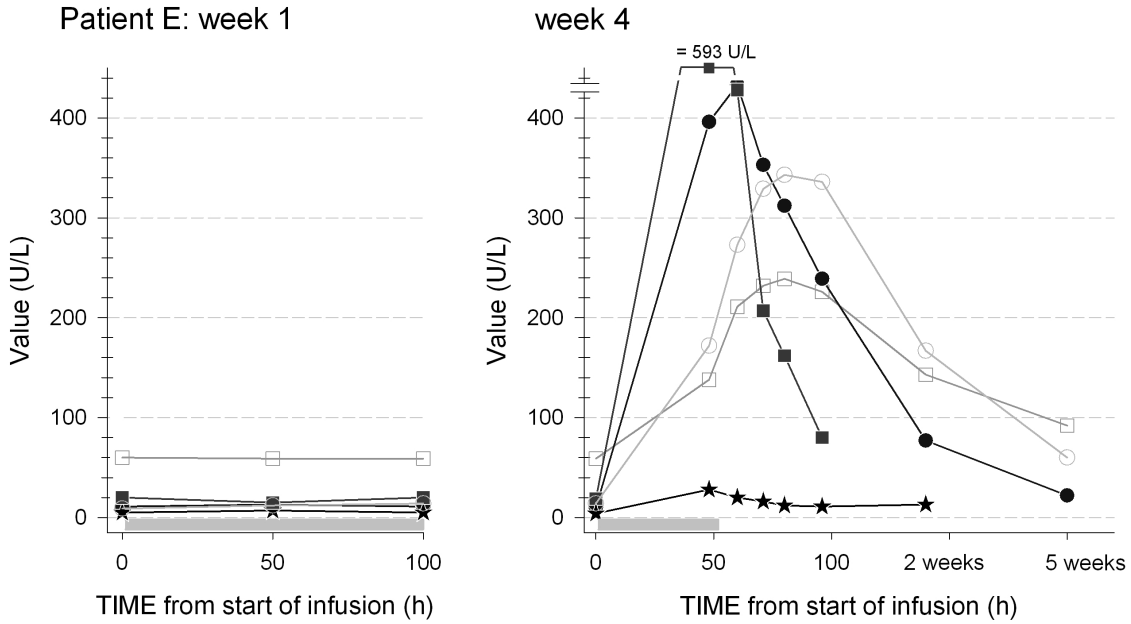

Figure 3 Serum liver enzymes in the first (**left**) and second (**right**) S-ketamine treatment week (= study week 4) of patient E. Alkaline phosphatase, alanine transaminase, aspartate transaminase, total bilirubin, and γ-glutamyltransferase exceeded the upper reference values (for reference values see Figure 2 legend). The gray bar indicates the ketamine infusion.

The following tests were performed: renal function; clotting time; serum concentrations of ammonia and lactate; serology for hepatitis A, B, and C; cytomegalovirus; Epstein-Barr virus; and antinuclear, antimitochondrial, and anti-smooth muscle antibodies. All tests were normal or negative except for the antinuclear antibody, which was weakly positive. An ultrasound of the liver, bile ducts, and related vasculature showed no abnormalities. On day 4 the patient developed a severe itch of both feet and petechiae. The CRPS seemed to flare up

with edema of the right foot and an increase in pain score to 6. The patient was discharged on day 5, after which liver enzymes slowly decreased and the itch and petechiae disappeared over a course of days to weeks. The liver enzymes normalized within 2 months.

Patient F: a 46-year-old man with CRPS of the left arm

Before treatment, the CRPS pain score was 8. Upon ketamine infusion, a slow decline in pain score occurred with no side effects apart from mild sedation. During the course of the week, the patient experienced various episodes of sub-febrile temperature (37.9 °C) without any signs of illness, infection, or allergy. At discharge, the pain score was 4. Upon admission in week 4, the CRPS pain score had increased to 7. The ketamine treatment was started, but terminated on the same day after 6 h of infusion, when the results of the blood screening became available: ALT was elevated to 77 U/L (normal range 5-34 U/L) and γGT was elevated to 267 U/L (5-40 U/L). The other enzymes remained within the range of normal. A second sample 8 h after the initiation of treatment gave similar values. The patient denied the use of alcohol, intake of any medication, or any episodes of epigastric pain in the period prior to his second admission. Blood hematology tests revealed the absence of gallstones or signs of infection. Since we discontinued ketamine treatment, the patient refused any follow-up blood measurements. Two additional blood samples were taken 1 week and 4 months later, by the patient's family doctor: γGT remained elevated (92 U/L) in the first sample but was normalized in the second.

The course of pain relief and ketamine infusion scheme, an average plot of the pain scores and ketamine infusion rates is given in Figure 4 for 5 patients of Group 1 (data from patient D are excluded in this graph). Since the study was single blind and not placebo-controlled, these data do not constitute efficacy data, but do give an impression of the effect of treatment on pain scores.

Discussion

Ketamine is increasingly used for the treatment of chronic pain. Data from recent trials suggest that prolonged or repetitive administrations of this agent are needed to induce long-term pain relief, that is, pain relief outlasting the treatment period for a period longer than 48 h.[4] In a previous study we showed that a single continuous 100-hour infusion of ketamine produces the relief of CRPS pain for up to 11 weeks, compared to placebo.[10] This is a somewhat disappointing effect and prompted us to examine the effect of a second 100-hour infusion period on pain relief in CRPS-1 patients.

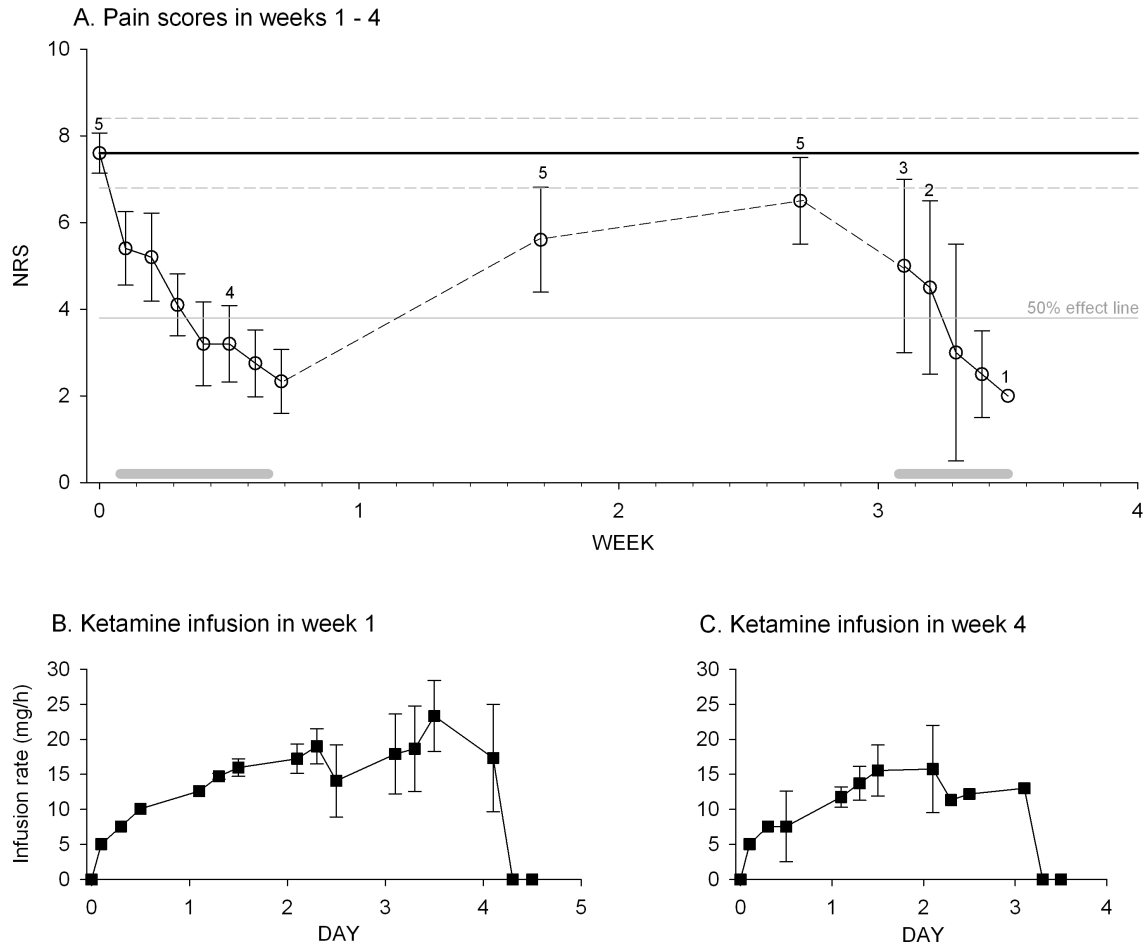

Figure 4 Pain data and infusion rates of 5 subjects showing an analgesic response during treatment with ketamine (given in a single-blind fashion). **A** Numerical rating scores from week 1 to the end of week 4. The continuous black line is the pretreatment baseline mean numerical rating score ± 95% confidence interval (dashed gray lines); the continuous gray line is the 50% effect line; the gray bars indicate the ketamine infusion. The numbers indicate the number of patients from which the pain data was obtained. **B** Ketamine infusion rates (mg/h) for the first treatment week (week 1). **C** Ketamine infusion rates for the second treatment week (week 4) Values are mean ± SEM.

A pilot study was designed to explore possible time frames for ketamine re-administration (4 weeks versus 13 weeks). Apart from the expected psychotropic and hypertensive side effects, we encountered a problem that so far was considered a rare side effect of ketamine administration -elevation of serum liver enzymes- at a relatively high frequency. In 3 patients in study Group 1, the increase in liver enzymes was detected just prior or during a second ketamine administration, 16 days after an initial 100-hour treatment. We relate the cause of elevated liver enzymes to ketamine-induced hepatotoxicity in patients A and E, as there was an evident chronological relation between the ketamine infusion and the development and resolution of the liver injury (e.g., liver enzymes declined

rapidly upon termination of treatment). The cause of the liver enzyme elevation in patient F is less clear and is possibly related to the ketamine treatment.

We made the decision to end the trial, as we argued that repetitive long-term ketamine infusions within a short time frame is a risk factor for liver cell damage and we therefore concluded that our study design (in Group 1 patients) is not acceptable. These data contrast findings in our previous study; patients in the Sigtermans et al. trial received a single 100-hour infusion of ketamine with an average ketamine dose of 2.5 g (range 1.7-3.3 g) without any signs of liver toxicity or side effects beyond the duration of treatment.[10] Some of the patients in the current study used co-medication that might have had an effect on liver function.

Ketamine-induced liver injury

The first reports on an association between ketamine and liver injury date from 1979-1980. In the isolated rat hepatocyte, supraclinical doses of ketamine inhibited gluconeogenesis, and urea formation from alanine caused a reduction in adenosine triphosphate concentration and a dose-dependent leakage of L-lactate dehyrogenase.[11] Dundee et al. described a higher incidence of significant elevations in liver enzyme levels in patients receiving 3-4 mg/kg ketamine for induction and maintenance of general anesthesia compared to "standard" techniques (involving halothane and thiopentone).[12] Fourteen (of 34) patients receiving ketamine and 7 (of 34) receiving standard treatment had signs of liver injury.

Most studies on the use of ketamine for chronic pain treatment either did not measure liver enzymes or found an absence of changes in plasma liver enzymes.[10,13,14] For example, in 30 patients receiving a 100-hour continuous infusion of ketamine (infusion rate between 10 and 20 mg/kg), no effect on liver enzymes was observed during the ketamine treatment period, but no measurements were made thereafter.[10] Sporadic reports of liver injury do appear.[15-18] For example, in refractory CRPS patients receiving ketamine in anesthetic dosages for 5 days, modest elevations in liver enzymes were noted in 16 (of 20) patients on the last days of treatment.[18] Following treatment, the enzymes returned to reference values within 10 to 14 days. In another study, low-dose ketamine given to CRPS patients caused elevated liver enzymes in 4 (of 33) patients during a first treatment period (duration of treatment ranged from 4 to 20 days; ketamine infusion rate 10-50 mg/h).[16] One of these patients who required additional treatments 3 months later developed immediate elevations of his liver-enzyme profile during 2 more treatment attempts. More frequent incidence of liver damage is observed following frequent recreational ketamine abuse.[6-8,19,20] These patients often present with kidney injury, elevated liver enzymes, and bladder dysfunction. Some of these patients have epigastric pain, but in most

cases the elevated liver enzymes were discovered upon blood examination when the patients came in for urinary tract symptoms.[19,20]

All of the above studies that reported liver injury in response to ketamine treatment used a racemic ketamine mixture. In our study we administered the S(+)-enantiomer indicating that the enantioselective use of ketamine will not protect the patient for possible liver injury.

Drug-induced liver injury (DILI)

Drug-induced liver injury is unpredictable (i.e., not dose related, and difficult to reproduce in animal models) and is considered to have a rare incidence.[21] Various forms have been described (hepatitis, cholestasis, cirrhosis, granulomas, steatosis, neoplasms, vascular). DILI may have immune-mediated (allergic) and non-immune-mediated (non-allergic) features. In the allergic form, the innate immune system responds to the drug or its metabolite as if it was a toxic foreign body or infectious organism causing a sterile inflammatory response. In the non-allergic form, mitochondrial impairment, oxidative stress, and cellular adaptation failure are causative factors. Allergic DILI has a latency period of 1-6 weeks, with a high incidence of fever, rash, and eosinophilia, is not dose related, and recurs upon drug re-challenge. Non-allergic DILI has a latency of 1 month to 1 year and is possibly dose related, while the occurrence of rash, fever, and eosinophilia is uncommon.

On the basis of the liver enzyme level, DILI is classified into hepatitis, cholestasis, or a mixed pattern.[21] DILI is defined as hepatitis when: ALT ≥ 3 ULN (where ULN = upper limit of the normal value) and (ALT/ULN)/(ALP/ULN) ≥ 5; as cholestatic when ALP ≥ 2 ULN and (ALT/ULN)/(ALP/ULN) ≤ 2; and mixed when 2 < (ALT/ULN)/(ALP/ULN) < 5. In severe cases of DILI, the patient may develop acute liver failure defined by coagulopathy (international normalized ratio ≥ 1.5) and hepatic encephalopathy occurring in the 6 months following the onset of DILI. On the basis of these definitions, the clinical features, and additional laboratory tests, we diagnosed patients A and E as having ketamine-induced hepatitis of the allergic form. While the cause of elevated enzymes in patient F is likely to be the administration of ketamine in week 1, the nature of the hepatotoxicity remains unknown. For patients A and E, it remains unclear whether co-medication played an additional role in the development of DILI. For example, patient A used paracetamol (acetaminophen). While this drug is associated with non-allergic DILI, it may have enhanced ketamine-induced liver injury. In a rat study, a synergistic hepatotoxic effect (increases in ALT and AST) was observed for ketamine and the solvent carbon tetrachloride.[22] A similar mechanism may possibly occur for paracetamol and ketamine. Further studies are needed to study paracetamol ketamine interaction on liver function. Also,

contraceptive pills are associated with elevated liver enzymes. Only patient C used these, without liver enzyme elevations during the ketamine infusions.

Warning signs for the development of DILI include abdominal pain, nausea/vomiting, and jaundice.[21] However, during ketamine treatment, nausea and vomiting may occur from ketamine itself, and abdominal pain may be absent due to analgesia. We therefore advise frequent testing during long-term ketamine treatment, especially when repeated ketamine infusions are given within short time frames. Treatment of ketamine-induced DILI is by prompt discontinuation of the exposure to ketamine and supportive/symptomatic treatment. In severe allergic hepatitis with no improvement upon drug removal, a 1-week treatment with steroids may be attempted, although proof for efficacy is limited at present.[23]

In summary, a study designed to explore the effect of repeated 100-hour ketamine infusions on pain relief in CRPS patients was ended prematurely due to the development of ketamine-induced hepatitis of allergic nature in 2 patients. In a third patient, liver injury was observed, although its origin cannot be confirmed with certainty. All affected patients (n = 3) received 2 ketamine exposures within 4 weeks' time, while patients receiving ketamine at a wider time interval (12 weeks, n = 2) had no signs of liver injury. Liver enzyme levels returned to normal in all patients within 2 months following the discontinuation of treatment. Patients that receive long-term or repetitive ketamine infusions for the treatment of chronic pain should receive regular monitoring of blood pressure and psychotropic side effects. Furthermore, as suggested by our current report of 3 cases, regular measurements of liver function are strongly advisable. Whether ketamine treatment should be extended to chronic pain patients other than CRPS type 1 requires further study, with the need for high-quality randomized trials, not only focusing on analgesic efficacy, but also carefully monitoring the myriad of short- and long-term side effects linked to ketamine treatment.

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Chapter 4

Absence of long-term analgesic effect from a short-term S-ketamine infusion on fibromyalgia pain:
A randomized, prospective, double blind, active placebo-controlled trial

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Eur J Pain, in press

Introduction

Fibromyalgia is characterized by chronic generalized musculoskeletal pain without a specific structural or inflammatory cause.[1] Prevalence is 2-3% with predominance for women (sex ratio 1:7).[1] There is debate whether fibromyalgia pain is neuropathic in origin. The IASP defines neuropathic pain as pain caused or initiated by a primary lesion or dysfunction in the nervous system.[2] Although no specific causes of fibromyalgia in the peripheral or central nervous system are apparent, fibromyalgia pain has been associated with central sensitization.[3,4] For example, Desmeules et al. observed reduced thermal pain thresholds and a reduced spinal nociceptive flexion reflex threshold in fibromyalgia patients.[3] Various mechanisms may underlie the process of central sensitization, most importantly, *N*-methyl-D-aspartate receptor (NMDAR) activation and up-regulation at spinal and supraspinal sites.[5] A role for NMDAR activation in fibromyalgia comes from studies in which the analgesic response to a low-dose intravenous ketamine (0.1 mg/kg) test is a positive predictor for a subsequent analgesic response to oral dextromethorphan.[6,7] Both ketamine and dextro-methorphan block the NMDAR.

In the current study we investigated the effect of a short-term S-ketamine infusion on fibromyalgia pain. We assessed the effect of a 30-min infusion with a relatively high dose of S-ketamine (0.5 mg/kg, equivalent to 1.0 mg/kg racemic ketamine)[8] on pain relief in the period following treatment. Studying the phase following treatment rather than during treatment is based on the following observations: (i) in animals, NMDAR antagonism in neuropathic pain states causes relief of spontaneous pain and allodynia outlasting the duration of in vivo NMDAR antagonism[9]; (ii) a short infusion with S-ketamine causes pain relief lasting for 24 h or longer in patients with Complex Regional Pain Syndrome type 1 (CRPS-1)[10]; (iii) treating patients with small-fiber neuropathy (due to diabetes and sarcoidosis) with a 1-h infusion with 0.5 mg/kg S-ketamine causes long-term pain relief (>> 24 h; M Niesters, unpublished observation); and (iv) a single infusion with 0.5 mg/kg ketamine produces antidepressant effect lasting one to seven days in therapy resistant major depression.[11] Although the latter patient population was not targeted for pain-related symptoms, we hypothesized with Zarate et al. and others that a common mechanism of action may cause both relief of the symptoms of depression and pain (i.e., blockade of the NMDAR).[11]

We hypothesize that (i) ketamine causes greater pain relief than placebo; (ii) ketamine induces pain relief beyond the treatment period (i.e., ketamine effect is not driven by pharmacokinetics). We compared the effect of ketamine to an active placebo (the benzodiazepine midazolam) to control for occurrence of side effects during treatment allowing a proper blinding of the study.

Methods

Patients were recruited after protocol approval was obtained from the local Human Ethics Committee (Commissie Medische Ethiek, Leiden University Medical Center, Leiden, The Netherlands) and the Central Committee on Research involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek, The Hague, The Netherlands). Informed consent was obtained according to the Declaration of Helsinki. The study was registered under number NTR1343 before recruiting at www.trialregister.nl.

Patients

Fibromyalgia patients were recruited through the outpatient clinics of the departments of Anesthesiology (pain clinic) and Rheumatology of the Leiden University Medical Center, as well as through an advertisement on the website of the Dutch Fibromyalgia Patient Association. Inclusion criteria were: fibromyalgia syndrome diagnosed according to the 1990 'American College of Rheumatology' criteria (presence of widespread pain and tenderness in at least 11 of 18 tender points on specific muscle-tendon sites)[12], age 18-75 years, spontaneous pain score of 5 or greater (based on a scale from 0 = no pain to 10 = most severe pain imaginable). Exclusion criteria were body weight > 100 kg or body mass index ≥ 35, use of strong opioids, a score of eight or greater on the subscale of the Hospital Anxiety and Depression Scale (HADS), pregnancy or lactation, severe cardiovascular disease, psychiatric disease, allergy or co morbidities incompatible with study medication (S-ketamine, midazolam), presence of factors or other diseases that could interfere with pain scores and functionality.

Patients were allowed to continue their medication and other therapies during the 8 weeks of the study, but changes in therapy were not allowed. They were asked not to eat or drink anything at least 4 hours prior to the treatment infusion and had to refrain from grapefruit and grapefruit juice for 7 days prior to the infusion.

Study design

This study had a randomized prospective, double blind, active placebo-controlled design. Randomization into S-ketamine (Ketanest, Pfizer BV, Capelle aan de IJssel, The Netherlands) or midazolam (Synthon BV, Nijmegen, The Netherlands) treatment groups was performed after subject screening and inclusion. An independent physician performed the randomization using an electronic randomization list (downloaded from www.randomization.com). An independent physician also prepared the study medication in a blinded syringe.

The study consisted of a single treatment day at the beginning of week 1 followed by an 8-week follow-up. On the study day two intravenous catheters were placed in the hands or arms of the patient, one for drug administration and one for blood sampling. After baseline measurements, patients received an infusion (starting at t = 0) with either 0.5 mg/kg S-ketamine or 5 mg midazolam given over 30 minutes. This was followed by 2.5 h of measurements after which the patients were discharged. Following treatment, weekly measurements of treatment effect were obtained. At the end of the study all patients were asked which treatment they thought they had received and were next informed of their treatment. Patients given midazolam were given the possibility of receiving an open-label ketamine treatment.

Measurements on the treatment day

Spontaneous (fibromyalgia) pain

Prior to infusion on the study day, a baseline pain score was obtained using a Visual Analogue Scale (VAS). The VAS was recorded by patients on a 10-cm paper scale that ranged from 0 (no pain) to 10 (worst pain). This was recorded 3 times and averaged for further analysis. After the infusion fibromyalgia pain scores were obtained at t = 45, 60, 75, 90, 120, 150 and 180 minutes following the start of intravenous treatment.

Heat pain test

To compare the effect of S-ketamine on a standardized nociceptive stimulus in the study population a noxious thermal stimulus was applied with the TSA-II NeuroSensory Analyzer (Medoc Ltd., Israel). A 3 x 3 cm thermode was placed on the skin of the volar side of the forearm. The temperature was gradually increased by 0.5 °C/s, from 32 °C to a peak temperature in the range of 46 to 52 °C. Peak temperature remained for one second, after which it rapidly decreased (10 °C/s) to 32 °C. After this stimulus, a VAS score for heat pain was obtained (scale 0 to 10). Peak temperature was determined by performing a set of test experiments prior to the drug infusion. To that end, peak temperature was set at 46 °C and the VAS score was obtained. When the VAS score was < 5 a subsequent test was performed with peak temperature increased by 1 °C (max. temperature allowed = 52 °C). The temperature at which the VAS score was 5 or greater was used in the remainder of the study. The test data were discarded. Next, three baseline tests were performed and the VAS data were averaged for further analysis. Heat pain tests were performed at t = 0 (baseline), 45, 60, 120, and 180 min after the start of the infusion. In order to prevent adaptation or sensitization, the location of the thermode was changed after every stimulus.

Side effects

S-ketamine side effects were measured using the Bowdle questionnaire.[13] This questionnaire consists of 13 psychotomimetic side effects that are typical for ketamine. These 13 items were scores on a numerical rating scale (NRS) ranging from 0 to 10 at t = 0 (baseline), 45, 60, 120 and 180 min after the start of treatment. The 13 items of the questionnaire were:

(i) my body or body parts seemed to change their shape or position (body);
(ii) my surroundings seemed to change in size, depth, or shape (surroundings);
(iii) the passing of time was altered (time);
(iv) I had feelings of unreality (reality);
(v) it was difficult to control my thoughts (thoughts);
(vi) the intensity of colors changed (colors);
(vii) the intensity of sound changed (sound);
(viii) I heard voices or sounds that were not real (voices);
(ix) I had the idea that events, objects, or other people had particular meaning that was specific for me (meaning);
(x) I had suspicious ideas or the belief that others were against me (suspicious);
(xi) I felt high (high);
(xii) I felt drowsy (drowsy);
(xiii) I felt anxious (anxious).

Monitoring

During and following infusion the following items were monitored: heart rate, blood pressure, breathing rate and arterial hemoglobin-oxygen saturation.

Blood sampling

Venous blood samples for measurement of plasma concentrations of S-ketamine and its active metabolite S-norketamine were obtained at t = 0 (baseline), 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 75, 90, 120, 150 and 180 min. Plasma was separated within 15 min of blood collection and stored at -25 °C until analysis. Analysis was by high performance liquid chromatography as described previously.[14] The lower limit of quantitation was 10 ng/ml, the lower limit of detection 3 ng/ml for both analytes.

Measurements during follow-up

The Fibromyalgia Impact Questionnaire (FIQ)

The FIQ was chosen to measure the effect of treatment.[15] This questionnaire is especially developed and validated for fibromyalgia patients and measures pain, stiffness, and activities in daily life over a period of one week. It consists of a total of 20 items that are quantified on numerical or visual analogue scales. Measurements were obtained on a weekly basis during the 8-week follow-up period (first measurement at baseline; second measurement at the end of week 1; last measurement at the end of week 8).

Data and statistical analysis

The study was powered to detect a 2-point difference in pain score during follow-up. Assuming a SD of 1.5, a power of 0.8 and α of 0.05 (two sided)[16], we calculated that 10 patients per group were needed per group. To compensate for possible loss of subjects during the study we enrolled 12 subjects per group. Statistical analysis included all patients according to the intention-to-treat principle.

The demographics of the treatment groups and baseline pain scores were compared using t-tests for continuous variables and Chi-square or Fisher's exact tests for categorical data. Patients that had a pain relief of their spontaneous fibromyalgia pain of > 50% were considered responders. A linear mixed model was used to analyze pain scores (on the study day and during the 8-week follow-up), side effects (derived from the Bowdle questionnaire) and heat pain scores. Finally the VAS scores relative to baseline (ΔVAS) were compared for experimental and fibromyalgia pain at t = 45 and 180 min. In the analyses the time is a within-subject factor and treatment level (or pain type) is a between-subject factor. Data analysis was performed with the statistical package SPSS 16.0. P-values < 0.05 were considered significant. Data are presented as mean ± SEM unless stated otherwise.

Results

Sixty-three patients were requested to participate in the study (Figure 1). Since 27 refused, 36 were screened of which 25 were randomized (11 did not meet the inclusion criteria). One patient refused further participation on the treatment day (prior to baseline measurements). The number of subjects treated was 24 subjects (12 in each group). The median time since diagnosis was 1.3 years (range 0.1 to 16 years), with on average 16 tender points. Patient demographics and baseline values are shown in Table 1.

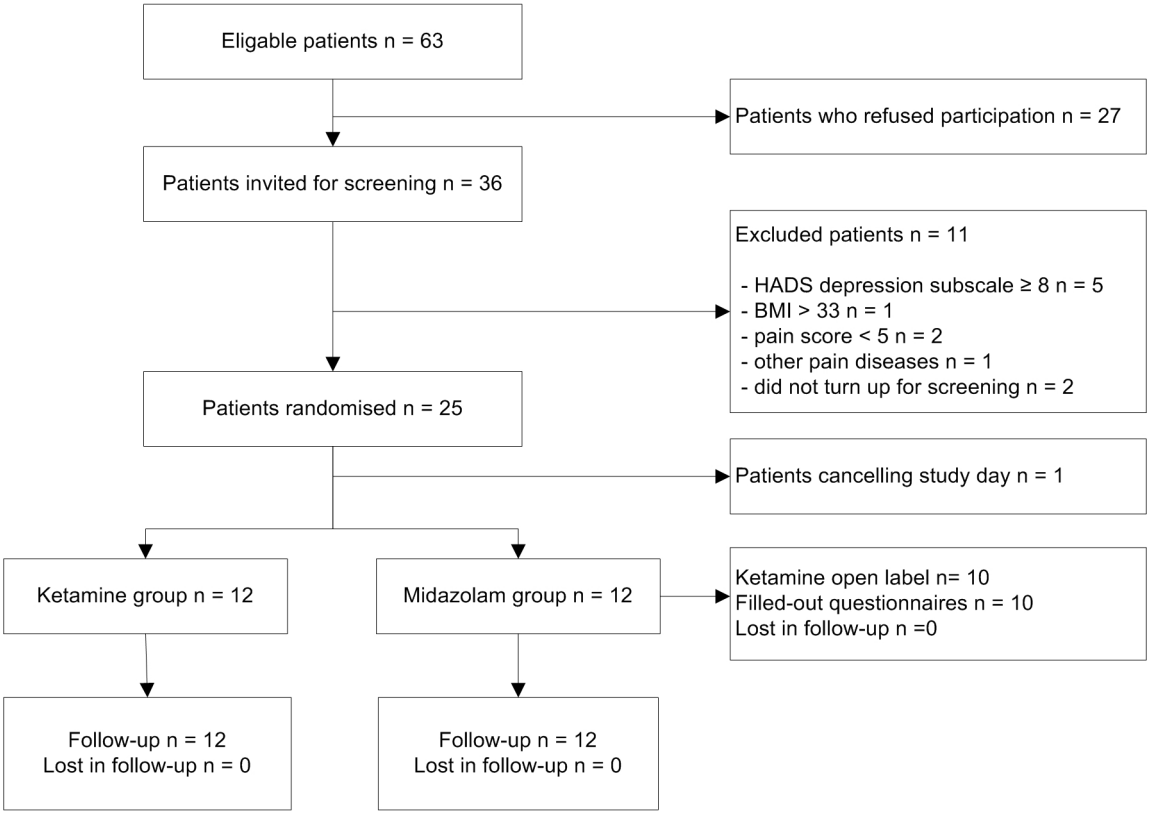

Figure 1 Study flow-chart.

Treatment effect on pain scores and side effects on the treatment day

Fibromyalgia pain

Baseline pain scores were 5.4 ± 0.6 cm in the S-ketamine group and 5.8 ± 0.4 cm in the midazolam group (ns). S-ketamine caused the reduction of fibromyalgia pain to 1.9 ± 0.8 cm ($P < 0.01$ versus baseline) at t = 45 and to 3.1 ± 0.8 cm at t = 180 min ($P < 0.01$ versus baseline) (see Figure 2). Midazolam pain scores at t = 45 min and t = 180 min were 4.4 ± 0.6 cm ($P < 0.01$) and 4.3 ± 0.7 cm ($P < 0.01$). Comparing treatments, a significant time effect was observed ($P < 0.001$) without significant treatment ($P = 0.09$) or interaction ($P = 0.10$) effects. This indicates the absence of a difference in pain relief between S-ketamine and midazolam in the treatment recovery period (i.e., t = 45 min to t = 180). In patients treated with S-ketamine VAS scores closely followed the S-ketamine plasma concentrations (Figure 2A) indicating that effect was driven by ketamine pharmacokinetics.

Table 1 Patient demographics and baseline characteristics.

	All patients	S-ketamine	Midazolam	
N women/men	23/1	11/1	12/0	ns
Age (year)	42.1 ± 11.0	39.1 ± 10.6	45.2 ± 10.9	ns
Weight (kg)	76.8 ± 13.0	79.5 ± 9.7	74.1 ± 15.6	ns
Height (cm)	170.9 ± 6.6	172.0 ± 5.6	169.8 ± 7.6	ns
Body mass index (kg/m^2)	26.2 ± 4.1	27.0 ± 4.4	25.4 ± 3.7	ns
Time since diagnosis (months)[#]	16 (1-192)	14 (1-168)	34 (1-192)	ns
Number of tender points	16.2 ± 1.9	16.7 ± 1.5	15.8 ± 2.2	ns
HADS total	9.6 ± 4.5	9.6 ± 4.1	9.6 ± 5.1	ns
HADS depression	3.5 ± 2.1	3.7 ± 2.3	3.4 ± 2.1	ns
HADS anxiety	6.0 ± 3.7	5.9 ± 3.3	6.2 ± 4.1	ns
Pain score at baseline (NRS)	7.0 ± 1.0	7.2 ± 1.2	6.8 ± 0.7	ns
Current pain medication (N)				
Paracetamol	7	3	4	ns*
NSAIDs	11	5	6	ns*
Tramadol	2	1	1	ns*
Amitriptyline	3	1	2	ns*
Duloxetine	1	1	0	ns*
Pregabaline	2	1	1	ns*

Data presented as mean ± SD.
[#] median (range); [*] Fisher exact test.
N = number

The number of responders did differ significantly between treatments from t = 45 to t = 60 min (Figure 3). At t = 45 min 8 patients receiving S-ketamine and 3 patients receiving midazolam had a pain reduction > 50%. Considering only the responders, S-ketamine caused pain reduction from 5.3 ± 0.8 (baseline) to 0.6 ± 0.2 cm (t = 45 min); midazolam caused pain reduction from 5.2 ± 1.9 to 1.8 ± 1.0 cm. In the S-ketamine group the number of responders declined. At t = 180 min the number of responders was 6 in the S-ketamine group (with pain score 0.8 ± 0.3 cm) versus 3 in the midazolam group (2.0 ± 0.3 cm).

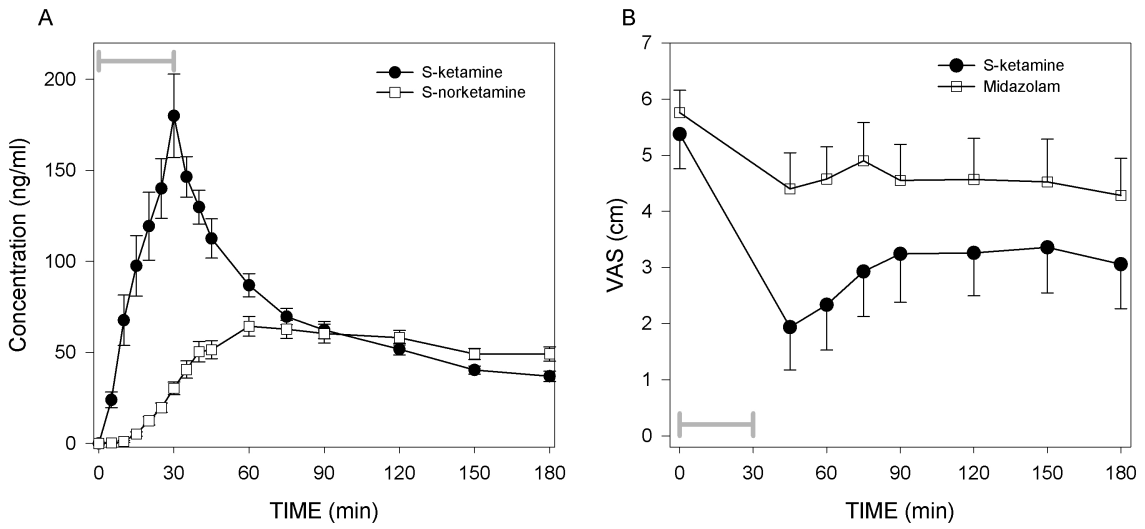

Figure 2 A Mean plasma concentrations of S-ketamine and S-norketamine in patients that received a 30-min S-ketamine infusion (grey bar). **B** Effect of a 30-min treatment with S-ketamine (0.5 mg/kg, closed circles, n = 12) and midazolam (5 mg, open squares, n = 12) on pain scores in fibromyalgia patients. No significant differences in pain relief between the two treatment was observed during the treatment recovery phase (t = 45 to t = 180 min). The grey bar indicates the treatment period. Data are mean ± SEM.

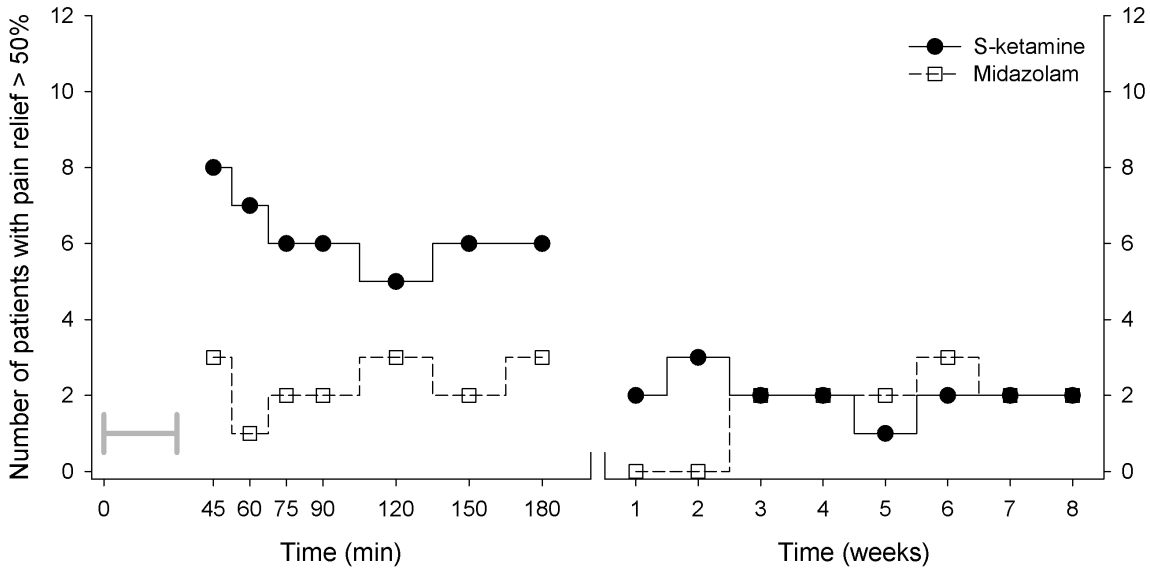

Figure 3 Number of fibromyalgia patients with pain relief > 50%. **Left** graph: data derived from the assessment of spontaneous fibromyalgia pain following treatment (t = 45 to 180 min following the 30-min treatment period). **Right** graph: data derived from the weekly Fibromyalgia Impact Questionnaire, which assess the mean pain score over the previous week. A difference in number of patients that had a pain score > 50% was significant at time t = 45 and t = 60 min only. The grey bar indicates the treatment period.

Experimental heat pain

Baseline heat pain intensity scores were 6.5 ± 0.3 cm and 5.4 ± 0.4 cm in the S-ketamine and midazolam groups, respectively (P = 0.046). Taken the difference in baseline values between groups, the treatment effects are presented relative to baseline (Figure 4). S-ketamine reduced the heat pain intensity score by 2.0 ± 0.9 cm at t = 45 min (P < 0.01 versus baseline) and 0.2 ± 0.7 cm at t = 180 min (ns). Midazolam effect at t = 45 and 180 min was -0.9 ± 0.7 cm (P < 0.05) and +0.5 ± 0.6 cm (ns). Comparing treatments, there was a significant main effect of time (P < 0.01; pain intensity was significantly different at t = 45 min relative to baseline (P = 0.01) but not at t = 180 min). Group and interaction effects were not significant. These data indicate no difference in effect between the two treatments on experimental heat pain in the 2.5 h period following treatment.

For both treatments, no differences in ΔVAS values were observed for experimental pain versus fibromyalgia pain at t = 45 and 180 min (S-ketamine: main effect P = 0.08; midazolam: main effect P = 0.09).

Side effects

Pre-treatment side effects as derived from the Bowdle questionnaire were not different between treatment groups. Following infusion a significant treatment effect was observed for 5/13 items of the questionnaire: surroundings, time, reality, thoughts and high, with a significant difference between treatments at t = 45 min (P < 0.05). The highest score was for reality: 6.5 ± 1.0 (S-ketamine versus midazolam = 1.1 ± 0.7) at t = 45 min. The other items were not different between groups. The effect of treatment on the items high and drowsy are shown in Figure 5; high but not drowsy differed between treatments. The total Bowdle scale showed no treatment effect (P = 0.08) but a significant time (P < 0.01) and time*treatment effect (P < 0.01) was observed.

Vital signs

No differences in baseline values were observed between treatment groups. Baseline heart rate was 70 ± 2 per min, respiratory rate 16 ± 0.3 per min, oxygen saturation 99 ± 0.3%, and mean arterial pressure 87 ± 3 mmHg. All values remained well within clinically acceptable ranges throughout the infusion and 150-min recovery period. Heart rate and blood pressure increased by about 20% during S-ketamine infusion while respiratory rate showed a 10% decrease. No changes were seen during midazolam infusion. Oxygen saturation remained > 98% during S-ketamine and midazolam infusions.

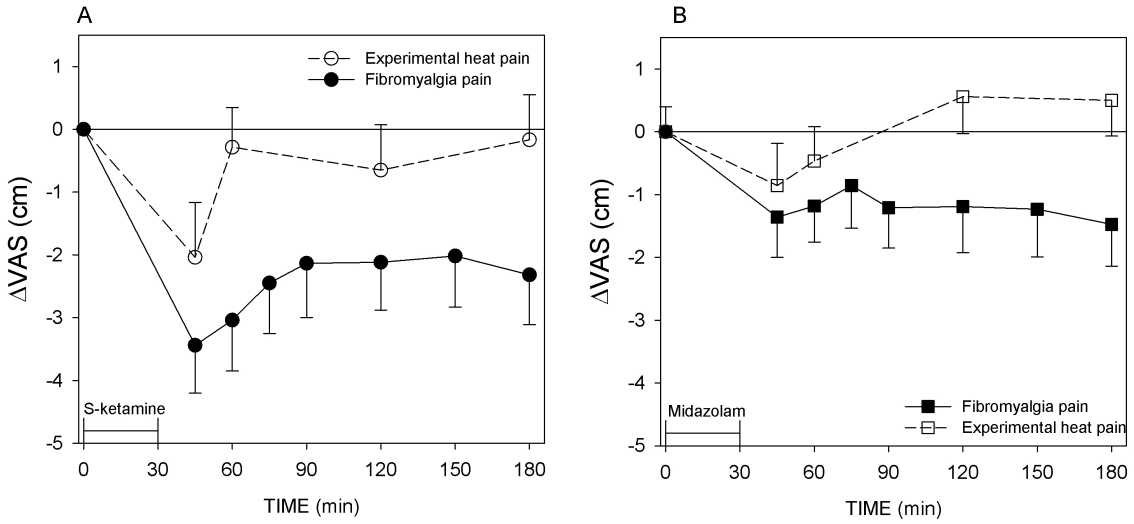

Figure 4 Effect of S-ketamine and midazolam treatment on experimental heat pain intensity scores. **A** Scores following S-ketamine treatment. **B** Scores following midazolam treatment. No differences between treatments on experimental pain scores were observed. For comparison, the effect of treatment of the fibromyalgia pain scores are included. The grey bar indicates the treatment period. Data are mean ± SEM.

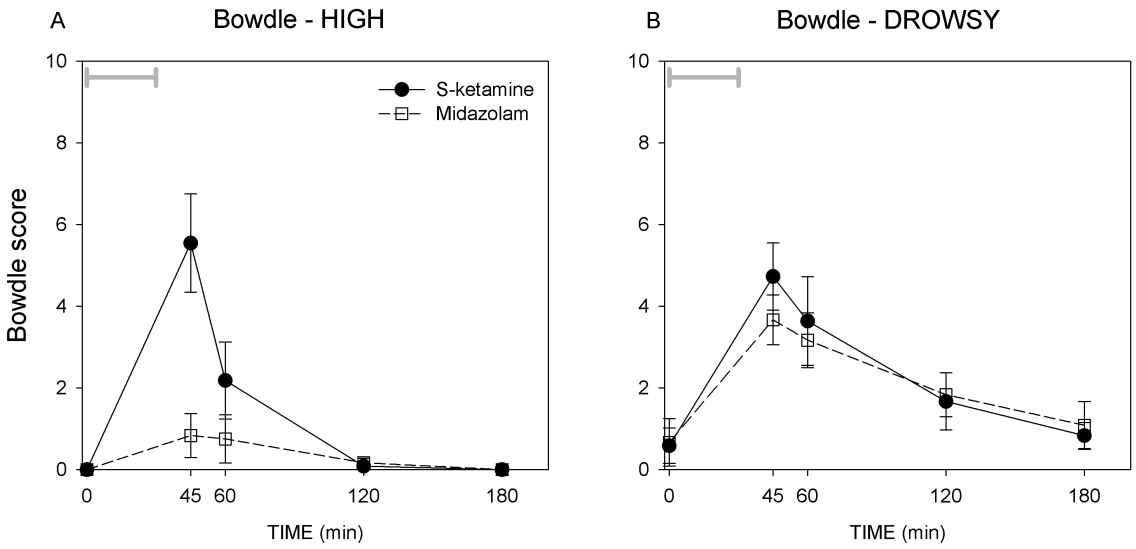

Figure 5 Effect of treatment on the Bowdle **A** high and **B** drowsy scores. A significant treatment effect was observed for high at t = 45 min. The grey bar indicates the treatment period. Data are mean ± SEM.

Effect of treatment on the Fibromyalgia Impact Questionnaire in weeks 1 to 8

Baseline FIQ scores were similar between treatments: 52 ± 4 and 50 ± 3 in S-ketamine and midazolam groups, respectively. No time (P = 0.07), group (P = 0.98) or interaction (P = 0.80) effects were observed in weeks 1 to 8 following treatment (Figure 6). Similarly, baseline pain scores (derived from the questionnaire) were not different between S-ketamine (6.7 ± 0.6) and midazolam

(7.6 ± 0.3) treatments. The FIQ pain scores were higher than the baseline fibromyalgia pain assessment on the treatment day (instantaneous sample). The FIQ pain score is the mean pain score over the past week and these results indicate that fibromyalgia pain varies considerably on a day-to-day basis. No time (P = 0.09), group (P = 0.55) or interaction (P = 0.57) effects were observed on FIQ pain scores in weeks 1 to 8 following treatment (Figure 6). The number of responders as assessed from the FIQ pain score did not differ between treatments and averaged to 2 patients irrespective of treatment (Figure 3).

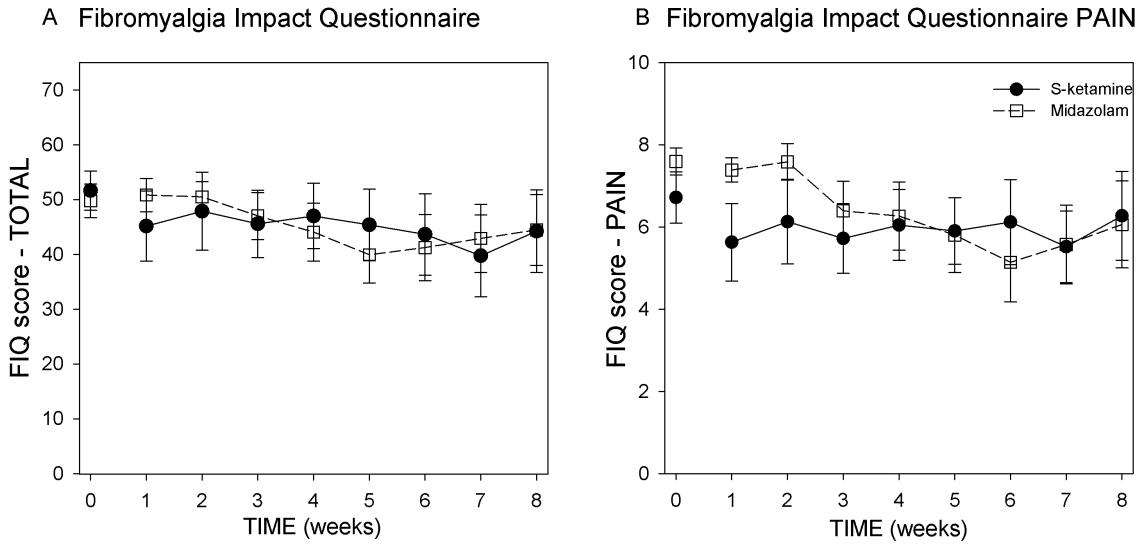

Figure 6 Effect of S-ketamine (closed circles) or midazolam (open squares) treatment on the Fibromyalgia Impact Questionnaire (FIQ). **A** Total scores. **B** FIQ pain scores. No treatment effect or time effect was observed during the 8-week follow-up period. Data are mean ± SEM.

Blinding

Seven patients (58 %) receiving ketamine guessed correctly which treatment they received (Cohen's kappa = 0.16). In contrast, 11 of 12 patients receiving midazolam stated the correct treatment (Cohen's kappa = 0.83).

Effect of open-label treatment on the FIQ

The FIQ values observed after open-label treatment with S-ketamine did not differ from that the values observed in the RCT (data not shown).

Discussion

We observed no significant effect from intravenous S-ketamine on spontaneous and experimental pain-parameters in fibromyalgia patients in the hours and 8 weeks following a 30-min intravenous treatment with 0.5 mg/kg compared to an

active placebo (5 mg midazolam). A small effect cannot be ruled out in the initial 45-min following treatment when taking into account the number of responders (eight responders in the S-ketamine group versus three in the midazolam group). Overall the efficacy of a 30 min infusion of S-ketamine in the relief of pain in fibromyalgia patients is disappointing despite the relatively large dose given. The effect of S-ketamine on fibromyalgia and experimental pain closely followed the measured plasma concentrations, indicating that the effect that was observed was driven by ketamine's pharmacokinetics.

Comparison with the literature

There are two previous RCTs on the efficacy of ketamine in fibromyalgia patients. Sörensen et al. studied the effect of 0.3 mg/kg racemic ketamine given intravenously over 30 min in 16 fibromyalgia patients and observed reduction in pain scores > 50% in 8 patients during treatment.[17] Four of the responders had a significant improvement of FIQ and pain scores (> 50% of baseline) for 1 to 5 days following treatment. Long-term follow-up (> 7 days) was not performed. The authors conclude that they cannot exclude a possible placebo effect. Graven-Nielsen et al. used a similar infusion regimen (0.3 mg/kg racemic ketamine given over 30 min) in 15 fibromyalgia patients (these 15 patients were responders to ketamine as tested in a previous assessment). They observed a significant analgesic effect during and up-to 150 min following treatment relative to placebo.[18] The results of these two studies are similar to ours, indicating a limited analgesic effect of ketamine in fibromyalgia patients after a single or short-term (30 min) infusion irrespective of dose.

Of interest is further the finding that a positive response to intravenous ketamine (dose around 0.1 mg/kg) in fibromyalgia patients may be used as predictive tool in treatment of pain with either oral ketamine or oral dextromethorphan.[6,7,19] These data indicate the usefulness of intravenous infusion tests with ketamine in the selection of chronic pain patients for long-term treatment with NMDAR antagonists. See for a critical review of the various intravenous infusion tests tool for prediction long-term treatment effect Cohen et al.[20] The observation that 8 of 12 of our patients had pain relief > 50% a t = 45 min suggests that these patients could respond in the long-term to either oral ketamine or oral dextromethorphan treatment.[6,7,19]

Ketamine efficacy in fibromyalgia

We tested an infusion of short duration (30 min) and administered a relatively high dose of S-ketamine (0.5 mg/kg) in that period. We based our study design on earlier observations from our laboratory that short infusions may produce long-term pain relief, i.e., pain relief lasting longer than S-ketamine's pharmacokinetics

predicts. In CRPS-1 patients and small-fiber neuropathy patients (Niesters, unpublished observations) pain relief lasting > 24 h is observed after a short infusion with 0.5 mg/kg S-ketamine.[10] Similar observations are made in animal models of neuropathic pain.[9] Furthermore, patients with therapy-resistant depression display long-term relief of symptoms following a single 0.5 mg/kg infusion with racemic ketamine.[11] All of these effects are thought to arise from a cascade of molecular changes at spinal and supraspinal sites that was initiated by blockade of the NMDAR by ketamine.[5,9,10,14,16,21,22] Our lack of effect of ketamine treatment may indicate the need for continuous ketamine infusions to obtain long-term analgesic benefit in fibromyalgia patients. For example, Amr, Sigtermans et al. and Schwartzman et al. performed RCTs on the efficacy of long-term ketamine administration (4 -14 days) in neuropathic pain patients from spinal cord injury and CRPS-1 and observed long-term pain relief (weeks to months).[14,16,23,24] This suggests that long-term ketamine exposure to affected spinal and supraspinal areas is required to cause long-term analgesia (due to a "reboot" of the affected central nervous system).[25] Alternatively, it may be that sub-populations of fibromyalgia patients have symptoms potentially not mediated via sensitized NMDAR. Guedj et al. found distinct brain functional SPECT patterns in responders versus non-responders to subsequent ketamine treatment in fibromyalgia patients.[26,27] Furthermore, the change in the midbrain regional cerebral blood flow after ketamine was highly correlated with the reduction in VAS pain scores during treatment.[27] A possible heterogeneity of pathophysiological profiles in fibromyalgia patients may have influenced the outcome of our study.[28]

Critical review of the protocol

Taken our negative and opposing stand regarding the efficacy of ketamine in the treatment of fibromyalgia pain a critical review of our protocol is needed. We choose an S-ketamine dose of 0.5 mg/kg. Since the S(+)-enantiomer of ketamine is about twice as potent as racemic ketamine our dose compares to 1.0 mg/kg racemic ketamine.[8] This dose is 2.5 times higher than used in the studies of Sörensen et al. and Graven-Nielsen et al.[17,18] We expected that the use of this relatively high dose would result in a long-term analgesic effect, at least longer than observed by Sörensen et al. and Graven-Nielsen et al. Our current results contradict this and suggest that it is not the dose but the duration of infusion that is the critical factor in producing pain relief.

In contrast to Sörensen et al. and Graven-Nielsen et al., we compared our treatment to an active placebo, the benzodiazepine midazolam.[17,18] It may well be that compared to an inactive placebo (such as normal saline) our results would have been more favorable towards an analgesic effect of S-ketamine. We choose this active placebo to induce a similar level of sedation and a similar occurrence of

psychotomimetic side effects during treatment. We did succeed in that during the 30-min infusion period, most subjects, irrespective of treatment, were sedated and unable to retain their consciousness for a sufficient extent of time to respond to questions. Following treatment side effects were mild to moderate in both study groups (see for an example of side effects drug high and drowsy Figure 5) and declined rapidly. Some (5 out of 13 tested) psychotomimetic side effects were greater in patients receiving S-ketamine at time point t = 45 min only. The occurrence of sedation during treatment together with the use of an active control adequately masked treatment allocation during the measurement period in our study. Consequently, the absence of a treatment difference is unrelated to a possible difference in side effect profile between the two treatment drugs. Furthermore the patients in the ketamine group were adequately blinded as just 58% of patients were correct in stating which treatment they received (Cohen's kappa = 0.16).

The active placebo that we used, the benzodiazepine midazolam, causes some muscle relaxation and anxiolysis. This may have affected the results of our study. Indeed pain relief from midazolam was observed at t = 45 and 180 min, however, the effects were small (a reduction by 1.5 cm only). This suggests just a small effect of muscle relaxation and anxiolysis on the study outcome. Still, the use of the active placebo may have reduced the contrast between treatments. We do argue, however, that by using midazolam we controlled for the sedation and other ketamine side effects without causing large effects on pain relief, as a priori intended.

Due to the occurrence of severe sedation during the 30-min S-ketamine and midazolam infusion, we were unable to obtain pain scores during treatment and during the initial part of the recovery period (i.e., t < 45 min). Because of this approach we may have missed a possible significant difference in pain scores between treatments. Indeed, Sörensen et al. and Graven-Nielsen et al. show large reductions in pain scores to values < 1 (on a scale from 0 to 10) at the end of their 30-min treatment period.[17,18] The lack of significant differences between ketamine and midazolam groups in the hours following treatment does indicate that our study was underpowered. A post hoc power analysis revealed that 30 patients per treatment group were required to observe a significant effect in the 3 hours following treatment.

In summary, we reject the hypothesis that a short-term infusion of relatively high-dose S-ketamine treatment produces long-term pain relief in fibromyalgia patients. Possibly, long-term analgesic effect is feasible with more prolonged or repetitive intravenous infusion regimens or daily treatment with oral ketamine or oral dextromethorphan.

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Chapter 5

Effect of rifampicin on S-ketamine and S-norketamine plasma concentrations in healthy volunteers after intravenous S-ketamine administration

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Introduction

Ketamine is an arylcycloalkylamine structurally related to phencyclidine and first synthesized in 1963.[1] At relatively high dose, ketamine produces anesthesia while at low, subanesthetic, dose it is a potent analgesic. Ketamine analgesia is thought to result from non-competitive antagonism at the ionotropic glutamatergic *N*-methyl-D-aspartate (NMDA) receptor.[2] NMDA receptors are excitatory and involved in enhanced nociceptive processing at spinal and supra-spinal sites.[3] By blocking the NMDA receptor, ketamine effectively reduces signal propagation in the pain circuitry from the spinal cord to the cortex and consequently produces analgesia. Ketamine-induced acute pain relief is driven by its pharmacokinetics, that is, upon termination of infusion (causing a brisk decrease in plasma concentration) acute pain relief ends rapidly[4,5], while in chronic pain patients the relief of spontaneous pain outlasts the treatment period by weeks to months.[6-9]

Ketamine undergoes extensive metabolism by hepatic cytochrome P450. In rat liver microsomes, norketamine, hydroxynorketamine, and hydroxyketamine account for 80%, 15% and 5%, respectively, of ketamine metabolism (Figure 1).[10] In vivo, rabbits convert 68% of ketamine to norketamine.[11] In humans, *N*-demethylation to norketamine is the major route of metabolism, which can undergo further metabolism via cyclohexanone ring hydroxylation to form 4-, 5,- and 6-hydroxynorketamine. Ketamine itself can be hydroxylated, forming 4-hydroxyketamine, although this is considered quantitatively insignificant.[10,12] Other minor metabolites have recently been reported.[13] Finally, norketamine and the hydroxy metabolic products are subsequently glucuronidated and eliminated via the kidney and bile.[14] Just 10-15% of ketamine is eliminated unchanged. Like ketamine, norketamine is a non-competitive antagonist at the NMDA receptor.[15,16] Non-human data indicate that norketamine passes the blood-brain barrier, has about one-fifth to one-third the potency of ketamine and is thought to contribute up to 30% of ketamine analgesia, and, albeit to a lesser extent, to the development of psychotomimetic side effects.[15,17,18] No pharmacological activity is attributed to the hydroxynorketamines.[17]

The major human hepatic cytochrome P450s catalyzing ketamine *N*-de-methylation in vitro are CYP2B6 and CYP3A4, although there is ambiguity as to their comparative contributions to clinical ketamine metabolism.[19,20] Rifampicin is an effective and non-selective inducer of multiple hepatic P450s, including those (CYPs 2B6 and 3A4) which catalyze ketamine *N*-demethylation.[21]

In the current study, we performed a compartmental pharmacokinetic analysis to quantify the effect of CYP induction on the elimination of S(+)-ketamine (S-ketamine) and formation and elimination of S(+)-norketamine (S-norketamine).

We took this approach (i.e., CYP induction) as norketamine is unavailable for testing in humans. Next, using a sigmoid E_{MAX} model with S-ketamine and S-norketamine contributions, we simulated the effect of rifampicin on analgesia to obtain an indication of the importance of variations in S-norketamine concentration on analgesic effect. We tested the effect of the S(+)-enantiomer of ketamine as it is the only registered ketamine product for human use in The Netherlands. S-ketamine has greater analgesic potency than either the R(-)-variant or the racemic mixture, with possibly fewer side effects than the racemic mixture.[16,22]

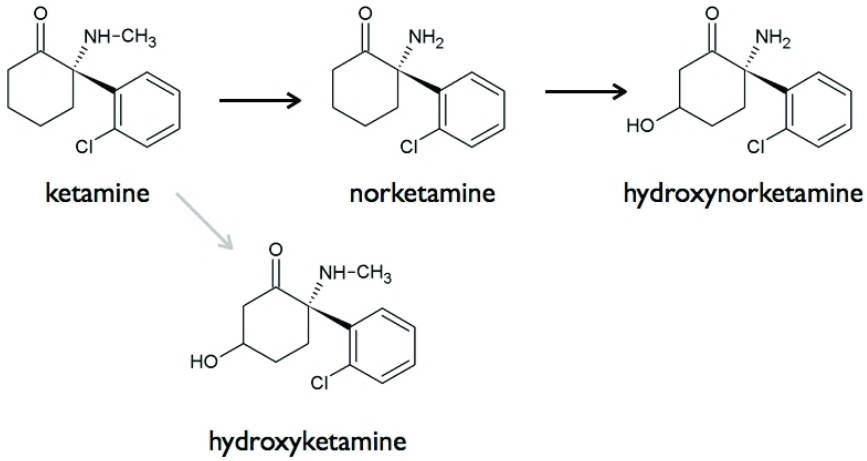

Figure 1 Metabolic pathways of ketamine. The black arrows indicate the major pathway; the grey arrow the minor pathway**.**

Materials and methods

Subjects

Twenty healthy male volunteers (age 19-29 years, body mass index < 30 kg/m^2) were recruited to participate in the study after approval of the protocol by the local Human Ethics Committee (Commissie Medische Ethiek, Leiden University Medical Center, Leiden, The Netherlands). Written informed consent was obtained prior to inclusion in the study according to the Declaration of Helsinki. All subjects were instructed not to eat or drink for at least 8 h before the study. Alcohol, coffee and chocolate were not allowed for 24 hours and grapefruit or grapefruit juice was not allowed 6 days prior to the study day. The study was registered at www.trialregister.nl under number NTR1328.

Study design: S-ketamine infusion and blood sampling

The study design was randomized single-blind, placebo-controlled and crossover. Subjects were treated with daily oral rifampicin 600 mg (Sandoz BV, Almere, The Netherlands) (Session I) or placebo (Session II; cellulose tablets produced by the local pharmacy) in the five days preceding the experimental testing (five 600 mg doses were given). Rifampicin/placebo was taken at bedtime. The time interval between the last dose and the ketamine administration was 10-12 hours. The order of the sessions was random with at least 3 weeks between sessions. On the study day the subjects received an arterial line in the radial artery of the non-dominant hand for blood sampling and a venous line in the contra-lateral arm for drug infusion. Total study duration was 5 h, of which S-ketamine (Ketanest S, Pfizer BV, Capelle aan de IJssel, The Netherlands) was administered during the first 2 h. The subjects were randomly allocated to receive a 20 mg/70 kg/h or 40 mg/70 kg/h S-ketamine infusion. The ketamine infusions were similar for Sessions I and II. Randomization was done by the pharmacy using computer-generated randomization lists.

Arterial blood sampling was performed at times t = 0 (pre-drug baseline), 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 125, 130, 135, 150, 180, 210, 240, 270 and 300 minutes after the start of the infusion. Five ml of blood was collected per sample. The analysis has been described previously.[4] In brief: the samples were centrifuged at a speed of 3000 rpm for 10 minutes. Two to three ml plasma was separated within 15 min of blood collection and stored at -25 ^{o}C until analysis. For the construction of S-ketamine and S-norketamine calibration lines, solid substances were obtained from Parke-Davis (Dallas, TX, USA) and Tocris (St. Louis, MO, USA), respectively. After extraction from the specific sample, S(+)-ketamine and S(+)-norketamine concentrations were determined by HPLC on a Gemini C18 column (Phenomenex, Utrecht, The Netherlands) at 40 ^{o}C. Monitoring of the eluent was performed at 195 nm with a photodiode-array-detector (PDA 100, Dionex, Amsterdam, The Netherlands). The lower limit of quantitation was 10 ng/ml, the lower limit of detection was 3 ng/ml for both drugs. All samples with concentrations > 3 ng/ml were included in the analysis. None of the samples had ketamine concentrations below 3 ng/ml while the initial 2 norketamine samples of all subjects were below the detection limit.

Pharmacokinetic analysis

A t-test was performed to compare the maximum plasma concentration of S-ketamine and S-norketamine (C_{MAX}), their time of occurrence (T_{MAX}), and the area-under-the-plasma concentration-curve (AUC) divided by the duration of the experiment ($AUC_{0 \rightarrow 300}$) of the placebo versus ketamine treatment. The analysis was performed in SPSS for Windows version 16.0 (SPSS Inc. Chicago, IL, USA). P-values < 0.05 were considered significant. Values reported are mean ± SEM.

The compartmental model used for pharmacokinetic analysis was identical to a previously published model.[4] The analysis was performed in two steps. Initially, only the ketamine data were analyzed. Subsequently, the combined ketamine norketamine data were analyzed. The model consisted of three ketamine compartments connected to two norketamine compartments (Figure 2). Since we had found that norketamine's formation and elimination (and inter-compartmental distribution) rates were not simultaneously estimable[4], the norketamine formation rate was set equal to the ketamine elimination rate. Volumes were scaled via WT/70 and clearances via $(WT/70)^{0.75}$, where WT is body weight in kg.[23] In order to test for a significant rifampicin effect on clearances, we introduced factor F as follows:

$$\text{Parameter}_{\text{RIFAMPICIN}} = \text{Parameter}_{\text{PLACEBO}} \cdot (1 + F)$$

For example, an F value of 2 indicates that the parameter increased by 200% after rifampicin treatment. Significance of factors was tested using forward selection. Since experiments were performed on two separate days we estimated both inter-individual (ω^2) and inter-occasion (ν^2) variances. Exponential error models were used for inter- and intraindividual variability.

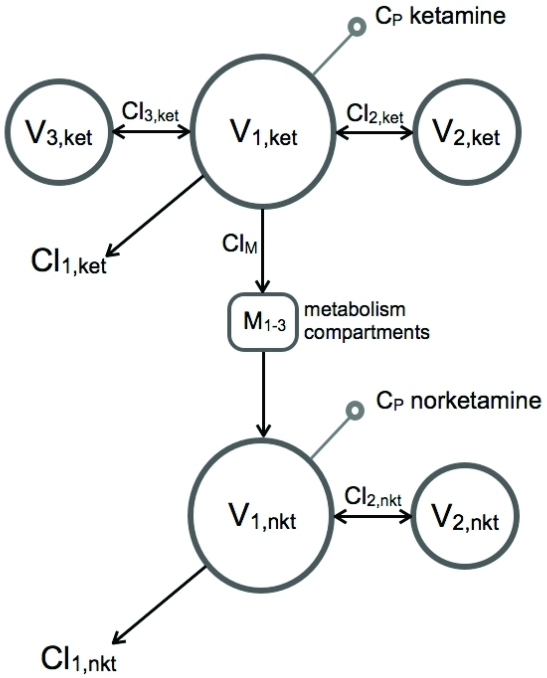

Figure 2 Schematic representation of the pharmacokinetic model used to analyze the combined S-ketamine and S-norketamine data. $V_{1,ket}$,$V_{2,ket}$ and $V_{3,ket}$ are the three ketamine (ket) compartments, $V_{1,nkt}$ and $V_{2,nkt}$ the two norketamine (nket) compartments. M_{1-3} represents three sequential metabolism compartments. $Cl_{1,ket}$ is the clearance from compartment $V_{1,ket}$, $Cl_{2,ket}$ the clearance from compartment $V_{2,ket}$, $Cl_{3,ket}$ the clearance from compartment $V_{3,ket}$, $Cl_{1,nkt}$ the clearance from compartment $V_{1,nkt}$ and $Cl_{2,nkt}$ is the clearance from compartment $V_{2,nkt}$. Cl_M is the S-ketamine clearance responsible for the S-norketamine formation.

The compartmental analysis was performed with the statistical package NONMEM VII with first-order conditional estimation with interaction method (ICON Development Solutions, Ellicott City, MD, USA).[24] Data presented are median and 95% confidence intervals. A bootstrap analysis was used to check the final model and to obtain 95% confidence intervals for the model parameters (from 1000 successful runs).

Side effects

Drug high and sedation were scored using an 11-point numerical rating scale ranging from 0 (= no effect) to 10 (= maximum possible effect) at regular intervals prior, during and following ketamine infusion.

Simulation study

Simulation studies enable the systematic exploration of inferences made in our study with respect to anticipated norketamine effect size. Here we performed simulations to estimate the effect of the large change in norketamine concentration and relatively modest change in ketamine concentration that we observed after rifampicin treatment on pain relief. Two sets of simulations were performed, one set on pain relief induced by short-term ketamine infusion and another set on pain relief induced by chronic ketamine administration. To that end we made a priori assumptions with respect to the norketamine contributions to ketamine effect: simulations with 0, 10 and 25% norketamine contribution were made. The difference in effect observed in the simulated pain relief with and without rifampicin treatment will give an indication of the norketamine contribution to effect.

Acute antinociception paradigm

Using the pharmacokinetic model parameters the effect of rifampicin treatment on acute antinociception was simulated for different norketamine contributions to effect. To that end, analgesic effect during and following a 2 hour S-ketamine infusion of 40 mg/h was simulated using the current pharmacokinetic data set linked to pharmacodynamic data previously obtained and modeled in a similar subject population in our laboratory.[4] Analgesia was simulated using a sigmoid E_{MAX} model assuming an additive effect of S-ketamine and S-norketamine as follows:

$$VAS(t) = \frac{BLN}{1 + \left(\frac{C_{ket}(t)}{C_{50,ket}} + \frac{C_{nkt}(t)}{C_{50,nkt}} \right)^{\gamma}}$$

where VAS is visual analogue score (ranging from 0 cm = no pain to 10 cm = severe pain), BLN is baseline (or predrug) VAS, γ a shape parameter, C_{ket} and C_{nkt} the plasma concentrations of S-ketamine and S-norketamine, respectively; $C_{50,ket}$ the plasma concentration S-ketamine causing 50% effect, $C_{50,nkt}$ the concentration S-norketamine causing 50% effect. The following model parameters where used (reference 4: BLN = 6.7 cm, γ = 2.5, and $C_{50,ket}$ = 375 ng/ml. The $C_{50,nkt}$ was varied in such a way that it contributed 0, 10 and 25% to total analgesic effect. We assumed no delay between blood concentration and acute antinociceptive effect (i.e., pain relief in response to an experimental heat pain stimulus) for both S-ketamine and S-norketamine.[4,5]

Chronic analgesia paradigm

Using pharmacokinetic model parameters (from references 6 and 7 on a study on the effect of ketamine on spontaneous chronic pain relief in complex regional pain syndrome type 1 patients), the effect of rifampicin treatment on chronic analgesia was simulated for different norketamine contributions to effect (0, 10 and 25%). To that end analgesic effect during and following a 4-day S-ketamine infusion of 5 mg/h on day 1, 10 mg/h on day 2, 15 mg/h on day 3 and 20 mg/h on day 4 was simulated.[6,7] Before the simulations were performed the pharmacokinetic data obtained in the healthy volunteer population and chronic pain patients were compared. The differences were sufficiently small (data not shown) to allow the application of a rifampicin effect as observed in our current study to the kinetic data obtained in chronic pain patients. The pharmacodynamic model was as given above with model parameters: BLN = 7 cm, γ = 1.9, and $C_{50,ket}$ = 10 ng/ml. The $C_{50,nkt}$ was varied in such a way that it contributed 0, 10 and 25% to total analgesic effect. We previously observed that in our group of chronic pain patients, the effect of ketamine on spontaneous pain relief lasted beyond the duration of treatment.[6,7] In a subgroup of these patients (i.e., responders to therapy) we estimated an effect half-life ($t_{½k}$) of about 11 days and used this value in the chronic pain simulations.[7]

Results

Subjects

All subjects completed the protocol without unexpected side effects. One subject (in the 20 mg/h ketamine group) failed to return, for unknown reasons, for his second experimental session. The subjects' ages ranged from 20 to 29 years (mean age 22.0 years), their body mass index (BMI) from 19 to 28 (mean BMI 22.2). There were no differences in age or BMI between treatment groups.

Descriptive pharmacokinetic analysis

In Figure 3 the mean S-ketamine and S-norketamine concentrations in plasma after rifampicin and placebo are plotted. Rifampicin decreased S-ketamine concentrations albeit the effects were modest in magnitude. The effect of rifampicin was most prominent in the lower S-ketamine infusion group: during 20 mg/h infusion rifampicin caused a 17% decrease in C_{MAX} ($P < 0.001$) and a 14% decrease in $AUC_{0 \rightarrow 300}$ ($P < 0.001$). At double the ketamine infusion dose rifampicin decreased C_{MAX} by 6% (ns) and $AUC_{0 \rightarrow 300}$ by 7% ($P = 0.02$; Table 1). S-ketamine T_{MAX} values remained unaffected by rifampicin (Table 1). In contrast, the effects of rifampicin on S-ketamine's metabolite S-norketamine concentrations in plasma were large with a 44% and 58% decrease in C_{MAX} for the infusion of 20 mg/h ($P = 0.001$) and 40 mg/h ($P < 0.001$), and a 53% and 62% decrease in $AUC_{0 \rightarrow 300}$ for the 20 mg/h and 40 mg/h infusions ($P < 0.001$), respectively (Table 1, Figure 3). S-norketamine T_{MAX} values remained unaffected by rifampicin (Table 1). The metabolite-to-parent drug $AUC_{0 \rightarrow 300}$ ratios (AUC_M/AUC_P in Table 1) were about 50% lowered by rifampicin compared to placebo irrespective of the S-ketamine infusion dose.

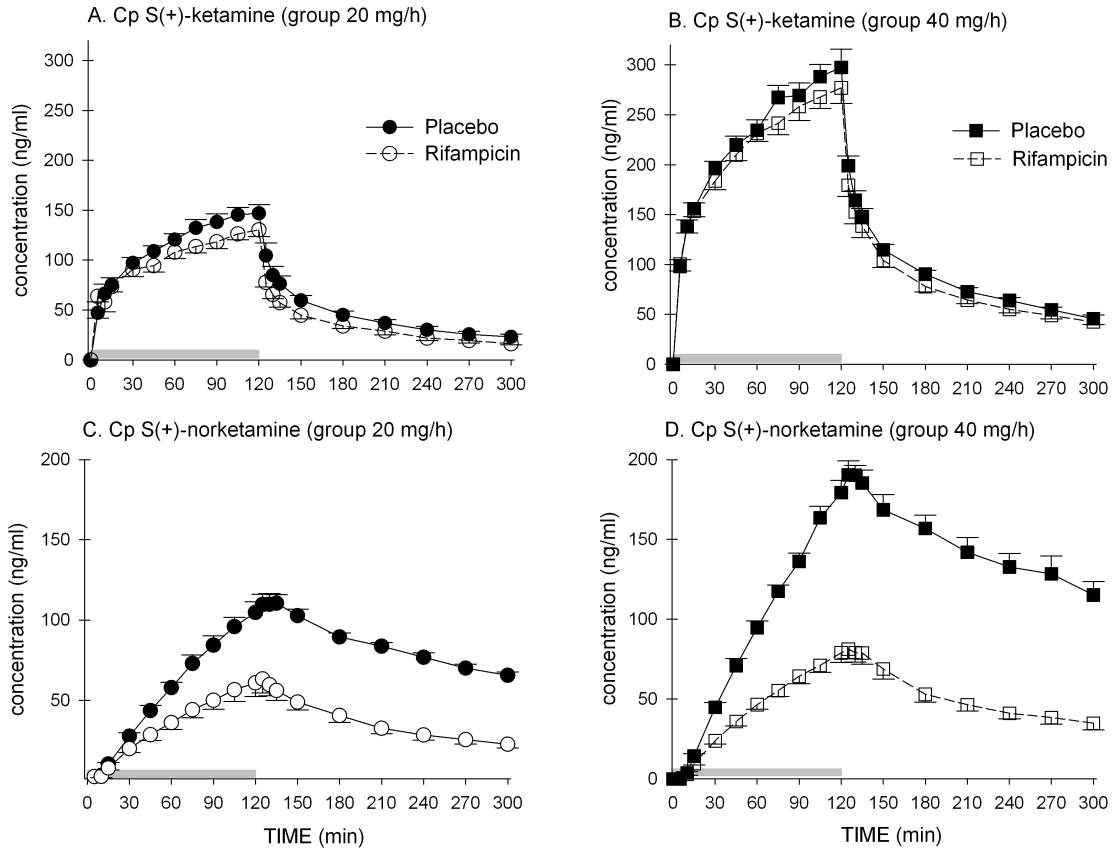

Figure 3 Effect of rifampicin treatment on plasma concentrations of S-ketamine (**A** and **B**) and S-norketamine (**C** and **D**) during and 3 h following a 2-h S(+)-ketamine infusion of 20 mg/h (**A** and **C**) and 40 mg/h (**B** and **D**). Values are mean ± SEM. The grey bars depict the 2-h infusion period.

Table 1 Effect of rifampicin on S-ketamine and S-norketamine concentrations: C_{MAX}, T_{MAX} and Area-Under-the-Curve (AUC) values.

	Placebo	Rifampicin	P value
$[S\text{-ketamine}]_{20}^{*}$			
C_{MAX} (ng/ml)	150.4 ± 3.9	132.6 ± 7.1	< 0.001
T_{MAX} (min)	114 ± 3	113 ± 4	0.72
$AUC_{0 \rightarrow 300}$ (ng/ml)	71.5 ± 5.4	60.8 ± 5.1	< 0.001
$[S\text{-norketamine}]_{20}^{*}$			
C_{MAX} (ng/ml)	113.8 ± 5.9	64.5 ± 8.7	0.001
T_{MAX} (min)	129 ± 2	125 ± 1	0.06
$AUC_{0 \rightarrow 300}$ (ng/ml)	73.3 ± 8.4	35.5 ± 11.7	< 0.001
AUC_M/AUC_P	1.03 ± 0.19	0.58 ± 0.19	< 0.001
$[S\text{-ketamine}]_{40}^{*}$			
C_{MAX} (ng/ml)	304.7 ± 17.5	285.6 ± 14	0.27
T_{MAX} (min)	110 ± 5	113 ± 3	0.51
$AUC_{0 \rightarrow 300}$ (ng/ml)	142.0 ± 15.8	132.0 ± 17.4	0.02
$[S\text{-norketamine}]_{40}^{*}$			
C_{MAX} (ng/ml)	198.3 ± 8.1	83.6 ± 6.2	< 0.001
T_{MAX} (min)	132 ± 2	128 ± 2	0.17
$AUC_{0 \rightarrow 300}$ (ng/ml)	124.8 ± 18.5	47.9 ± 11.6	< 0.001
AUC_M/AUC_P	0.90 ± 0.12	0.36 ± 0.10	< 0.001

Values are mean ± SEM.

* $_{20}$ and $_{40}$ denote data obtained during and after the 2-h infusion of 20 mg/h and 40 mg/h S-ketamine infusion (both per 70 kg), respectively.

AUC_M = metabolite $AUC_{0 \rightarrow 300}$ (S-norketamine); **$AUC_{0 \rightarrow 300}$** = the area-under-the-curve determined for the 300 min study period divided by 300 min; **AUC_P** = parent $AUC_{0 \rightarrow 300}$ (S-ketamine); **C_{MAX}** = peak concentration; **T_{MAX}** = time of occurrence of C_{MAX}.

Compartmental pharmacokinetic analysis

The pharmacokinetic model is given in Figure 2. In agreement with an earlier observation[4], extending the three compartmental model (in which just the ketamine data were modeled) with a part describing the norketamine data did not cause any change in S-ketamine pharmacokinetic parameter values (data not shown). This indicates that adding the metabolism compartments ($M_{1\text{-}3}$ in Figure 2) and the two norketamine compartments did not affect the analysis of ketamine. As judged by the eye, the model adequately described the data. Best, median and worst data fits as determined by the coefficient of determination (R^2) for the combined S-ketamine/S-norketamine data fits are given in Figure 4.

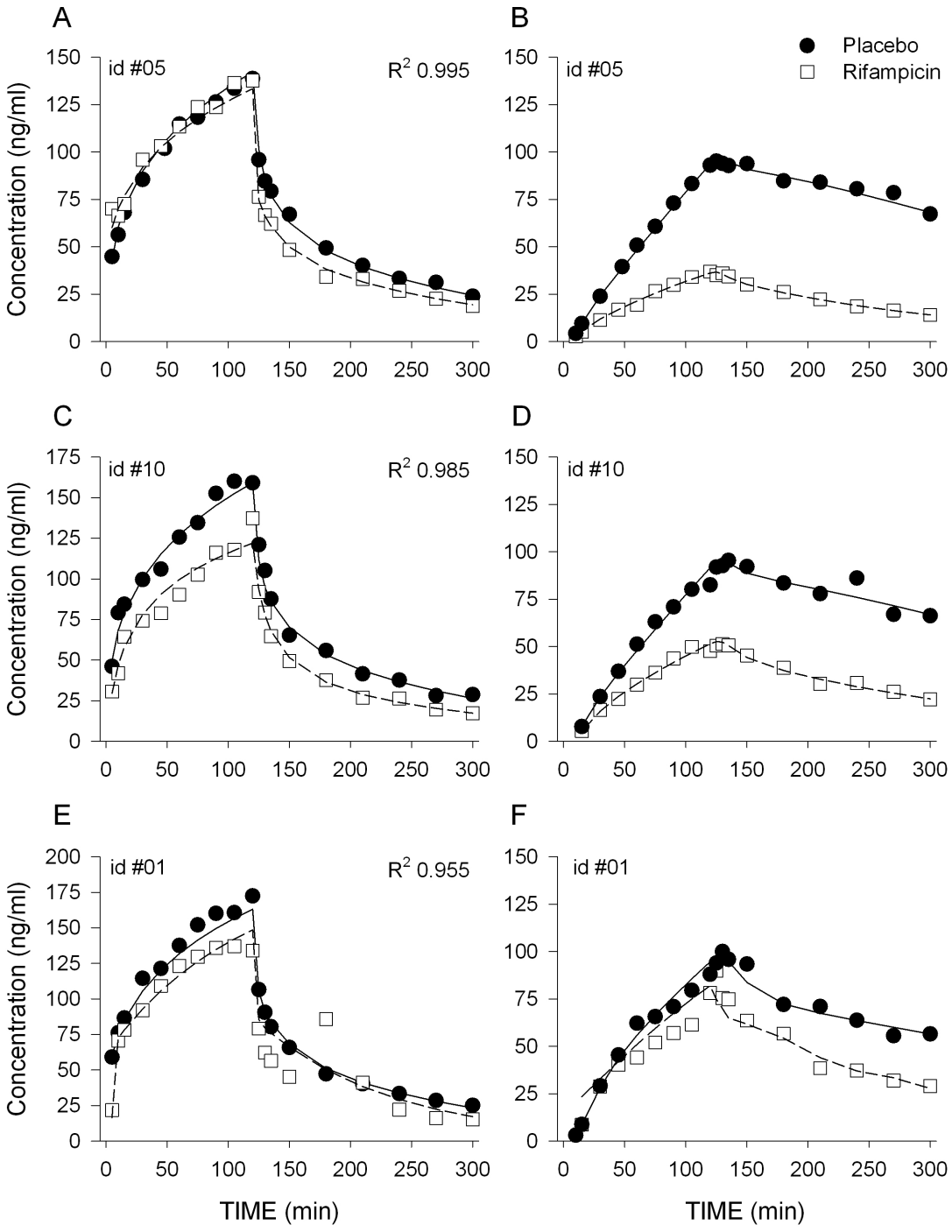

Figure 4 Pharmacokinetic data fits of the combined S-ketamine/S-norketamine model.
Best **A** S-ketamine and **B** S-norketamine fits of subject #05; median **C** S-ketamine and **D** S-norketamine data fits of subject #10; worst **E** S-ketamine and **F** S-norketamine data fits of subject #01. Closed circles and continuous lines are the placebo data; open squares and dashed lines are the rifampicin data. Goodness of fit was determined by the coefficient of determination (R^2).

Goodness of fit plots are given in Figure 5 (individual predicted versus measured concentration). Model parameter estimates together with their 95% confidence intervals are given in Table 2. The pharmacokinetic model parameters were in agreement with previous findings.[4,25-27] Rifampicin affected S-ketamine elimination (and consequently S-norketamine formation) modestly: clearance from the central compartment ($V_{1,ket}$) increased by 13% (F $Cl_{1,ket}$ = 0.13 in Table 2). In contrast, rifampicin had a much greater effect on S-norketamine elimination: S-norketamine clearance from its central compartment ($V_{1,nkt}$) increased by about 200% (F $Cl_{1,nkt}$ = 2.02 in Table 2).

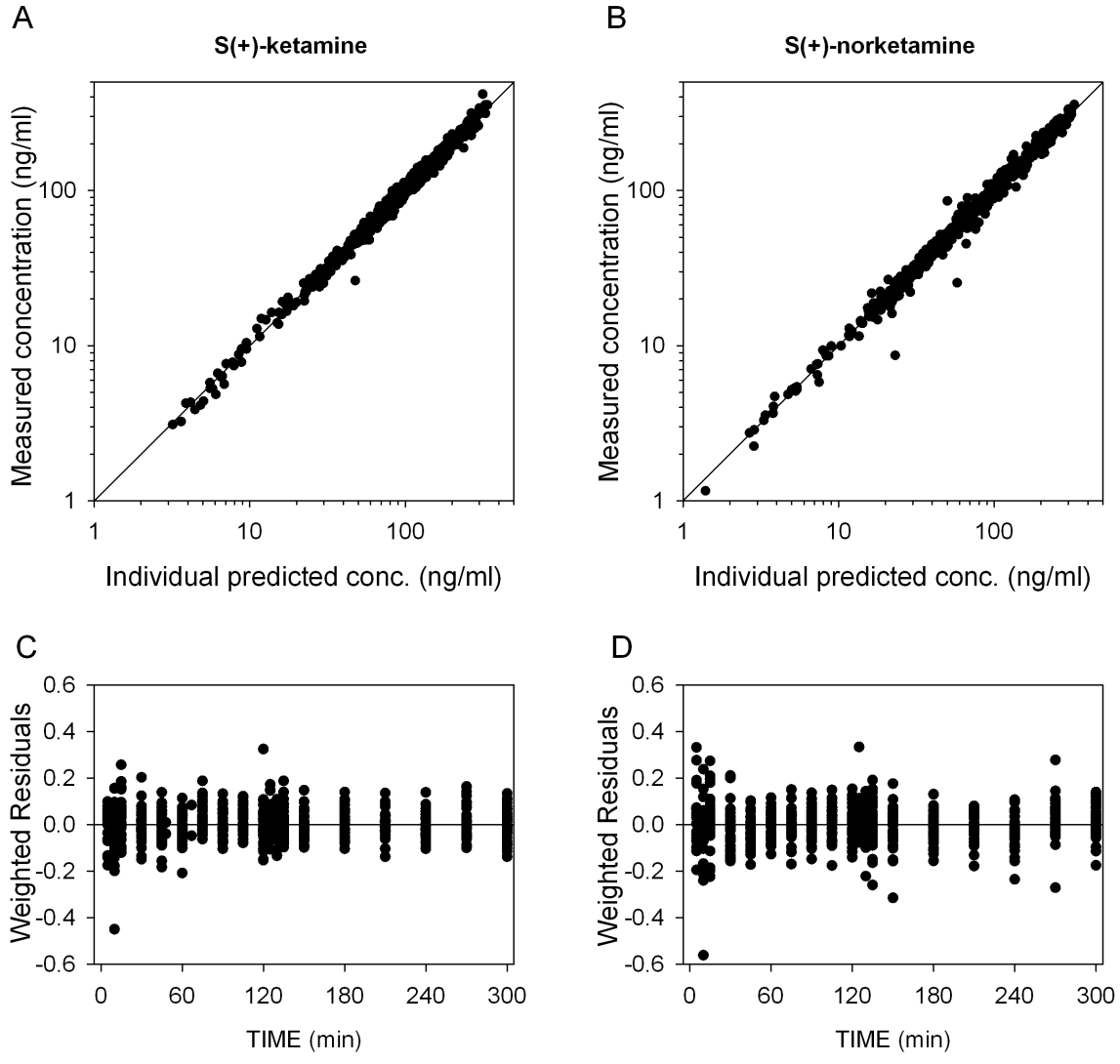

Figure 5 Goodness of fits plots: individual predicted PK data versus measured data for **A** S-ketamine and **B** S-norketamine. Weighted residuals, $(Y_{measured} - Y_{predicted})/Y_{predicted}$, versus time for **C** S-ketamine concentrations and **D** S-norketamine concentrations.

Table 2 Pharmacokinetic model parameters of the combined ketamine-norketamine model.

S(+)-ketamine	**Estimate**	**SEM**	**ω^2**	**SEM**	**ν^2**	**SEM**
$V_{1,ket}$ (L)	17.0	1.91	-	-	0.148	0.048
95% CI	13.6 - 21.0				0.06 - 0.25	
$V_{2,ket}$ (L)	28.3	2.97	-	-	-	-
95% CI	23.3 - 34.1					
$V_{3,ket}$ (L)	147	10.2	0.065	0.019	0.029	0.020
95% CI	129 - 172		0.03 - 0.10		0.00001 - 0.08	
$Cl_{1,ket}$ (L/h)	93.5	2.70	0.007	0.004	0.006	0.002
95% CI	87.6 - 98.8		0.001 - 0.02		0.002 - 0.01	
F $Cl_{1,ket}$[#]	0.13	0.03				
95% CI	0.07 - 0.20					
$Cl_{2,ket}$ (L/h)	127	12.3	-	-	-	-
95% CI	107 - 148		-		-	
$Cl_{3,ket}$ (L/h)	91.9	5.10	0.02	0.006	-	-
95% CI	82 - 101		0.01 - 0.03		-	
σ^2	0.008	0.002				
S(+)-norketamine	**Estimate**	**SEM**	**ω^2**	**SEM**	**ν^2**	**SEM**
MTT (h)	0.25	0.02	0.05*	0.03*	0.02	0.01
95% CI	0.21 - 0.28		0.01 - 0.14		0.004 - 0.05	
$V_{2,nkt}$ (L)	193	10.6	-	-	0.14	0.03
95% CI	173 - 217		-		0.09 - 0.19	
$Cl_{1,nkt}$ (L/h)	64.9	2.81	0.03	0.01	0.03	0.01
95% CI	58.5 - 70.8		0.01 - 0.06		0.01 - 0.05	
F $Cl_{1,nkt}$[#]	2.02	0.19				
95% CI	1.67 - 2.41					
$Cl_{2,nkt}$ (L/h)	334	30.5	0.05*	0.03*	0.14	0.04
95% CI	285 - 411		0.01 - 0.14		0.07 - 0.22	
σ^2	0.007	0.001				

Units of the volumes are L at 70 kg; units of the clearances are L/h at 70 kg. Factor F denotes the significant effect of rifampicin treatment on model parameters $Cl_{1,ket}$ and $Cl_{1,nkt}$ ($P < 0.01$).

[*] One parameter was sufficient for the inter-individual error of $Cl_{2,nket}$ and MTT.

[#] $P < 0.01$.

$Cl_{1,ket}$ = clearance from compartment 1,ket; **$Cl_{2,ket}$** = clearance from compartment 2,ket; **$Cl_{3,ket}$** = clearance from compartment 3,ket; **$Cl_{1,nket}$** = clearance from compartment 1,nket; **$Cl_{2,nket}$** = clearance from compartment 2,nket; **F** = factor F; **ket** = S-ketamine; **MTT** = mean transit time; **nket** = S-norketamine; **$V_{1,ket}$** = volume of compartment 1,ket; **$V_{2,ket}$** = volume of compartment 2,ket; **$V_{3,ket}$** = volume of compartment 3,ket; **$V_{2,nkt}$** = volume of compartment 2,nket; **ν^2** = interoccasion variability (in the log-domain); **ω^2** = intersubject variability (in the log-domain); **σ^2** = within-subject variability (in the log domain).

Side effects

Side effects were present during ketamine infusion and resolved promptly upon termination of infusion. The highest scores measured during ketamine infusion all occurred at the end of the infusion period and were for drug high: 6.5 ± 2.3 (placebo, low-dose ketamine) versus 5.5 ± 3.0 (rifampicin, low-dose ketamine; $P > 0.05$), 8.1 ± 2.0 (placebo, high-dose ketamine) versus 7.8 ± 2.6 (rifampicin, high-dose ketamine; $P > 0.05$); and for sedation 3.8 ± 3.6 (placebo, low-dose ketamine) versus 3.8 ± 2.6 (rifampicin, low-dose ketamine; $P > 0.05$), and 5.1 ± 3.6 (placebo, high-dose ketamine) versus 4.5 ± 3.8 (rifampicin, high-dose ketamine; $P > 0.05$).

Simulation study

Acute antinociception paradigm

The simulation study was based on the current pharmacokinetic model parameters (Table 2) linked to a pharmacodynamic model in which both S-ketamine and S-norketamine contribute to effect. The results of the simulations are shown in Figure 6. At increasing norketamine contributions to analgesic effect, peak VAS is reduced by a maximum of 0.7 cm at 25% contribution. At 0, 10 and 25% norketamine contribution, rifampicin treatment causes an increase in peak VAS of 0.3 cm (= 5% of peak VAS), 0.4 cm (10%) and 0.7 cm (21%), respectively (Figure 6).

Chronic analgesia paradigm

The study was based on the application of the rifampicin effect to pharmacokinetic and pharmacodynamic data obtained in chronic pain patients. Hence, the effect of S-ketamine infusion mimics the effect observed in chronic pain patients (Figure 7).[7] The effect of rifampicin was relatively small with maximum increases in VAS of 0.4 cm (0% norketamine contribution), 0.8 cm (10% contribution) and 1.3 cm (25% contribution, Figure 7). A biphasic effect of rifampicin on analgesia was observed with an initial peak during infusion (peak on day 2 of the infusion) and a second peak between days 20 and 30, irrespective of the magnitude of the norketamine contribution.

Discussion

Rifampicin caused a 10% reduction in S-ketamine plasma concentrations coupled to a 50% reduction in plasma concentrations of ketamine's metabolite S-norketamine. We relate these changes to the induction of S-ketamine and S-norketamine elimination. The compartmental pharmacokinetic analysis indicated a 13% increase in S-ketamine elimination, and a 200% increase in S-norketamine elimination.

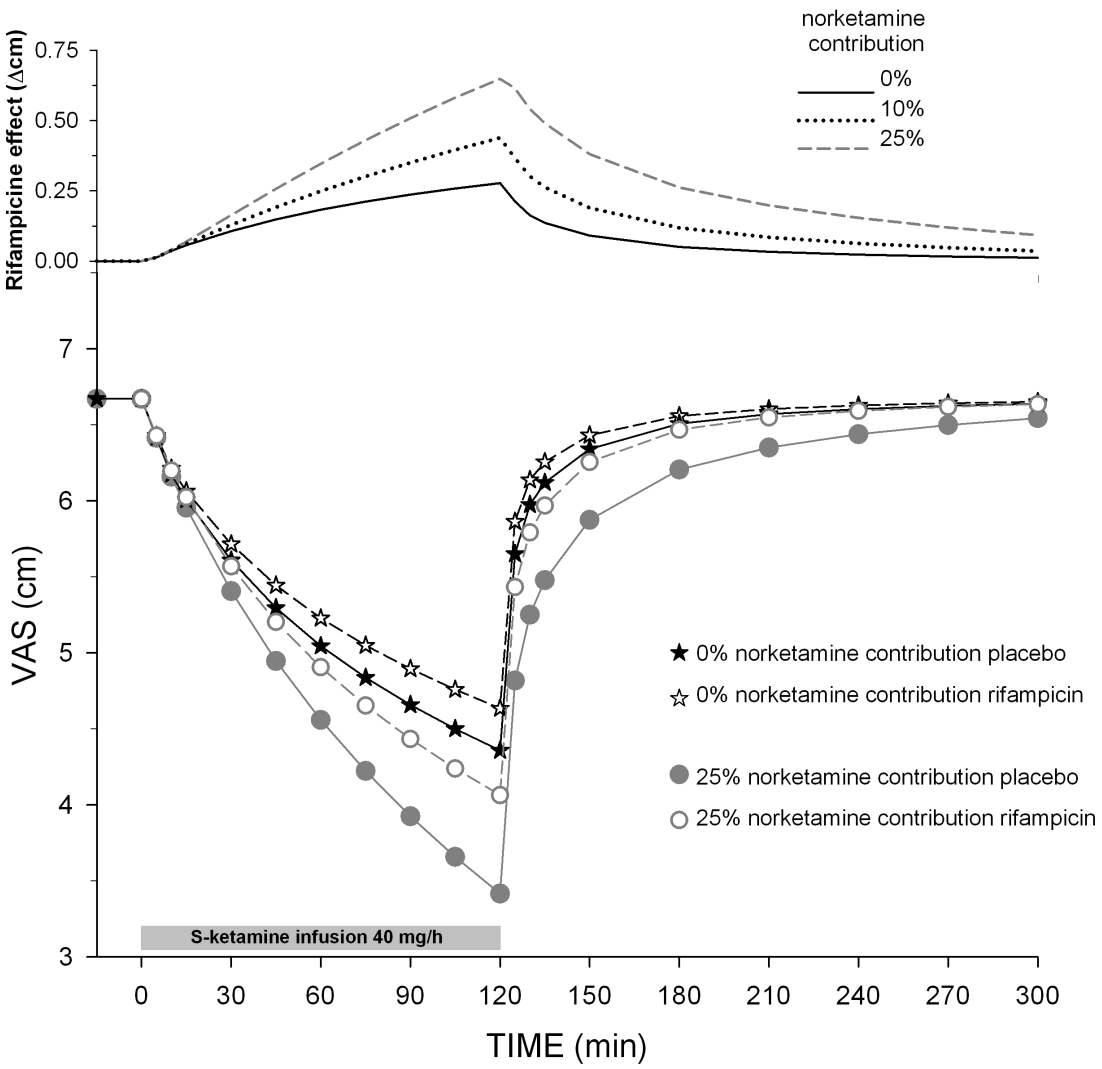

Figure 6 Simulation study for an acute antinociception paradigm. Simulation studies enable the systematic exploration of inferences made in our study with respect to anticipated norketamine effect size. We performed simulations to estimate the effect of the large change in norketamine concentration and relatively modest change in ketamine concentration that we observed after rifampicin treatment on acute pain relief induced by a 2-h 40 mg/h ketamine infusion (grey bar). We made a priori assumptions with respect to the norketamine contributions to ketamine effect: simulations with 0, 10 and 25% norketamine contribution were made. The difference in effect observed in the simulated pain relief with and without rifampicin treatment will give an indication of the norketamine contribution to effect. The top diagram shows the difference in VAS between placebo and rifampicin data for the three norketamine contribution to effect: 0, 10 and 25%. VAS = visual analogue score.

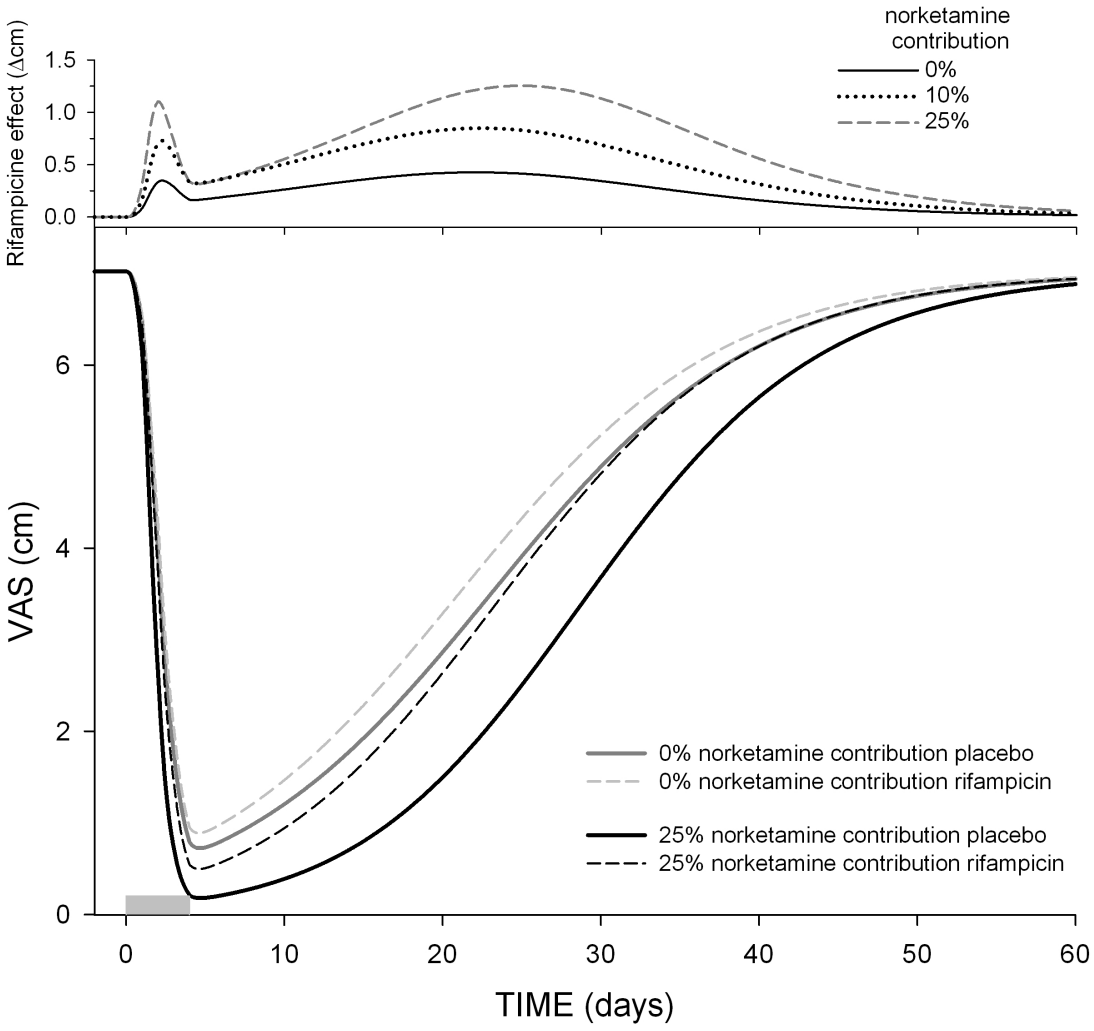

Figure 7 Simulation study for a chronic analgesia paradigm. We performed simulations to estimate the effect of the large change in norketamine concentration and relatively modest change in ketamine concentration that we observed after rifampicin treatment on chronic pain relief, induced by a 4-day intravenous ketamine infusion (grey bar: day 1 = 5 mg/h, day 2 = 10 mg/h, day 3 = 15 mg/h and day 4 = 20 mg/h). Simulations with 0, 10, and 25% norketamine contribution were made. The difference in effect observed in the simulated pain relief with and without rifampicin treatment will give an estimate of the norketamine contribution to effect. The top diagram shows the difference in VAS between placebo and rifampicin data for the three norketamine contribution to effect: 0, 10, and 25%. VAS is visual analogue score.

The significant reduction in norketamine plasma concentrations in rifampicin-treated subjects was an unexpected finding. Both norketamine C_{MAX} and ratio AUCnorketamine/AUCketamine were diminished. Several explanations, not necessarily mutually exclusive, are possible. The first is a diminished rate of norketamine formation. This seems unlikely. CYP2B6 and CYP3A4 are the major human hepatic cytochrome P450s catalyzing ketamine *N*-demethylation in vitro, although their relative contributions in vivo remain unknown.[19,20] At therapeutic

substrate concentrations, both the rate and intrinsic clearance of *N*-demethylation to norketamine by CYP2B6 were found to be more than 10-fold greater than by CYP3A4 in two different studies.[19,20] Based on this observation, using individual S(+)- and R(-)-ketamine enantiomers, Yanagihara et al.[19] concluded that CYP2B6 mainly mediates ketamine *N*-demethylation. In contrast, Hijazi et al.[20] concluded that CYP3A4 is the predominant CYP responsible for ketamine *N*-demethylation, which is based in part on the greater hepatic expression of CYP3A4 than CYP2B6. At clinical ketamine concentrations (< 5 μM) there is no evidence for other important CYPs involved. At supraclinical ketamine concentrations (50 μM) other CYP involvement has been described[28], although also then CYP2B6 and 3A4 are the enzymes with the highest activity. The clinical involvement of CYP3A4 was supported by the effect of the CYP3A4 inhibitor clarithromycin, which caused a 3.6-fold increase in S-ketamine C_{MAX} while norketamine C_{MAX} was reduced by 54%.[29] Regardless of the relative contributions of CYPs 2B6 and 3A4 to ketamine *N*-demethylation, since rifampicin (600 mg daily for 5 days) induces the hepatic expression and catalytic activity of CYP2B6 and CYP3A4 several-fold, as well as the clinical metabolism of numerous CYP2B6 and CYP3A4 substrates[21], induction of ketamine metabolism by rifampicin is expected. Patients treated with chronic barbiturates (another inducer of hepatic CYP enzymes) have lower plasma ketamine concentrations.[30] Together these findings do not suggest that rifampicin inhibits ketamine *N*-demethylation in vivo.

Another possible explanation for reduced norketamine concentrations is increased ketamine metabolism via an alternate pathway, and metabolic switching. Human liver microsomes form 4- and 5-hydroxyketamine.[10] Although ketamine hydroxylation is considered minor compared with *N*-demethylation, the enzymes responsible for ketamine hydroxylation and the effect of rifampicin induction are unknown.

A third potential explanation for rifampicin reduction of plasma norketamine concentrations is increased secondary metabolism of norketamine. The major secondary metabolite is 6-hydroxynorketamine, with lesser formation of 4- and 5-hydroxynorketamine. At clinical ketamine concentrations the enzymes responsible for norketamine hydroxylation and the effect of rifampicin induction are unknown. A recent study performed at supraclinical concentrations (50 μM norketamine) indicates involvement of CYP2B6 and 2A6.[28] Our compartmental analysis indicates that this possible mechanism is most likely. While norketamine formation may have increased by 13%, its elimination was increased by more than 200% (Table 2). Assuming that also at clinical concentrations norketamine metabolism is predominantly regulated via CYP enzymes 2B6 and 2A6, it is highly probable that rifampicin caused induction of these enzymes and consequently increased the elimination of S-norketamine and caused the 50% lower S-norketamine plasma concentrations that we observed (Figures 3 and 4).

Finally, another potential explanation is an effect of hepatic and/or renal drug transporters on ketamine and/or norketamine disposition. In addition to induction of several CYP isoforms, rifampicin is an effective inducer of several drug transporters, including the efflux transporters P-glycoprotein, breast cancer resistance protein, and multi-drug resistance proteins 1 and 2, and an inhibitor of the hepatic uptake transporter organic anion-transporting polypeptide.[21,31] Inhibition of hepatic ketamine uptake or induction of efflux might reduce intracellular concentrations and hence the extent of *N*-demethylation, despite an accelerated rate. No information is available on ketamine or norketamine and hepatic drug transport.

Previously we modeled the contribution of norketamine to ketamine effect in a study on the effect of a ketamine infusion on acute antinociception using an experimental heat pain model.[4] In that study we were unable to estimate a contribution of norketamine to ketamine antinociception, suggesting that, in humans, norketamine is not analgesic. However, since norketamine concentrations were not manipulated there was considerable uncertainty in the estimation of norketamine contribution to effect. In order to get an indication of the pharmacodynamic effect of the variations in S-norketamine concentration, we performed a simulation study with acute analgesia as end-point. Animal data show that S-norketamine is a non-competitive NMDA receptor antagonist in the spinal cord and cortex.[15] Its affinity to the NMDA receptor is weaker than that of S-ketamine, but there seems consensus that, at least in animals, norketamine does contribute significantly to the antinociceptive properties of ketamine (up to 30%), irrespective whether the S(+)-isomer or the racemic mixture is tested.[14,15,17,18]

Extrapolating the animal data to our simulations we assumed that S-norketamine contributed to overall analgesic effect in a range of 0 to 25%. Our simulation indicates that rifampicin caused a reduction in acute pain responses with a maximum in effect in VAS increase of less than 1 cm (Figure 6). Part of this effect is attributed to changes in S-ketamine concentration (7%) while the remainder is related to the changes in the concentration of S-norketamine (up to 16%). In actual practice, such an effect is small and may not be clinically detectable or important. This then suggests that the dose of ketamine for treatment of acute pain in patients on rifampicin needs little or no adjustment.

We infused S-ketamine for 2 hours and observed a ratio AUC_M/AUC_P of about 1 after placebo treatment (Table 1). Similar ratios are observed after long-term intravenous S-ketamine infusion in patients with chronic pain.[6] Our simulation study revealed only a relatively small effect of variations in norketamine concentration on analgesia in chronic pain patients (maximum increase in VAS = 1.3 cm, Figure 7). However, a prolonged exposure to norketamine may cause a greater passage of the drug into the central nervous system than occurred

during our short-term infusion and this may then increase its contribution to ketamine's pharmacodynamics.[18] We therefore may have underestimated the norketamine effect in our estimation of analgesia from S-ketamine in chronic pain patients. Also the route of administration may play a role in norketamine's contribution to effect. For example, after oral and sublingual ketamine administration the concentration S-norketamine exceeds that of S-ketamine due to the first pass-effect in the liver and enterocyte.[27] These higher concentrations may increase norketamine's bioavailability to the brain compartment with a corresponding increase in contribution to ketamine's analgesic effect. Further studies are needed to assess the effect of the induction of cytochrome P450 enzymes on norketamine's contribution to ketamine-induced analgesia.

The two simulations studies that we performed used different values for effect-half life (no delay for acute pain and a $t_{½k}$ of 11 days for chronic pain relief). The absence of a delay for acute pain is derived from various studies on the acute effects of ketamine (measuring ketamine-induced electroencephalographic changes, arousal and recall during anesthesia and acute pain relief)[4,5,32,33], and suggests a rapid passage of ketamine across the blood-brain-barrier. The equilibration half-life ($t_{½k}$) of 11 days used in the chronic study is not a blood-effect-site equilibration constant, but rather a disease modulatory factor. Various studies indicate that ketamine produces long-term analgesic effects beyond the treatment period with a half-life of many days.[5-9] We recently estimated a value for $t_{½k}$ of about 11 days in patients with chronic pain from complex regional pain syndrome type 1.[7] Interestingly, side effects in chronic pain patients treated with ketamine still show a rapid onset and offset suggesting that the drug rapidly crosses the blood-brain barrier and that the persistent analgesia observed is unrelated to the brain ketamine concentration.[5,6] We recently argued that ketamine initiates a cascade of events (of which NMDA receptor antagonism is a first step) resulting in persistent analgesia.[34]

In conclusion, using rifampicin as inducer of specific cytochrome P450 enzymes, we observed that rifampicin induces the elimination of S-ketamine's metabolite, S-norketamine, probably via induction of the CYP3A4, CYP2B6, and/or CYP2A6 enzymes.

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Chapter 6

Negative contribution of norketamine to ketamine-induced acute pain relief but not neurocognitive impairment in healthy volunteers

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Introduction

The *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine is used as anesthetic and at low-dose for the treatment of (acute and chronic) pain or combined with opioids in the treatment of perioperative and cancer pain.[1-3] Ketamine is rapidly metabolized into the NMDAR antagonist norketamine.[4] Animal data indicate that norketamine passes the blood-brain barrier, has about 20-30% the potency of ketamine and is thought to contribute significantly to ketamine (side) effects.[5-8] No human data are available on norketamine's contribution to ketamine effect. We previously showed that pretreating humans with rifampicin (an antibiotic that induces multiple hepatic cytochrome P450 enzymes, including CYP 2B6 and 3A4, involved in the ketamine *N*-demethylation into norketamine) caused a small (10%) reduction in S-ketamine concentration, but a large (50%) reduction in S-norketamine concentrations during and following a 2-h S-ketamine infusion (Figure 1).[9] Simulation studies were performed (as no pharmacodynamic measures were obtained), and by using data on norketamine's contribution to effect derived from animal studies, we predicted a 10-20 % contribution of norketamine to ketamine effect.

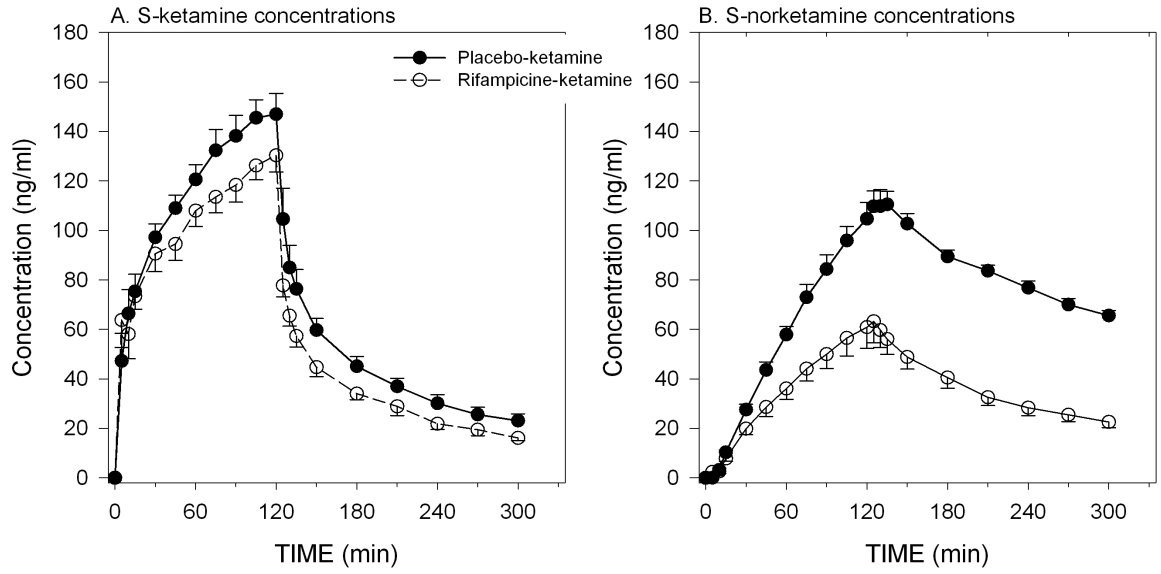

Figure 1 Effect of placebo (closed symbols) and rifampicin (open symbols; pretreatment 600 mg po per day for five days) on **A** S-ketamine and **B** S-norketamine concentrations, during and following a 2-h S-ketamine infusion (from t = 0 to 120 min; dose = 20 mg/h). Values are mean ± SEM. Data are from Noppers et al.[9]

In the current study we measured the effect of rifampicin pretreatment on pain responses and cognitive impairment during and following a 2-h ketamine infusion using a placebo controlled randomized cross-over and single blind

design. This design and the application of an additive ketamine-norketamine pharmacokinetic-pharmacodynamic (PK-PD) model allows the estimation of the norketamine versus ketamine contribution to changes in effect observed after infusion of just ketamine.

The main aims of this study were: (i) to assess the effect of low-dose ketamine on pain responses and cognition during and following a 2-h infusion; and (ii) to get an estimate of the contribution of norketamine to ketamine effect. We hypothesize that, in agreement with our previous simulation study, norketamine contributes 10-20 % to ketamine-induced effect. In order to assess the contribution of norketamine, we performed a population PK-PD analysis using the PK data from our previous study.[9]

Methods

After the protocol was approved by the local Human Ethics Committee and the Central Committee on Research involving Human Subjects, participants were recruited and informed consent was obtained according to the Declaration of Helsinki. The study was registered (www.trialregister.nl) under number NTR1328.

Participants

Twelve healthy male volunteers aged 18-37 were enrolled in the study. Participants were excluded from participation in the presence of one or more of the following criteria: body mass index > 30 kg/m^2; presence or history of major heart, lung, liver, kidney, neurological or psychiatric disease; history of chronic alcohol or illicit drug use; medication use, allergy to study medication; use of contact lenses during the study (to prevent damage by rifampicin) and color-blindness. All participants were subjected to a medical history and physical examination before participation. Participants had to refrain from food and drinks 8 hours prior to the start the study day. Alcohol, coffee and chocolate were not allowed for 24 hours and grapefruit or grapefruit juice was not allowed for 6 days prior to the study day.

Study design

This study had a randomized single-blind, placebo-controlled, crossover design. Participants were studied on three occasions, with at least three weeks between sessions (Figure 2). In the five days before study occasion 1, six subjects took rifampicin 600 mg tablets (Sandoz BV, Almere, The Netherlands) (1 tablet/day taken just before going to sleep), six others took placebo tablets (cellulose tablets

produced by the local pharmacy). On the study day all 12 subjects received a 2-h treatment with normal saline (NaCl 0.9%) (treatment R/PP). In the five days before study occasion 2, all 12 subjects took rifampicin 600 mg tablets (1 tablet/day, taken before going to sleep). On the study day all subjects received a 2-h treatment with S-ketamine (Ketanest S, Pfizer BV, Capelle aan de IJssel, The Netherlands) (treatment RK). Finally, in the five days before study occasion 3, all 12 subjects took placebo tablets (1 tablet/day, taken before going to sleep). On the study day all subjects received a 2-h treatment with S-ketamine (treatment PK). The S-ketamine intravenous infusion dose was 0.29 mg/kg/h (= 20 mg/h for a volunteer of 70 kg). The order of the three occasions was random. Randomization was performed upon inclusion of the subject by the local pharmacy that provided the blinded study material (rifampicin/placebo tablets and S-ketamine/saline infusion).

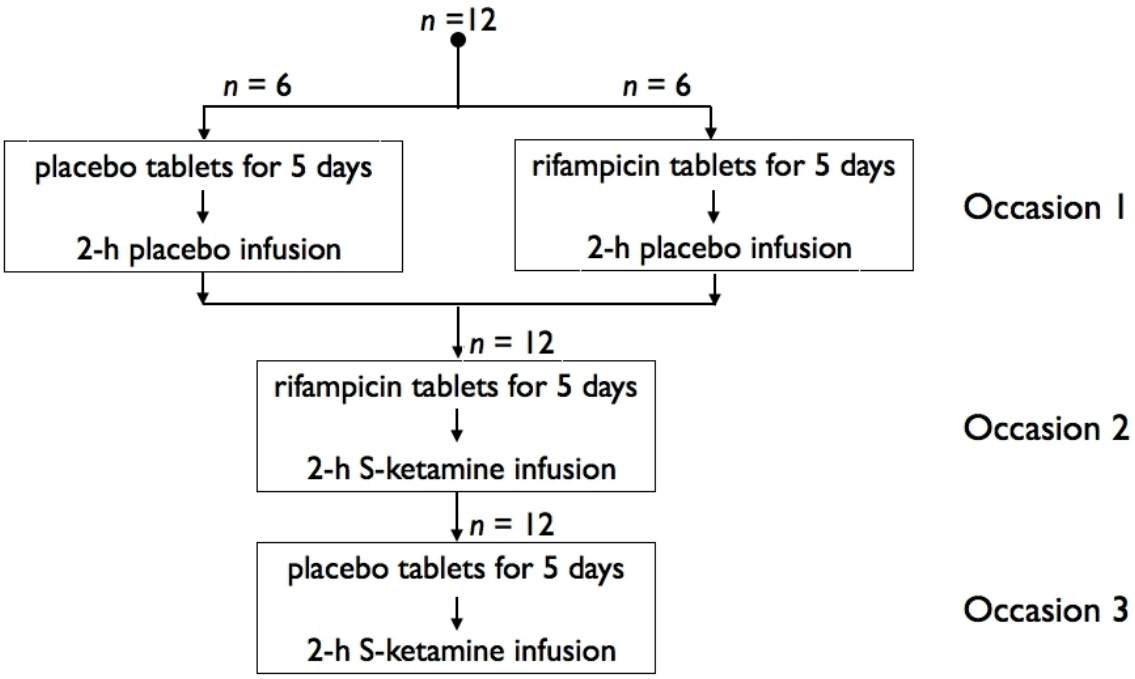

Figure 2 Flow chart of the study. The occasion sequence was random.

Prior to the first study occasion all subjects participated in two training sessions to get accustomed to the cognitive function tests. On the study day, baseline parameters were obtained (pain tests, cognitive function tests, and side effect score: drug high) before treatment. Next, during the 2-h treatment and 3 h following infusion, all tests and scores were performed at regular intervals, except for drug high which was determined at the end of the infusion.

Heat pain

The response to a noxious heat pain stimulus (scores for pain intensity ('strength' of the pain stimulus) and pain appreciation ('unpleasantness' of the pain stimulus)), was obtained. Heat pain was induced with the TSA-II NeuroSensory Analyzer (Medoc Ltd, Ramat Yishai, Israel). A 3 × 3 cm thermode was placed on the skin of the volar side of the forearm. The temperature was increased from 32 °C by 0.5 °C/s to 'peak temperature', after which the temperature was rapidly returned to 32 °C. After each stimulus the Visual Analogue Score (VAS) for pain intensity and pain appreciation was obtained using a 10 cm scale ranging from 0 (= no pain) to 10 (= most severe pain). 'Peak temperature' was determined for each subject individually during a test phase. 'Peak temperature' was varied from 46 to 52 °C at 1°C intervals. The lowest temperature that caused a VAS of 6 or greater was used in the study. Pain tests were performed at t = 0 (baseline), 5, 10, 15 min following the start of drug infusion and subsequently at 30-min intervals. In order to prevent sensitization of the skin, the thermode was repositioned after each stimulus.[10]

Cognition

Cognition was measured with a neurocognitive test battery (CNS Vital Signs LLC, Morrisville, NC, USA) and performed on a laptop computer.[11] The battery consisted of seven tests: 1 symbol digit coding; 2 Stroop test; 3 shifting attention test; 4 finger tapping; 5 continuous performance test; 6 verbal and visual memory test; 7 verbal and visual memory delay test. See for an explanation of the tests Appendix 1. All tests were in the Dutch language. The full battery (i.e., all 7 tests) was performed prior to drug infusion (baseline) and at t = 120 and 300 min following the start of infusion (the duration of the battery was approximately 30 min). At t = 30, 60, 90, 150, 180, 210, 240 and 270 min a short battery was performed that included symbol digit coding, Stroop test and shifting attention test. The full battery generates scores on 5 separate domains: memory, psychomotor speed, reaction time, complex attention and cognitive flexibility (see Appendix 1). The short battery generates scores on the domains: reaction time and cognitive flexibility. Data analysis was performed on the domain scores.

Domain scores are reported as standard scores (*z*-scores standardized to a mean of 100 and a standard deviation of 15).[11] The average of the *z*-scores for the five domains generates a summary score, the NeuroCognition Index (NCI), which is reported as a standard score as well. The NCI is similar to an IQ score, and is generated by averaging the *z*-scores of different subtests, (an NCI score of 100 is at the 50th percentile; 80% of the population scores between 80 and 120, 90% between 75 and 125). The NCI score gives an indication of the impact of treatment on the cognitive functions altogether.

Side effects

Drug high was scored at the end of the infusion on a 10-point numerical rating scale from 0 = no effect to 10 = maximal effect. Only integers were allowed as scores.

Statistical analysis

Descriptive analysis

Prior to the group comparisons the placebo-placebo and rifampicin-placebo data were compared. Since no significant differences were present, these two groups were combined (R/PP) in the remainder of the analysis. The area-under-the-curve divided by the 300 min duration of the study ($AUC_{0 \rightarrow 300}$) of pain intensity and appreciation were calculated. These AUCs of the three treatments were compared with an analysis of variance (and post-hoc Bonferroni's test) or Kruskal-Wallis test (and post-hoc Dunnett's test). The NCI and the five cognition domains were analyzed with a repeated measures analysis of variance (factors: time and treatment) with post-hoc Bonferroni's test. Drug high scores at the end of infusion were compared with an analysis of variance (and post-hoc Bonferroni's test). Data analysis was performed with SPSS 16.0. P-values < 0.05 were considered significant. Data are presented as mean ± standard error of the mean (SEM) unless otherwise stated.

Pharmacokinetic-pharmacodynamic analysis

Since blood sampling has stimulatory effects that may interfere with the measurement of pain, cognition, and side effects, we decided to perform this study without blood sampling. To be able to perform a PK-PD analysis, we assumed that S-ketamine and S-norketamine concentrations under these conditions are well described by earlier established pharmacokinetic models. The pharmacokinetic model that we used has three compartments for S-ketamine and two for S-norketamine linked by three metabolism compartments.[4,9]

To eliminate a possible hysteresis between plasma concentration and effect, an effect compartment was postulated that equilibrates with the plasma compartment with a half-life $t_{½}k_{e0}$ (i.e., the blood-effect-site equilibration half-life). A similar value of $t_{½}k_{e0}$ was assumed for S-ketamine and S-norketamine.

To estimate the contribution of S-norketamine on S-ketamine-induced changes in pain responses, cognition (reaction time and cognitive flexibility) and side effects (drug high) the following linear model was fitted to the data:

$$Y_E(t) = Y_0 + F_K \cdot C_{E,K}(t) + F_N \cdot C_{E,N}(t)$$

where $Y_E(t)$ = the effect at time t, Y_0 = predrug baseline effect, F_K the ketamine contribution to effect, $C_{E,K}(t)$ = the ketamine effect-site concentration at time t, F_N = the norketamine contribution to effect and $C_{E,N}(t)$ = the norketamine effect-site concentration at time t. F_N is parameterized as fraction of F_K, as follows: $F_N = F_{N^*} \cdot F_K$. For example, when F_K = 0.2 and $C_{E,K}$ = 100, the ketamine contribution to effect = 20%. When F_{N^*} = 1 the value of F_N = 1 × 0.2 = 0.2 indicating that norketamine contributes as much to the effect as ketamine (both cause a 20% change in effect).

The pharmacokinetic-pharmacodynamic data were analyzed with the statistical package NONMEM VII (ICON Development Solutions, Ellicott City, MD, USA).[12] Model parameters were assumed to be log-normally distributed. Residual error was assumed to be additive with variance σ^2. P-values less than 0.01 were considered significant.

Results

All subjects completed the protocol without major or unexpected side effects. The subjects' age, weight, height and body mass index averaged to 23 ± 5 years, 183 ± 6 cm, 75 ± 12 kg and 22 ± 3 kg/m^2, respectively (values are mean ± SD).

Descriptive analysis - comparison to placebo

The population averages are given in Figure 3. Based on the AUCs (Table 1), S-ketamine produced antinociception to a greater extent than placebo (R/PP). No differences in antinociception were observed between the PK and RK treatments. As determined from the measurement at the end of infusion, drug high was reduced in the subjects pretreated with rifampicin (RK) compared to those treated with placebo (PK; Table 1). S-ketamine produced cognitive impairment greater than placebo (R/PP) for all measures at t = 120 min (difference ranging between 17 and 24%, except for reaction time where the differences ranged from 5 to 12%) with no difference between treatment groups PK and RK. Most domains showed a decline over time, possibly caused by fatigue. An exception is psychomotor speed which showed an increase over time, which may be related to a learning effect. The results of the full battery are given in Table 2, the results of the short battery in Figure 3. These latter data were used in the PK-PD analysis.

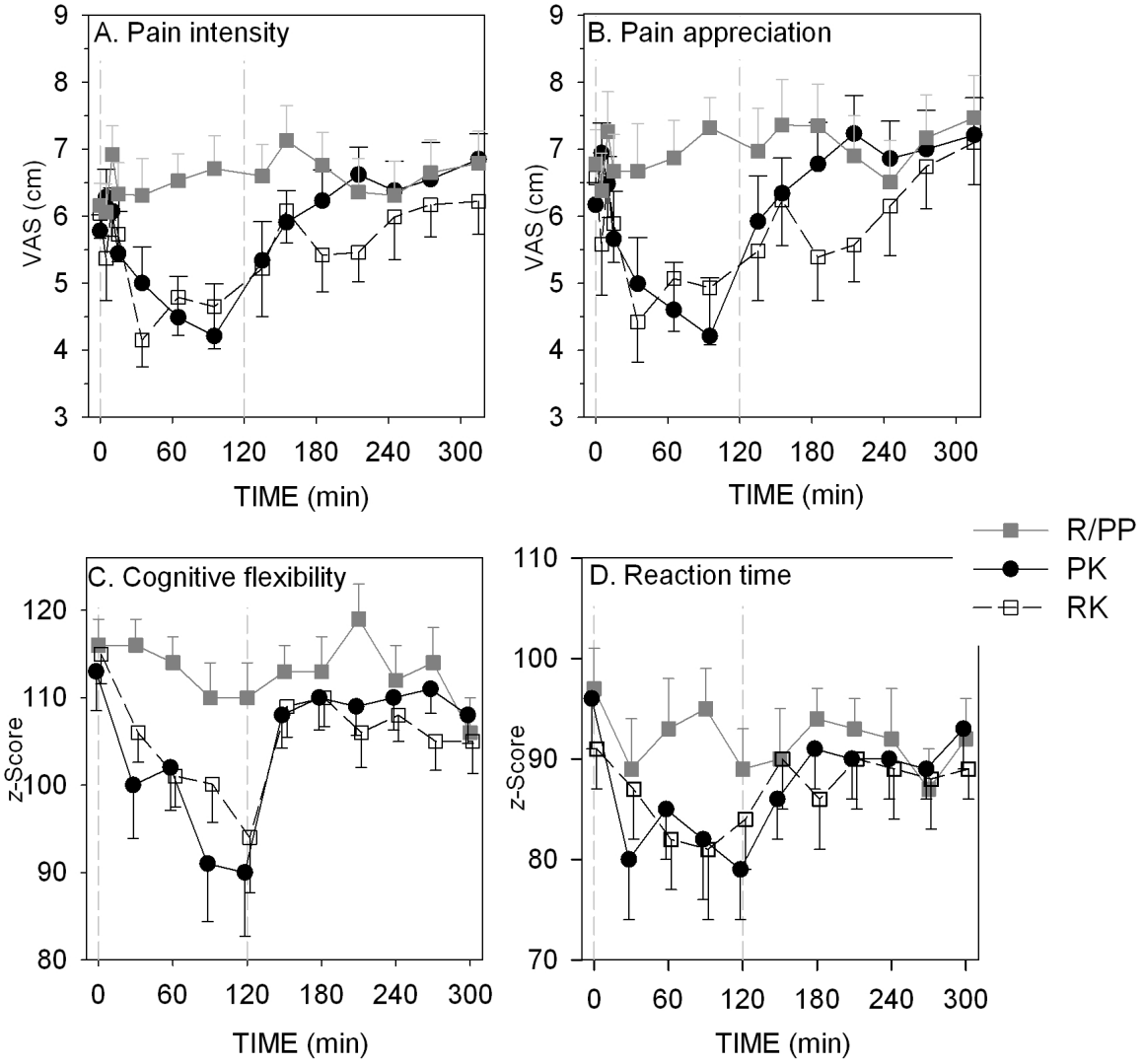

Figure 3 Average responses of the influence of rifampicin or placebo pretreatment on **A** pain intensity, **B** pain appreciation, **C** cognitive flexibility and **D** reaction time. The responses were measured during and 3 h following a 2-h S-ketamine infusion of 20 mg/h from t = 0 to t = 120 min. Grey squares are the placebo infusion data following a 5-day pretreatment with placebo or rifampicin (R/PP); black circles are the S-ketamine infusion data following a 5-day pretreatment with placebo; open squares are the S-ketamine infusion data following a 5-day pretreatment with rifampicin. Values are mean ± SEM.

Pharmacokinetic-pharmacodynamic analysis

An initial analysis was performed in which the S-norketamine contribution to S-ketamine effect was constrained to behave in a similar direction as S-ketamine (e.g., ketamine and norketamine are both analgesic and produce both drug high). This yielded no contribution of norketamine to effect in any of the tested end-points (i.e., $F_N = 0$). Since we observed that in some of the end-points the RK data following infusion remained below the PK data (e.g., pain intensity and pain

appreciation, Figure 3A and B), any constraint on F_N was removed, and F_N was allowed to have values causing an effect in the same as well opposite direction as S-ketamine.

Table 1 Descriptive analysis of the ketamine-induced pain relief and side effects (drug high).

	Rifampicin/ Placebo-Placebo	Rifampicin-Ketamine	Placebo-Ketamine
Pain intensity			
$AUC_{0 \rightarrow 300}$ (cm)	6.8 ± 0.4	5.7 ± 0.4 *	6.0 ± 0.4 *
Pain appreciation			
$AUC_{0 \rightarrow 300}$ (cm)	7.5 ± 0.6	6.0 ± 0.4 *	6.4 ± 0.5 *
Drug high			
Score at end of infusion	0 ± 0	5.2 ± 0.6 ‖ #	7.0 ± 0.4 ‖

Values are mean ± SEM.
* P < 0.05 versus Rifampicin/Placebo-Placebo; ‖ P < 0.001 versus Rifampicin/Placebo-Placebo;
# P = 0.01 versus Placebo-Ketamine.

Examples of best, median and worst data fits for effect of S-ketamine on pain intensity after placebo and rifampicin treatment are given in Figure 4. The population PD parameter estimates are given in Table 3. Goodness of fit plots for all end-points are given in Figure 5. Overall, the data were adequately described by the linear model. For pain intensity and pain appreciation the value of F_{N^*} indicates an effect of S-norketamine opposite to that of S-ketamine (i.e., an anti-analgesic effect of S-norketamine) (Table 3). For the cognitive end-points (cognitive flexibility and reaction time) no contribution of S-norketamine to effect could be estimated.

As an example we will further discuss pain intensity (Figure 6). For pain intensity the S-ketamine contribution F_K is -0.038 cm.(ng/ml)$^{-1}$. This indicates that at an effect-site S-ketamine concentration of 100 ng/ml, the effect due to just ketamine will be a 3.8 cm decrease in VAS. The S-norketamine contribution F_N is +0.03 (= $F_K \times F_{N^*}$ = -0.038) cm.(ng/ml)$^{-1}$ × -0.824, which indicates that at a S-norketamine concentration of 50 ng/ml (assuming that this is the S-norketamine effect-site concentration that coincides with an effect site S-ketamine concentration of 100 ng/ml in short-term infusion paradigms), the contribution of just S-norketamine is +1.5 cm VAS increase resulting in a total VAS change of -2.3 cm (= -3.8 + 1.5 cm).

Table 2 Descriptive analysis of the neurocognitive data.

	Rifampicin/ Placebo-Placebo	Rifampicin-Ketamine	Placebo-Ketamine
Neurocognitive Index [II]			
0 min	105.6 ± 1.9	104.6 ± 2.4	104.3 ± 3.0
120 min	99.7 ± 2.9	83.8 ± 3.3 [*]	77.6 ± 4.2 [*]
300 min	99.6 ± 2.6 [#]	98.1 ± 2.7[#]	101.0 ± 2.3 [#]
Memory [II]			
0 min	101.3 ± 5.2	106.6 ± 4.5	104.7 ± 5.0
120 min	88.9 ± 6.1	65.1 ± 4.7 [*]	55.5 ± 5.7 [*]
300 min	90.7 ± 5.3 [#]	93.8 ± 5.5 [#]	96.1 ± 4.1 [#]
Psychomotor speed [II]			
0 min	108.2 ± 5.2	107.8 ± 5.8	108.7 ± 4.8
120 min	112.6 ± 7.0	90.6 ± 3.9 [*]	86.8 ± 5.2 [*]
300 min	117.0 ± 5.8 [#]	114.8 ± 5.9 [#]	113.9 ± 5.2 [#]
Reaction time [II]			
0 min	97.4 ± 3.8	90.9 ± 4.4	95.9 ± 5.3
120 min	88.6 ± 3.6	83.8 ± 4.8 [*]	78.8 ± 4.8 [*]
300 min	91.5 ± 4.2	88.6 ± 2.6	93.1 ± 3.6
Complex attention [II]			
0 min	104.0 ± 4.4	102.9 ± 3.2	99.4 ± 4.3
120 min	97.2 ± 3.7	85.8 ± 5.4 [*]	77.3 ± 7.5 [*]
300 min	91.3 ± 2.9 [#]	88.2 ± 4.1 [#]	94.4 ± 3.5 [#]
Cognitive flexibility [III]			
0 min	116.3 ± 3.2	114.8 ± 3.3	112.6 ± 4.5
120 min	110.1 ± 4.0	94.2 ± 6.3 [§] [**]	89.5 ± 7.3 [§] [**]
300 min	106.4 ± 4.0 [**]	104.8 ± 3.7 [**]	107.6 ± 3.2 [**]

Values are mean ± SEM.
Significant main treatment, time and time[*] treatment effects at [II] $P < 0.001$ and [III] $P < 0.05$.
Post-hoc analysis: treatment: [*] $P < 0.01$ versus Rifampicin/Placebo-Placebo (at 120 min);
[§] $P < 0.05$ versus Rifampicin/Placebo-Placebo (at 120 min);
Post-hoc analysis: time: [*] $P < 0.01$ versus t = 0, [#] $P < 0.01$ versus t = 0, [**] $P < 0.05$ versus t = 0.

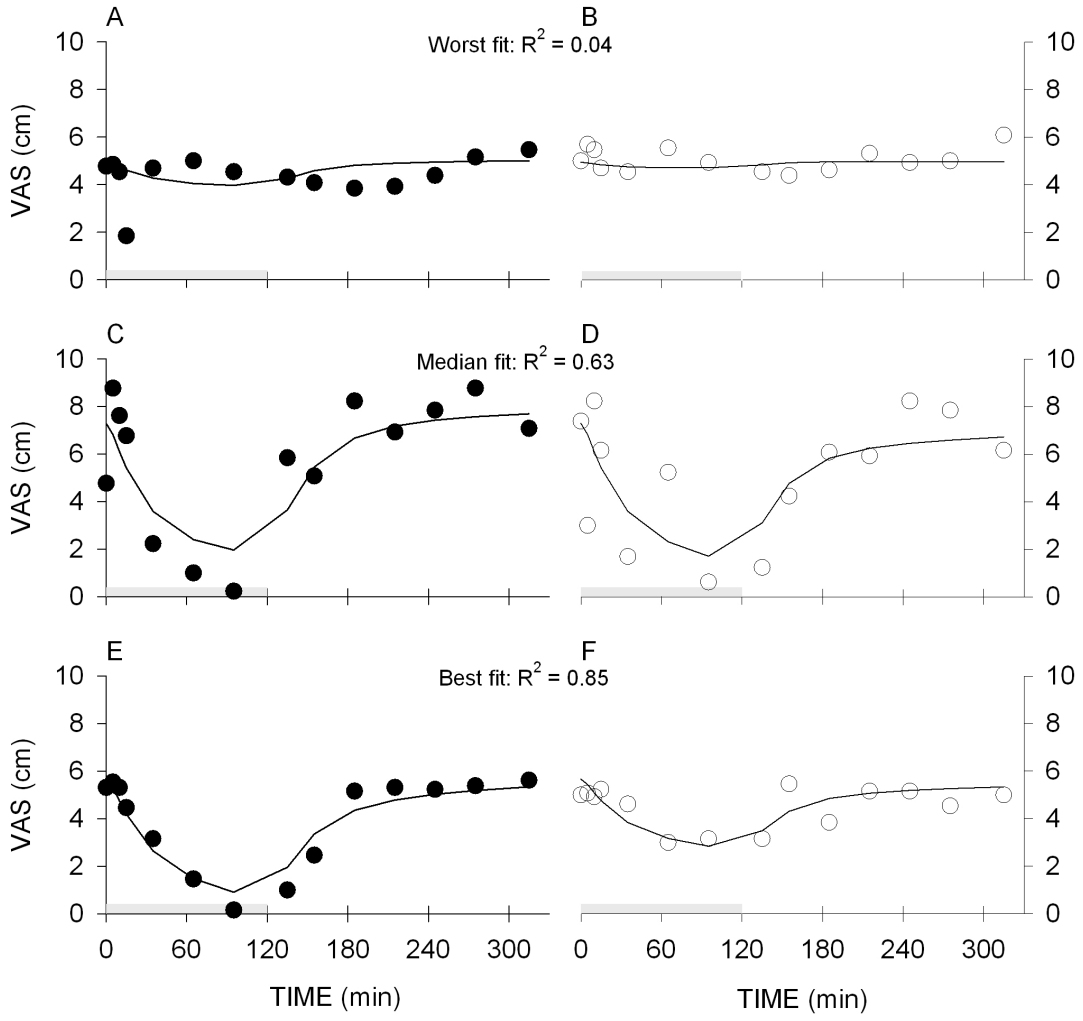

Figure 4 Examples of data fits from three subjects, showing worst (**A** and **B**), median (**C** and **D**) and best (**E** and **F**) data fits for the effect of S-ketamine on pain intensity following placebo (A, C and E) or rifampicin (B, D and F) pretreatment.

In Figure 6 the relative contributions of S-ketamine and S-norketamine to the changes in VAS score and their sum (the measured response) are simulated, using the model parameters of Table 3 for the two test conditions (placebo pretreatment, panels A and C; and rifampicin pretreatment, panels B and D). It shows the anti-analgesic effect of norketamine on the change in VAS (relative to S-ketamine's effect) with hyperalgesia following S-ketamine infusion when S-norketamine levels are high (panels A and C). When S-norketamine levels are relatively low (panels B and D) the negative effect on analgesia is less and no hyperalgesia is observed following the 2-h S-ketamine infusion.

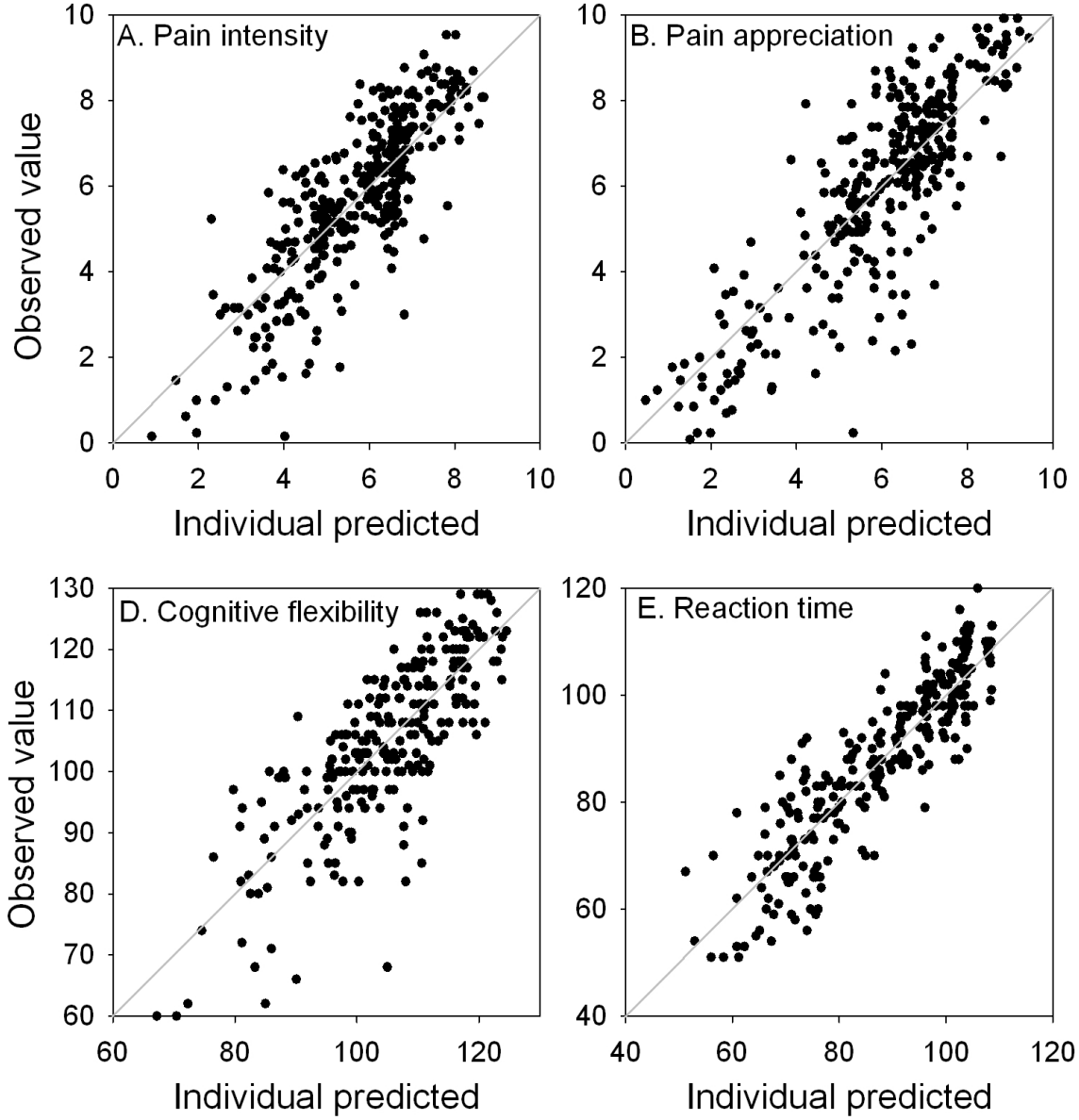

Figure 5 Goodness of fit plots for **A** pain intensity, **B** pain appreciation, **D** cognitive flexibility and **E** reaction time. Individual predicted values are plotted against the observed values. The grey lines are the lines of identity.

The blood-effect-site equilibration half-life ($t_{½}k_{e0}$) ranged from 0 min (cognitive flexibility) to 11.8 min (pain intensity). For cognitive flexibility no hysteresis between arterial plasma concentrations and effect was estimated, indicating that the effect instantaneously followed arterial plasma concentrations. The value of $t_{½}k_{e0}$ averaged across all end-points was 6.1 min.

Table 3 Pharmacodynamic model parameters.

	θ ± SEM	**ω^2 ± SEM**	**ν^2 ± SEM**
Pain intensity			
F_K (cm.(ng/ml)$^{-1}$)	$-3.80 \cdot 10^{-2} \pm 1.17 \cdot 10^{-2}$	$1.26 \cdot 10^{-2} \pm 4.94 \cdot 10^{-4}$	$2.0 \cdot 10^{-4} \pm 7.9 \cdot 10^{-5}$
F_{N^*}	-0.824 ± 0.266	$5.12 \cdot 10^{-4} \pm 4.02 \cdot 10^{-4}$	-
Y_0 (cm)	6.11 ± 0.38	1.46 ± 0.56	-
$t_{½}k_{e0}$ (min)	11.8 ± 0.2	-	-
ε	1.28 ± 0.26		
Pain appreciation			
F_K (cm.(ng/ml)$^{-1}$)	$-4.35 \cdot 10^{-2} \pm 1.20 \cdot 10^{-2}$	$1.30 \cdot 10^{-2} \pm 4.89 \cdot 10^{-4}$	$3.76 \cdot 10^{-4} \pm 1.48 \cdot 10^{-4}$
F_{N^*}	-0.785 ± 0.208	$4.95 \cdot 10^{-4} \pm 3.06 \cdot 10^{-4}$	-
Y_0 (cm)	6.55 ± 0.42	1.95 ± 1.20	-
$t_{½}k_{e0}$ (min)	10.0 ± 2.1	-	-
ε	1.71 ± 0.40		
Cognitive flexibility			
F_K (cm.(ng/ml)$^{-1}$)	$-0.245 \pm 5.67 \cdot 10^{-2}$	$3.12 \cdot 10^{-2} \pm 1.48 \cdot 10^{-2}$	$5.72 \cdot 10^{-3} \pm 2.66 \cdot 10^{-3}$
F_{N^*}	0.00 ± 0.00	-	-
Y_0 (cm)	113.0 ± 2.3	$4.17 \cdot 10^{-3} \pm 1.27 \cdot 10^{-3}$	$5.35 \cdot 10^{-4} \pm 3.24 \cdot 10^{-4}$
$t_{½}k_{e0}$ (min)	0.0[#]	-	-
ε	0.976 ± 0.175		
Reaction time			
F_K (cm.(ng/ml)$^{-1}$)	$-0.166 \pm 3.42 \cdot 10^{-2}$	$8.66 \cdot 10^{-3} \pm 5.81 \cdot 10^{-3}$	-
F_{N^*}	0.00 ± 0.00	$4.06 \cdot 10^{-2} \pm 3.68 \cdot 10^{-2}$	-
Y_0 (cm)	92.0 ± 3.8	$2.01 \cdot 10^{-4} \pm 8.86 \cdot 10^{-3}$	$1.21 \pm 9.10 \cdot 10^{-4}$
$t_{½}k_{e0}$ (min)	2.4 ± 2.2	-	-
ε	62.4 ± 9.5		

ε = a residual error term; **F_K** = the parameter that describes the contribution of ketamine to total effect; **F_{N^*}** = the fraction of F_K that describes the contribution of norketamine to total effect; **θ** = the typical parameter value; **ν^2** = interoccasion variability (in the log-domain); **$t_{½}k_{e0}$** = the blood-effects-site equilibration half-life; **Y_0** = baseline value; **ω^2** = the between-subject variability (in the log-domain).
[#] no hysteresis between blood-concentration and effect observed.

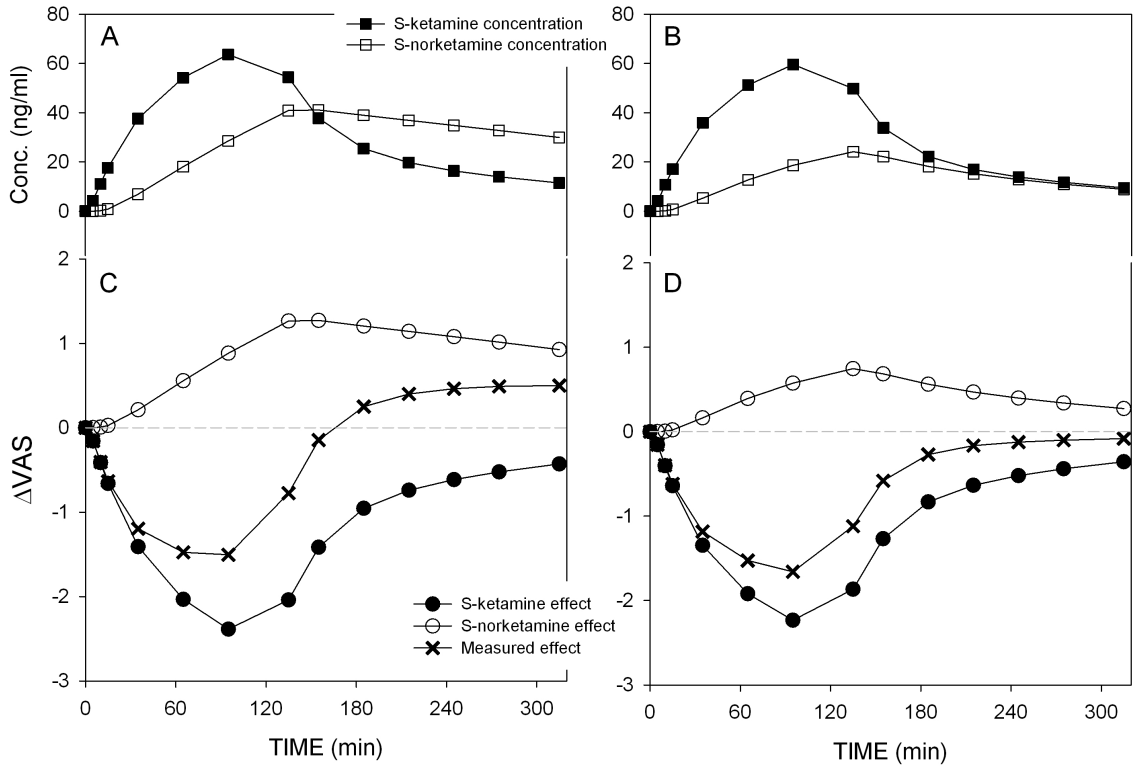

Figure 6 Simulation showing the relative contribution of S-ketamine and S-norketamine to measured effect. Simulated **A** PK and **C** PD data assuming placebo pretreatment. Simulated **B** PK and **D** PD data assuming rifampicin pretreatment.

Discussion

Many drugs used in clinical anesthesia and pain medicine are metabolized into active compounds. Often it is unknown how parent and metabolite contribute to the observed effects. One way to determine their relative contributions is to administer the metabolite and assess its potency. Next, PK-PD modeling is required to obtain a precise estimate of the relative contributions as steady-state conditions are seldom reached after infusion of the parent drug.

An illustrious example of a drug and its active compound is morphine, that is metabolized into the active morphine-6-glucuronide (and the inactive morphine-3-glucuronide). While early (descriptive) human and animal studies suggested a relative large contribution of morphine-6-glucuronide to the effects of morphine, later PK-PD studies performed in humans that combined data on the separate infusions of morphine and morphine-6-glucuronide, showed just a minor contribution of morphine-6-glucuronide to effect (at least in people with normal renal function).[13,14]

Another example of the unknown contribution of metabolites to effect is ketamine. Ketamine is metabolized by *N*-demethylation into norketamine via cytochrome P450 enzymes in the liver, and norketamine is further metabolized into hydroxynorketamine. Ketamine and norketamine are centrally acting NMDAR antagonists, hydroxynorketamine is without pharmacological activity.[4-9] Although ketamine is in use for half a century, the relative contribution of parent and active metabolite to effect remains unknown in humans. Animal studies indicate that norketamine has about 20 to 60% the potency of ketamine and is thought to contribute up to 30% of ketamine analgesia, and, to a lesser extent, to the development of psychotomimetic side effects.[5-8,15] Since norketamine is not available for human use, we assessed the contribution of S-norketamine to S-ketamine effect by measuring S-ketamine's pharmacodynamics under two specific pharmacokinetic conditions: 1 a condition in which the metabolism of S-ketamine and S-norketamine was not influenced, and 2 a condition in which the metabolism of both compounds was induced by rifampicin. These two conditions lead to variations in plasma concentration of S-ketamine (rifampicin causes a reduction in S-ketamine's C_P AUC by about 10%, Figure 1) and S-norketamine (S-norketamine C_P AUC reduced by 50%) and allow determination of their relative contributions to effect.[9]

Ketamine is a drug that causes a myriad of side effects.[16] Consequently the use of ketamine is not always without discomfort to the patient. Side effects include nausea/vomiting, cardiovascular effects and effects due to interaction of ketamine with NMDARs within the central nervous system. These latter side effects include psychotomimetic (psychedelic) effects and cognitive impairment, while animal but not human studies associate ketamine with neurotoxicity. Knowledge on the contribution of norketamine to ketamine analgesia and any of these side effects is of importance as it may lead to further drug development or adaptation of dosing regimens aimed at optimizing analgesia while minimizing side effects. Our current study was aimed at quantifying S-norketamine contribution to S-ketamine's analgesic and cognitive effects. In an initial descriptive analysis we observed that S-ketamine infusion produced analgesia, impairment of cognition and psychotomimetic effects (drug high) to a greater extent than placebo infusion (Tables 1 and 2). These findings are in close agreement with earlier studies.[17,18] As expected, the PK-PD analysis of the S-ketamine infusion data, using a linear additive model of the S-ketamine and S-norketamine contribution to effect, enabled estimation of the S-norketamine contribution. For pain intensity and pain appreciation a negative rather than a positive contribution to effect was observed (negative meaning an effect opposing the direction of the S-ketamine effect). The magnitude of these opposing effects is not easily quantified as they depend on the pertaining S-ketamine and S-norketamine concentrations. To visualize their relative contributions to measured (simulated) effect, we plotted the magnitude of

S-ketamine and S-norketamine effect versus time in Figure 6 for two conditions: placebo (Figure 6 A and C) and rifampicin (Figure 6 B and D) pretreatment. This simulation further shows that following S-ketamine infusion, when S-norketamine concentrations exceed S-ketamine concentrations, the VAS response is hyperalgesic (Figure 6 C). This observation is realistic and in close agreement with earlier studies on the effect of ketamine on pain responses in healthy volunteers and chronic pain patients.[4,19-21]

There are various indications in the literature that ketamine, under specific circumstances, is associated with pain facilitation.[4,19-23] In healthy volunteers ketamine has a dose-dependent antinociceptive effect on static nociceptive pain (repetitive noxious heat pain stimuli), while pain responses following infusion were perceived as more painful (by about 1 cm VAS) for more than 3 h compared to pretreatment pain responses.[21] In agreement with these findings, Mitchell described a cancer patient that developed severe hyperalgesia and allodynia directly following treatment with ketamine.[19] Recently we showed that endogenous modulation of pain, as assessed by the Diffuse Noxious Inhibitory Control (DNIC) paradigm, displayed pain facilitation following a 1-h infusion with S-ketamine (dose 40 mg/70 kg).[20] These findings, together with our current observations, indicate that norketamine may be anti-analgesic and produce pain facilitatory effects, especially when ketamine concentrations are low and norketamine concentrations are elevated, as occurs following a short-term infusion.

It has been argued that the hyperalgesic effects from NMDAR antagonists are related to activation of non-NMDA excitatory receptors (metabotropic or non-NMDA ionotropic glutamate receptors) activated by excitatory amino acids released from spinal or supraspinal sites, or are related to a rebound increase in NMDAR activity following the rapid decrease in ketamine concentration.[4,20-23] Our data indicate that norketamine may be an additional contributor to the hyperalgesic or anti-analgesic effects of ketamine. One possible mechanism of the excitatory behavior of norketamine on pain responses may be activation of excitatory receptors (other than the excitatory glutamate receptors), such as the σ-, κ- and muscarinic receptors.[24] For example, known agonists of the σ-receptor include the NMDAR-antagonists phencyclidine and ketamine, and σ_1-receptor activation has been associated with pronociceptive and psychotomimetic responses.[25] Assuming higher affinity and intrinsic activity of norketamine for the σ-receptor compared to ketamine, this then suggests that when norketamine concentrations are relatively low (as occurs in the rifampicin treatment group) 1 relatively more analgesia will be present (see above and Figure 6), but also that 2 psychotomimetic side effects will be of lesser intensity compared to a condition in which the norketamine concentrations are relatively higher. Indeed, in our experiments we did observe a significantly lower score for drug high at the end of

the infusion period during the RK treatment (Table 1). How much this may be attributed to the lower S-ketamine concentration or S-norketamine concentrations remains presently unknown (as no PK-PD analysis was performed on the drug high data). Our data are consistent in that they suggest that norketamine acts at a non-NMDAR that is associated with excitatory responses, including hyperalgesia, and that it enhances psychotomimetic side effects, possibly via the σ-receptor. However, no human data are available on the activity of norketamine at the σ-receptor or any of the other receptors mentioned above, and further studies are warranted to better understand our observations. The absence of effect of variations in norketamine concentration on cognitive function, suggests absence of involvement of norketamine in these ketamine-related effects. However, the changes in cognition were large and variable (Figure 3). We therefore may have missed subtle changes in cognition related to norketamine.

The PK-PD model that we applied did not make a distinction between S-ketamine and S-norketamine onset/offset times ($t_{½}k_{e0}$). The blood-effect-site equilibration half-lifes of the two compounds were assumed to be similar, as reliable estimates of ketamine's $t_{½}k_{e0}$ and that of its metabolite are not available from animal studies and separate estimations were not possible from the data we collected. The estimated values of $t_{½}k_{e0}$ ranged from 0 (absence hysteresis between plasma concentration and effect) to 11.8 min (overall mean = 6.1 min; Table 3). There are just two earlier studies that report estimates of ketamine's $t_{½}k_{e0}$. Schüttler et al. showed no hysteresis between S-ketamine plasma concentration and median frequency changes of the electroencephalogram from an anesthetic induction dose of S-ketamine in five healthy volunteers.[26] Similarly, Herd et al. estimated a value of $t_{½}k_{e0}$ of 11 s in a pediatric population during induction and recovery from general anesthesia (end-point arousal and recall memory) using racemic ketamine.[27] While these data are difficult to compare to ours (we used a much lower S-ketamine dose and measured different end-points), these data together with ours clearly point towards a rapid onset/offset of S-ketamine's effect following a short-term infusion paradigm (i.e., ketamine's pharmacodynamics is driven by its pharmacokinetics). In contrast, long-term ketamine infusion (100 h or longer) has a much more prolonged effect. In chronic pain patients we recently estimated a half-life for onset/offset of pain relief of 11 days (95% confidence interval 5-21 days).[28] These long-term effects are independent of the passage of ketamine to a postulated receptor site in the central nervous system and most probably reflect a modulatory effect of ketamine with central sensitized chronic pain pathways.

In the current study we did assess the pharmacodynamics of S-ketamine without obtaining S-ketamine and S-norketamine pharmacokinetic data. Instead, we relied on previously obtained pharmacokinetics in a similar group of volunteers that

received a similar pretreatment with rifampicin.[9] The use of simulated PK data in PK-PD modeling studies has been applied with success before when we modeled the effect of opioids on the control of breathing and recently on naloxone reversal of opioid-induced respiratory depression.[29,30] The main reason for not obtaining ketamine PK data is that frequent blood sampling from an arterial line may cause arousal and stress, which may interfere with obtaining reliable data such as pain responses and cognition. A second issue is that the ethics committee of our institution has a restrictive policy regarding the use of arterial lines when reliable PK data is available from earlier studies.[31] As indicated before, we agree that the lack of PK data is a potential drawback of our study; we do believe, however, that taken the quality of our PK data set, that our approach is valid and allows reliable assessment of the relevant PD model parameters.

The observation from our PK-PD study that S-norketamine has anti-analgesic effects opposite to its parent and co-NMDAR antagonist S-ketamine, is an intriguing finding. While it may explain some of the observations made in human studies on the development of pain facilitation following ketamine infusion[4,19-21], we believe that one has to be careful with the interpretation of these data derived from "complex" PK-PD modeling using simulated PK data. Further proof is required before we can conclude that norketamine has a negative contribution to ketamine-induced analgesia and side effects. A careful conclusion at present is that norketamine contribution to ketamine analgesia is limited and that we cannot exclude a small anti-analgesic effect from norketamine.

Appendix 1: Cognition tests

The CNS Vital Signs cognition tests have been described in full elsewhere.[11] In short:

> *Symbol digit coding*: the test consists of serial presentations of screens, each of which contains a bank of 8 symbols above and 8 empty boxes below. At the top of the screen a bank of 8 symbols is depicted with the corresponding numbers below. The subject types the number into the empty box that corresponds to the symbol that is highlighted. Each time the test is administered, the program randomly chooses eight new symbols to match to the eight digits. Scoring is the number of correct responses generated in 2 minutes.
>
> *Stroop test*: the test has three parts. **A** The words RED, YELLOW, BLUE and GREEN appear at random on the screen in black. The subject has to press a button as the word appears. **B** The words RED, YELLOW, BLUE and GREEN appear on the screen in color. The subject has to press a button when the color of the word matches the meaning of the word. **C** The words RED, YELLOW, BLUE and GREEN appear on the screen in color. The subject is asked to press a button when

the color and the meaning of the word do not match. Each test generates a separate reaction time score (part A generates a simple reaction time, parts B and C complex reaction times), which combined give an indication of information processing speed. The value of the Stroop reaction time is on average 120 ms longer than the complex reaction time generated in part B of the test (range 78-188 ms). Part C also generates an error score. The test requires about 4 minutes.

Shifting attention test (SAT): in the shifting attention test subjects are instructed to match geometric objects either by shape or color. The test measures the ability to shift from one instruction to another quickly and accurately. Three figures appear on the screen, one on top and two on the bottom. The top figure is either a square or a circle. The bottom figures are a square and a circle. These figures are either red or blue; the colors are mixed randomly. The subject is asked to match one of the bottom figures to the top figure, either by color or by shape. The rules of the matching change at random. This goes on for 90 seconds. The goal is to make as many correct matches as possible. The scores generated by SAT are: correct matches, errors and response time in ms.

Finger tapping: the test generates relevant data about fine motor control, which is based on motor speed, as well as kinesthetic and visual-motor ability. The subjects press the space bar with the index finger as many times as they can in 10 s; this test is performed 3 times with the right index finger and 3 times with the left index finger. The score is the average number of taps.

Continuous performance: this test is a measure of vigilance or sustained attention over time. The subject is asked to respond to a target stimulus, e.g. the letter B, but not to any other letter, by pressing the space bar. In 5 min, the test presents 200 letters; 40 of the letters are the target B, 160 are non-targets (any other letter). The stimuli are presented at random, although the target stimulus only appears 8 times during each minute of the test. The scores generated are: correct matches, commission errors (pressing when no B is shown, e.g., impulsive responding) and omission errors (not pressing when a B appears, e.g., inattention).

Immediate and delayed verbal memory: This is an adaptation of the Rey Auditory Verbal Learning Test. Fifteen words are presented, one by one, on the screen. A new word is presented every two seconds. The subject is asked to remember these words. Then a list of thirty words is presented. The fifteen target words are mixed randomly among 30 words of which 15 new words. When the subject recognizes a word from the original list, he or she presses the space bar. This is a recognition test, however, not a test of recall. After finishing the other tests, a delayed recognition test is performed. The 15 targets remain the same for the delayed memory testing; the 15 distractors are different between the immediate and delayed challenges.

Immediate and delayed visual memory: this test is the same as the verbal memory test, but instead of words geometric figures are used.

These tests generate scores on 5 separate domains: memory, psychomotor speed, reaction time, complex attention and cognitive flexibility.

- The Memory domain is calculated from the correct scores of the verbal and visual (immediate and delayed) memory tests.
- Psychomotor speed is derived from number of taps in the finger tapping test and number of correct answers in the symbol digit coding tests.
- The domain score for Reaction time is made up by combining two reaction time scores (B and C) of the Stroop test.
- The domain score for Complex attention is generated by adding the number of errors in the continuous performance test, the shifting attention test and the Stroop test.
- The domain score for Cognitive flexibility is generated by taking the number of the correct responses on the shifting attention test and subtracting the number of errors on the shifting attention and Stroop tests.

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Chapter 7

Summary, conclusions and future perspectives

Summary

The balance between safety and efficacy is important in pharmacotherapy, and registration authorities emphasize the need for pre- and post-registration safety studies. Sometimes, the indication of a registered drug shifts to another disease or a different patient population. Apart from the need for studies on efficacy in this 'new' indication and 'new' population, also safety needs to be addressed. Unfortunately safety is often not studied as clinicians (and the pharmaceutical industry) are convinced that safety can be extrapolated from one population, one disease or one specific mode of administration to the other. An example that such thinking is not justified comes from an important observation in this thesis (Chapter 3). A trial on the safety and efficacy of repeated long-term infusions of S-ketamine in CRPS-1 patients was terminated early because of a high and unexpected incidence of liver damage in patients receiving S-ketamine at 3-week intervals.

Ketamine is a relatively 'old' drug and used for almost 50 years as an anesthetic, but recently there has been a renewed interest for the treatment of therapy-resistant chronic pain. S-ketamine at subanesthetic concentrations produces potent analgesia. Consequently, low-dose ketamine is used perioperatively to improve opioid analgesia, and in chronic pain patients where other more conventional therapies are without significant effect. In this thesis the effects of the *N*-methyl-D-aspartate receptor (NMDAR) antagonist S-ketamine in patients with chronic pain (CRPS-1 and fibromyalgia patients) and healthy volunteers are described. Low-dose ketamine has, apart from its intended effect (analgesia), a variety of side effects, including effects on the cardiovascular system (e.g., hypertension), psychotomimetic effects, cognitive dysfunction and liver damage (see above).

This thesis has three main topics: efficacy, safety and metabolism of low-dose S-ketamine. These three topics were addressed in the different studies as efficacy and safety are inherently linked, and as such naturally part of the complex pharmacokinetics and pharmacodynamics of S-ketamine.

Chapter 2 is a review of the efficacy of ketamine treatment in chronic non-cancer pain. Worldwide the number of patients affected by chronic pain is increasing and conventional treatment is often insufficient. Recently the importance of NMDAR in the mechanisms and maintenance of chronic pain was established. Ketamine is the most studied NMDAR antagonist in the treatment of various chronic pain syndromes. This review focuses on the efficacy, safety, pharmacology and toxicology of ketamine. Electronic databases were scanned for prospective, randomized controlled trials that assessed ketamine's analgesic effect in patients with chronic pain, with a particular focus on trials published after 2008

that applied long-term intravenous infusions. While most studies on intravenous ketamine show acute analgesic effects, three recent trials on long-term ketamine treatment (days to weeks) demonstrated the effectiveness of ketamine in producing long-term (months) relief of chronic pain. Despite these positive results, further studies are needed on safety/toxicity issues. Other administration modes, i.e., short-term intravenous administration, are less effective in causing long-term pain relief. Therefore, there is now evidence from a limited number of studies that pain relief lasting for months can occur after long-term intravenous ketamine infusion, suggesting a modulatory effect of ketamine in the process of chronic pain, possibly via blockade of upregulated NMDAR.

Chapter 3 As reviewed in Chapter 2, studies on the efficacy of ketamine in the treatment of chronic pain indicate that prolonged or repetitive infusions are required to ensure prolonged pain relief. Few studies address ketamine-induced toxicity. In this chapter data are presented on the occurrence of ketamine-induced liver injury during repeated administrations of S-ketamine for treatment of chronic pain in patients with complex regional pain syndrome type 1. This study was designed to explore possible time frames for ketamine re-administration. Six patients were planned to receive two continuous intravenous 100-h S-ketamine infusions (infusion rate 10-20 mg/h) separated by 16 days. Three of these patients developed hepatotoxicity. Patient A, a 65-year-old female, developed an itching rash and fever during her second exposure. Blood tests revealed elevated liver enzymes (ALT, APT, AST and γGT, all ≥ 3 times the upper limit of normal) and moderately increased eosinophilic leucocyte counts. Patient E, a 48-year-old female, developed elevated liver enzymes of similar pattern as Patient A during her second ketamine administration and a weakly positive response to antinuclear antibodies. In a third patient, Patient F, a 46-year-old male, elevated liver enzymes (ALT and γGT) were detected on the first day of his second exposure. In all patients the ketamine infusion was promptly terminated and the liver enzymes slowly returned to normal values within two months. These data suggest an increased risk for development of ketamine-induced liver injury when the infusion is prolonged and/or repeated within a short time frame. Regular measurements of liver function are therefore required during such treatments.

Chapter 4 Prolonged and/or repeated ketamine infusions can cause serious side effects. In contrary to the evidence from Chapter 2, short S-ketamine infusions seem to produce good effect on pain in fibromyalgia according to non-experimental data. To explore the efficacy of a short-term infusion with S-ketamine on fibromyalgia pain, a randomized double blind, active-placebo controlled trial was performed. Twenty-four fibromyalgia patients were randomized to receive a 30-min intravenous infusion with S-ketamine (total dose 0.5 mg/kg, n = 12) or the active placebo, midazolam (5 mg, n = 12). Visual Analogue Pain Scores (VAS) and ketamine plasma samples were obtained for

2.5 h following termination of treatment; pain scores derived from the Fibromyalgia Impact Questionnaire (FIQ) were collected weekly during an 8-week follow-up. Fifteen minutes after termination of the infusion the number of patients with a reduction in pain scores of > 50% was 8 versus 3 in the placebo group ($P < 0.05$), at t = 75 min 6 versus 2 (ns), at the end of week-1 2 versus 0 (ns), and at end of week-8 2 patients in each of the ketamine and midazolam groups. The effect of S-ketamine on VAS closely followed the changes in ketamine plasma concentrations. For VAS and FIQ scores no significant differences in treatment effects were observed in the 2.5-h following infusion or during the 8-week follow-up. Side effects, as measured by the Bowdle questionnaire (which scores for 13 separate psychedelic symptoms), were mild to moderate in both groups and declined rapidly, indicating adequate blinding of treatments. Efficacy of S-ketamine was limited and restricted in duration to its pharmacokinetics. In common with earlier findings (see Chapter 2) a short infusion of S-ketamine has no long-term effects on fibromyalgia pain. Alternatively, there may be a subset of patients in which sensitized NMDAR play only a minor role in the development of chronic fibromyalgia pain.

Chapter 5 Ketamine is metabolized in the liver to norketamine via cytochrome P450 enzymes (CYP enzymes). There are few human data on the involvement of CYP enzymes on the elimination of norketamine and norketamine's possible contribution to the analgesic effect. The aim of this study was to investigate the effect of cytochrome P450 enzyme induction by rifampicin on the pharmacokinetics of S-ketamine and its major metabolite, S-norketamine, in healthy volunteers. Twenty healthy male subjects received 20 mg/70kg/h (n = 10) or 40 mg/70kg/h (n = 10) intravenous S-ketamine twice for 2 h, following either 5 days of oral rifampicin (once daily 600 mg) or placebo treatment. During and 3 h following drug infusion arterial plasma concentrations of S-ketamine and S-norketamine were obtained at regular intervals. The data were analyzed with a compartmental pharmacokinetic model consisting of three compartments for S-ketamine, three sequential metabolism compartments and two S-norketamine compartments using NONMEM. Rifampicin caused a 10% and 50% reduction in the area-under-the-curve of the plasma concentrations of S-ketamine and S-norketamine, respectively. The compartmental analysis indicated a 13% and 200% increase in S-ketamine and S-norketamine elimination from their respective central compartments by rifampicin. A novel observation is the large effect of rifampicin on S-norketamine concentrations, and indicates that rifampicin induces the elimination of S-ketamine's metabolite, probably via induction of the CYP3A4 and/or CYP2B6 enzymes.

Chapter 6 As described in the previous chapters, S-ketamine has analgesic, cognitive and psychotomimetic effects. The contribution of S-norketamine to these effects is unknown and is explored in this chapter. Twelve healthy young

male volunteers participated in this randomized, single blind, cross-over study. Volunteers were studied on 3 occasions and received 20 mg/70kg/h intravenous S-ketamine or placebo for 2 h, following either 5 days of oral rifampicin (once daily 600 mg) or placebo treatment. Before, during, and after the infusion the subjects performed computerized neurocognitive tests (e.g. memory and reaction time), and pain responses to a painful stimulus and side effects (drug high) were recorded. S-ketamine infusion caused pain relief, drug high and impaired cognition during infusion. All of the effects decreased within the three hours following infusion, with parameters not different between treatments at t = 3 h following the termination of the ketamine infusion. Using the pharmacokinetic data obtained in Chapter 5, the contribution of norketamine to ketamine effect was modeled using a linear, additive population pharmacokinetic-pharmaco-dynamic model. Modeling showed that S-norketamine diminished S-ketamine analgesia, but had no effect on cognitive impairment. These findings are intriguing, but should be considered with care as this is the first study, using "complex'' modeling and simulated PK data, showing an excitatory effect of norketamine on pain, but an antagonistic effect on psychotomimetic side effects. A more sensible conclusion would be that norketamine does not contribute to ketamine's effects, although a small negative or antagonistic effect relative to ketamine's effects cannot be excluded. No conclusions can be extrapolated to clinical use, because of the difference in population and administration duration.

Conclusions

The conclusions that may be drawn from this thesis are:

1. There is evidence from a limited number of studies (n = 3) that chronic non-cancer pain relief can last for months after long-term intravenous ketamine infusions (duration at least 35 h). *This is insufficient to warrant the clinical (i.e., non-experimental) use of S-ketamine in chronic neuropathic pain patients*;

2. S-ketamine may cause liver enzyme elevations when a repeated dosing regimen is used with just 2 weeks between treatments and the dosing duration is long-term (100 h). *This observation warrants the measurement of liver enzymes in all patients receiving long-term ketamine treatment;*

3. A 30-min infusion with S-ketamine has no long-term efficacy in the treatment of fibromyalgia patients. *Treatment of fibromyalgia pain with a short-term exposure to ketamine is currently not advisable;*

4. Rifampicin has a large impact on the pharmacokinetics of S-norketamine and a lesser impact on the pharmacokinetics of S-ketamine, causing a 50 and 10% reduction of their respective area-under-plasma concentration-time-curves;

5. A 2-h infusion with low-dose S-ketamine causes neurocognitive impairment that rapidly dissipates upon the termination of the infusion;

6. S-norketamine does not contribute to ketamine's effects, although a small negative or antagonistic effect relative to ketamine's effect, cannot be excluded. *These data suggest that pursuing of this agent as an alternative to ketamine is not warranted.*

Future perspectives

Since ketamine is currently the most potent NMDAR antagonist available, it will remain popular in the (experimental) treatment of diseases in which the sensitized NMDAR plays a crucial role. Such a disease is chronic neuropathic pain. In fact, this is a rather new indication for a drug that was originally developed as an anesthetic, and leaves considerable room for further exploration. Future studies should be directed in two directions:

1. Further explore the S-ketamine safety-efficacy balance in chronic pain patients, possibly in outpatient or ambulatory settings, and with new administration modes (such as intranasal ketamine or iontophoretic cutaneous applications);

2. Since the prolonged S-ketamine treatment is a serious disadvantage with respect to patient discomfort (the side effects are often such that patient compliance is limited, even during low-dose administration), and cost to health care (in-patient treatment is expensive and currently not reimbursed by healthy insurance), it is advisable to seek affordable alternatives. Alternatives may be aimed at the NMDAR, an example is traxoprodil (Pfizer BV), a NR1/NR2B selective NMDAR antagonist, possible with lesser psychotomimetic and cognitive side effects, or at other processes that enhance pain, such as the spinal inflammatory response at the spinal and supraspinal level involving astrocytes and microglia cells. An example of agents that may be used to target spinal inflammation include anti-TNFα-drugs or erythropoietin-derivatives (erythropoietin is the natural anti-TNFα produced during inflammation in the affected tissues to control excessive tissue damage from TNF). There is now evidence from our laboratory that ARA290 (a peptide that mimics the three dimensional structure erythropoietin), when given after nerve injury in a rat, is able to prevent allodynia for weeks to months depending on the treatment regimen.

Possibly combinations of pharmacological agents aimed at different parts of the chronic pain process are optimal (e.g., S-ketamine together with ARA290), but should be looked upon in further studies.

Chapter 8

Samenvatting, conclusies en toekomstperspectieven

Samenvatting

De balans tussen veiligheid en werkzaamheid is belangrijk in de farmacotherapie en de registratie instanties benadrukken het belang van pre- en post-registratie veiligheidsstudies. Soms verschuift de indicatie voor een geregistreerd geneesmiddel naar een andere aandoening of patiëntenpopulatie. Behalve de noodzakelijke studies voor de werkzaamheid bij deze nieuwe indicaties en nieuwe populaties, moet er ook aandacht worden besteed aan de veiligheid. Helaas wordt de veiligheid vaak niet onderzocht omdat clinici (en de farmaceutische industrie) ervan overtuigd zijn dat de veiligheid kan worden geëxtrapoleerd van één populatie, één aandoening of één specifieke toedieningsvorm naar een andere. Dat deze gedachtegang niet altijd juist is, wordt duidelijk na een belangrijke observatie die wordt beschreven in dit proefschrift (Hoofdstuk 3). Een studie naar de werkzaamheid en veiligheid van herhaaldelijke toediening van lange infusies met S-ketamine in CRPS type 1 patiënten werd voortijdig gestaakt in verband met een hoge en onverwachte incidentie van leverschade bij patiënten die 2 maal S-ketamine infusies ontvingen binnen een tijdsbestek van 3 weken.

Ketamine is een relatief 'oud' geneesmiddel en het wordt al bijna 50 jaar gebruikt als een anestheticum. Recent is er hernieuwde belangstelling ontstaan voor ketamine in de behandeling van therapieresistente chronische pijn. Bij subanesthetische concentraties is ketamine een potent analgeticum. Derhalve wordt een subanesthetische dosering ketamine perioperatief gebruikt ter verbetering van opioïde pijnstilling en bij patiënten met chronische pijn waarbij conventionele therapieën zonder significant effect zijn gebleven. Dit proefschrift beschrijft de effecten van de *N*-methyl-D-aspartaat receptor (NMDAR) antagonist S-ketamine in patiënten met chronische pijn (CRPS type 1 en fibromyalgie) en gezonde vrijwilligers. Bij subanesthetische doseringen ketamine kunnen naast het beoogde effect (analgesie) ook een verscheidenheid aan bijwerkingen optreden, zoals effecten op het cardiovasculaire systeem (bijvoorbeeld hypertensie), psychotomimetische effecten, cognitieve disfunctie en leverschade (zie hierboven).

Dit proefschrift heeft 3 thema's: werkzaamheid, veiligheid en het metabolisme van subanesthetische doseringen S-ketamine. Deze 3 thema's komen aan bod in de verschillende studies omdat werkzaamheid en veiligheid onlosmakelijk verbonden zijn en daarom vanzelfsprekend onderdeel zijn van de complexe farmacokinetiek en farmacodynamiek van S-ketamine.

Hoofdstuk 2 is een overzichtsartikel over de werkzaamheid van ketamine behandelingen bij chronische niet-kanker pijn. Het aantal patiënten met chronische pijn groeit wereldwijd en conventionele behandelingen zijn vaak onvoldoende. Recent is het belang van de NMDAR in de ontstaansmechanismen

en het onderhoud van chronische pijn vastgesteld. Ketamine is de meest bestudeerde NMDAR antagonist in de behandeling van verschillende chronische pijnsyndromen. Dit overzichtsartikel richt zich op de werkzaamheid, veiligheid, farmacologie en toxicologie van ketamine. In elektronische databases werd gezocht naar prospectieve, gerandomiseerde controle-interventie studies, die het analgetische effect van ketamine in patiënten met chronische pijn onderzochten, met specifieke aandacht voor studies gepubliceerd na 2008 die lange intraveneuze infusies toepasten. Terwijl de meeste studies acute analgetische effecten laten zien bij intraveneus ketamine, laten 3 recente studies zien dat na lange ketamine behandeling (dagen tot weken) langdurige (maanden) pijnstilling van chronische pijn kan optreden. Ondanks deze positieve resultaten zijn er studies nodig die zich richten op veiligheid en toxiciteit. Andere toedieningsvormen, zoals korte intraveneuze toediening, zijn minder effectief in het bereiken van langdurige pijnstilling. Er is nu bewijs uit een beperkt aantal studies dat langdurige pijnstilling (maanden) optreedt na lange intraveneuze ketamine toediening, dit suggereert een modulerend effect van ketamine op het chronische pijn mechanisme, mogelijk via blokkade van opgereguleerde NMDAR.

Hoofdstuk 3 Zoals beschreven in Hoofdstuk 2, geven studies naar de effectiviteit van ketamine in de behandeling van chronische pijn aan dat lange of herhaaldelijke infusies nodig zijn om langdurige pijnstilling te bewerkstelligen. Weinig studies besteden aandacht aan toxiciteit veroorzaakt door ketamine. In dit hoofdstuk wordt het optreden van ketamine geïnduceerde leverschade beschreven tijdens herhaaldelijke toediening van S-ketamine voor de behandeling van chronische pijn in patiënten met complex regionaal pijnsyndroom type 1. In dit onderzoek werd gekeken naar mogelijke tijdstippen voor de herhaaldelijke toediening van ketamine. Zes patiënten waren gerandomiseerd voor 2 continue intraveneuze S-ketamine infusies van 100 uur (infusiesnelheid 10-20 mg/uur) gescheiden door 16 dagen. Drie van deze patiënten ontwikkelden levertoxiciteit. Patiënt A, een 65 jarige vrouw, ontwikkelde een jeukende huiduitslag en koorts tijdens de tweede toediening. Bloedonderzoek liet verhoogde leverenzymen zien (ALAT, alkalische fosfatase, ASAT en γGT, allen ≥ 3 keer boven de bovengrens van de referentiewaarden) en geringe stijging in het eosinofiele leukocyten getal. Patiënt E, een 48 jarige vrouw, ontwikkelde verhoogde leverenzymen, met een zelfde patroon als Patiënt A, tijdens de tweede toediening en had een zwak positieve reactie op antinucleaire antilichamen. Bij een derde patiënt, Patiënt F, een 46 jarige man, werden verhoogde leverenzymen (ALAT en γGT) vastgesteld op de eerste dag van de tweede toediening. Bij alle patiënten werd de ketamine infusie direct gestaakt en de leverenzymen daalden langzaam tot binnen de referentiewaarden binnen twee maanden. Deze gegevens suggereren een verhoogd risico op het ontwikkelen van ketamine geïnduceerde leverschade bij lange en/of herhaaldelijk infusies binnen een kort tijdsbestek. Frequente metingen van de leverfunctie is daarom noodzakelijk tijdens deze behandelingen.

Hoofdstuk 4 Lange en/of herhaaldelijke ketamine infusies kunnen ernstige bijwerkingen veroorzaken. In tegenstelling tot het bewijs uit Hoofdstuk 2, lijken, afgaande op non-experimentele gegevens, korte S-ketamine infusies een goed effect te hebben op pijn bij fibromyalgie. Het effect van een korte S-ketamine infusie op fibromyalgie pijn werd onderzocht met een gerandomiseerde, dubbelblinde, actief placebo controle-interventie studie. Vierentwintig fibromyalgie patiënten werden gerandomiseerd voor een 30 minuten durende intraveneuze infusie met de NMDAR antagonist S-ketamine (totale dosering 0,5 mg/kg, n = 12) of het actieve placebo, midazolam (5 mg, n = 12). Visual Analogue Scale (VAS) pijn scores werden gemeten na de infusie en bloed voor de bepaling van ketamine plasmaconcentraties werd gedurende en na de infusie afgenomen. Deze metingen werden verricht tot 2,5 uur na einde van de infusie; pijn scores uit de Fibromyalgie Impact Questionnaire (FIQ) werden wekelijks gedurende de 8 weken follow-up verzameld. Vijftien minuten na het einde van de infusie waren er 8 patiënten in de ketamine groep met een pijnreductie van > 50% versus 3 in de controlegroep ($P < 0.05$), op t = 75 minuten 6 versus 2 (ns), aan het eind van week 1 2 versus 0 (ns) en aan het eind van week 8 in zowel de ketamine als de midazolam groep 2 patiënten. Het ketamine effect op de VAS hing nauw samen met de veranderingen in ketamine plasmaconcentraties. In de VAS en FIQ scores werden geen significante behandelingseffecten aangetoond in de 2,5 uur na het einde van de infusie of tijdens de 8 weken follow-up. Bijwerkingen, gemeten met de Bowdle vragenlijst (deze lijst scoort 13 verschillende psychedelische symptomen), waren mild tot matig in beide groepen en namen snel af na stoppen van de infusie, dit duidt op adequate blindering van de behandelingen. De werkzaamheid van ketamine was beperkt in effect en duur door de farmacokinetiek. Overeenkomend met eerdere bevindingen (zie Hoofdstuk 2) geeft een korte infusie met S-ketamine geen langdurige pijnstilling bij fibromyalgie patiënten. Aan de andere kant is het mogelijk dat bij een gedeelte van de patiënten een gesensitiseerde NMDAR maar een kleine rol speelt bij het ontstaan van chronische fibromyalgie pijn.

Hoofdstuk 5 Ketamine wordt in de lever gemetaboliseerd tot norketamine via de cytochroom P450 enzymen (CYP enzymen). Er zijn bij mensen weinig gegevens bekend over de betrokkenheid van CYP enzymen bij de eliminatie van norketamine en de mogelijke bijdrage die norketamine levert aan het analgetische effect. Het doel van deze studie was om het effect van cytochroom P450 enzym inductie door rifampicine te onderzoeken op de farmacokinetiek van S-ketamine en de belangrijkste metaboliet, S-norketamine, in gezonde vrijwilligers. Twintig gezonde mannelijke proefpersonen kregen tweemaal een 2 uur durende intraveneuze infusie S-ketamine van 20 mg/70 kg/uur (n = 10) of 40 mg/70 kg/uur (n = 10), na een voorbehandeling met 5 dagen rifampicine oraal (eenmaal daags 600 mg) of een placebo. Tijdens en 3 uur na de infusie werden op vaste tijden arteriële bloedmonsters afgenomen voor het bepalen van de S-ketamine en

S-norketamine plasmaconcentraties. De gegevens zijn met NONMEM geanalyseerd met een farmacokinetisch compartimenten model bestaande uit 3 compartimenten voor S-ketamine, 3 opeenvolgende metabolisme compartimenten en 2 S-norketamine compartimenten. Rifampicine veroorzaakte een 10% en 50% afname in de 'area-under-the-curve' (oppervlakte onder de grafieklijn) van de plasmaconcentraties van respectievelijk S-ketamine en S-norketamine. De analyse gaf aanwijzingen voor een 13% en 200% toename in respectievelijk S-ketamine en S-norketamine eliminatie vanuit hun centrale compartimenten door rifampicine. Een opmerkelijke observatie is het grote effect van rifampicine op de S-norketamine concentraties en duidt erop dat rifampicine de eliminatie van de S-ketamine metaboliet induceert, mogelijk via inductie van de CYP3A4 en/of CYP2B6 enzymen.

Hoofdstuk 6 Zoals beschreven in de voorgaande hoofdstukken heeft S-ketamine analgetische, cognitieve en psychotomimetische effecten. De bijdrage van S-norketamine aan deze effecten is onbekend en werd onderzocht in dit hoofdstuk. Twaalf gezonde mannelijke vrijwilligers namen deel aan deze gerandomiseerde, enkelblinde, cross-over studie. Alle vrijwilligers namen deel aan 3 verschillende onderzoeksdagen, en kregen een 2 uur durende intraveneuze infusie van 20 mg/70 kg/uur S-ketamine of placebo, voorafgaand door een voorbehandeling met 5 dagen rifampicine oraal (eenmaal daags 600 mg) of een placebo. Voor, tijdens en na de infusie maakten de vrijwilligers neurocognitieve testen op een computer (bijvoorbeeld geheugen en reactiesnelheid testen) en werden pijn na een pijnstimulus en bijwerkingen ('drug high') vastgelegd. De S-ketamine infusie gaf pijnstilling, 'drug high' en lagere cognitie scores tijdens de infusie. Alle effecten verdwenen binnen de 3 uren na de infusie en er waren geen verschillen tussen de verschillende behandelingsgroepen 3 uur na het stoppen van de S-ketamine infusie. Met de farmacokinetische gegevens uit Hoofdstuk 5, werd de bijdrage van norketamine aan het ketamine effect gemodelleerd met een lineair, additief populatie farmacokinetisch-farmacodynamisch model. Het model liet zien dat S-norketamine het analgetisch effect van S-ketamine verkleint, maar dat het geen invloed had op de cognitieve effecten. Deze bevindingen zijn fascinerend, maar moeten voorzichtig worden geïnterpreteerd omdat dit de eerste studie is, gebruikmakend van complexe modellering en gesimuleerde PK gegevens, die een excitatoir effect van norketamine op pijn laat zien, maar een antagonistisch effect op de psychotomimetische bijwerkingen. Een meer correcte conclusie zou zijn dat norketamine niet bijdraagt aan de ketamine effecten, maar dat een klein negatief of antagonistisch effect tegengesteld aan het ketamine effect niet kan worden uitgesloten. Er kunnen geen conclusies worden 'doorgetrokken' naar de klinische situatie omdat er sprake is van een andere populatie en toedieningsduur.

Conclusies

De conclusies die uit dit proefschrift getrokken kunnen worden zijn:

1. Er is bewijs uit een beperkt aantal studies (n = 3) bij chronische niet-kanker pijn, dat pijnstilling gedurende maanden kan optreden na lange intraveneuze ketamine infusies (duur minimaal 35 uur). *Dit is echter niet voldoende om de klinische toepassing (met andere woorden niet-experimentele toepassing) van S-ketamine bij chronische neuropathische pijnpatiënten te rechtvaardigen;*

2. S-ketamine kan leverenzym stijgingen veroorzaken bij herhaaldelijke toedieningen met maar 2 weken tussen behandelingen met een lange toedieningsduur (100 uur). *Deze observatie rechtvaardigt het bepalen van leverenzymen in alle patiënten die een lange ketamine behandeling krijgen;*

3. Een 30 minuten durende infusie met S-ketamine heeft geen langdurige werkzaamheid bij fibromyalgie patiënten. *Behandeling van fibromyalgie pijn met een korte ketamine infusie is momenteel niet aan te bevelen;*

4. Rifampicine heeft een grote invloed op de farmacokinetiek van S-norketamine en kleinere invloed op de farmacokinetiek van S-ketamine, het veroorzaakt een 50 en 10 % reductie van de respectievelijke oppervlakte-onder-de-plasmaconcentratie-tijd-curve;

5. Een 2 uur durende infusie met subanesthetische dosering S-ketamine veroorzaakt lagere neurocognitieve scores die snel verbeteren na stoppen van de infusie;

6. S-norketamine draagt niet bij aan de ketamine effecten, al kan een klein negatief of antagonistisch effect, ten opzichte van het ketamine effect, niet worden uitgesloten. *Deze gegevens suggereren dat verder onderzoek naar dit middel als alternatief voor ketamine niet gerechtvaardigd is.*

Toekomstperspectieven

Aangezien ketamine momenteel de meest potente NMDAR antagonist is, zal het populair blijven in de (experimentele) behandeling van aandoeningen waarbij de gesensitiseerde NMDAR een cruciale rol speelt. Onder deze aandoeningen valt chronische neuropathische pijn. In feite is dit een redelijk nieuwe indicatie voor een geneesmiddel dat oorspronkelijk was ontwikkeld als anestheticum, en laat

aanzienlijke ruimte voor verder onderzoek. Toekomstige studies dienen zich te richten op 2 richtingen:

1. Aanvullend onderzoek naar de balans tussen veiligheid en werkzaamheid van S-ketamine bij chronische pijnpatiënten, mogelijk in de thuissituatie of poliklinische behandeling en naar nieuwe toedieningsvormen (zoals intranasale of iontoforetische toediening);

2. Omdat lange S-ketamine behandelingen belangrijke nadelen hebben in de vorm van ongemak voor de patiënt (de bijwerkingen zijn vaak zodanig dat therapietrouw beperkt is, zelfs tijdens subanesthetisch gedoseerde toedieningen) en de kosten voor de gezondheidszorg (klinische behandeling is kostbaar en wordt momenteel niet vergoed door de ziektekostenverzekeraars), is het raadzaam om te zoeken naar betaalbare alternatieven. Deze alternatieven kunnen gericht zijn op de NMDAR, een voorbeeld is traxoprodil (Pfizer bv), een NR1/NR2B selectieve NMDAR antagonist met mogelijk minder psychotomimetische en cognitieve bijwerkingen, of op andere processen die een rol spelen in de versterking van pijn, zoals de spinale inflammatoire reactie op spinaal en supraspinaal niveau waarbij astrocyten en microgliacellen betrokken zijn. Voorbeelden van geneesmiddelen die mogelijk gebruikt kunnen worden tegen spinale inflammatie zijn onder andere anti-TNFα geneesmiddelen of erytropoëtine derivaten (erytropoëtine is de natuurlijke anti-TNFα die wordt geproduceerd tijdens inflammatie in de aangedane weefsels om buitensporige weefselschade door TNF te voorkomen). Er is bewijs uit onze onderzoeksgroep dat ARA290 (een eiwit dat de driedimensionale structuur van erytropoëtine nabootst), wanneer dit wordt gegeven na zenuwbeschadiging in de rat, allodynie gedurende weken of maanden kan voorkomen, afhankelijk van het behandelingsschema.

Het is mogelijk dat combinaties van geneesmiddelen, gericht op specifieke delen van het chronische pijnproces, optimaal zijn (bijvoorbeeld S-ketamine samen met ARA290), dit moet worden onderzocht in aanvullende studies.

Curriculum Vitae

Ingeborg Marieke Noppers was born on the 29th of October 1981 in Emmen, the Netherlands. She obtained her VWO diploma at the Hondsrug College in Emmen in June 2000. Later that year she entered Medical School at the Rijksuniversiteit Groningen. She received her Medical Degree in September 2006 and began working as a resident in the Intensive Care department of the University Medical Center Groningen. Early 2008 she was appointed as a PhD student at the department of Anesthesiology in the Leiden University Medical Center under the supervision of Prof. Dr. A Dahan and Dr. EY Sarton and started the investigations described in this thesis. She started her residency in Anesthesiology in October 2010. As of April 2011 she started her medical specialist training at the department of Anesthesiology in the Leiden University Medical Center (Chairman: Prof. Dr. LPHJ Aarts).

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