CHAPTER 5

COMPARISON OF RESPIRATORY AND ANALGESIC EFFECTS OF MR30365/07 AND FENTANYL IN MEN

A double blind placebo controlled randomized phase 1 study

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5.1 INTRODUCTION

Opioid analgesics form the cornerstone of contemporary treatment of moderate to severe (acute and chronic) pain. Opioids are associated with a series of side effects including opioid-induced respiratory depression (OIRD), which is potentially life threatening.[1] In recent years the number of lethal opioid-related respiratory complications has increased significantly,[2,3] mainly due to the misuse or abuse of legally prescribed opioids for moderate to severe chronic pain (most importantly lower back pain). Opioids produce respiratory depression via activation of μ-opioid receptors (MORs) expressed on pontine neurons involved in the respiratory control.[4] Full MOR agonists produce a dose dependent respiratory depression with apnea at high doses.[5] Few studies address the (positive or negative) contribution of the other opioid receptors on respiratory depression. We recently showed both in rodents and humans that buprenorphine (a partial agonist at the MOR, antagonist at the κ-opioid receptor (KOR), and with activity at the opioid-receptor-like (ORL1) receptor) produces a ceiling in respiratory depression; ceiling is defined as an apparent maximum effect regardless of drug dose tested.[5,6] This is a major advantage over other opioids and implicates some protective effect at high doses. The molecular mechanism underlying the ceiling in respiratory effect has not yet been elucidated. In mice, Lutfy *et al*. showed that the ceiling in analgesia is related to the activation of the ORL1 receptor.[7] Extrapolation of these findings to the respiratory system would suggest that some of the opioid receptors may counteract, at least in part, the respiratory depression induced by the activation of the MOR.[5,6]

In the current phase 1 study, we assessed the respiratory and analgesic effects of the experimental drug MR30365/07, an opioid with high affinity for the MOR, δ-opioid receptor (DOR), KOR and lower affinity for the ORL1 receptor (confidential data, Mundipharma Research Ltd.). We compared MR30365/07 to fentanyl, a selective and high affinity MOR agonist that produces dose-dependent respiratory depression and apnea at high doses (2-3 $\mu g.kg^{-1}$ and greater).[5,6,8] Experiments were performed in healthy male volunteers. The study consisted of two parts. Part 1 was a single blinded, placebo controlled pilot study on the respiratory effects of a range of MR30365/07 doses, designed to select the MR30365/07 dose most suitable for use in the main phase of the study (Part 2). Part 2 was a double-blind, randomized, placebo-controlled study, which compared the respiratory and analgesic effects of MR30365/07 and fentanyl.

5.2 METHODS

This phase 1 study had two parts. Initially a MR30365/07 dose-ascending, cohort group, single-blinded pilot study (part 1) was performed for dose-finding. After the pilot study was completed, the MR30365/07 doses were selected for the main study (part 2), a randomized, double-blind, placebo- and active comparator (fentanyl)-controlled parallel group study was performed.

5.2.1 SUBJECTS

One hundred and two healthy male volunteers (10 in the pilot study and 92 in the main study) participated in the study after approval of the protocol was obtained from the Leiden University Medical Center (LUMC) Human Ethics Committee and the Central Committee on Research Involving Human Subjects (CCMO, The Hague). Written and oral informed consent was obtained prior to enrolment into the study. All volunteers provided a medical history, and a physical examination, 12-lead ECG and blood screening was conducted before enrollment. The eligible volunteers were between the ages of 18 and 45 years, weighed between 60 and 100 kg, had a body mass index between 18 and 30 $kg.m^{-2}$, and a forced expired lung volume in 1 s of > 85% of predicted. Study subjects were healthy with no history of major medical disease, alcohol abuse, illicit drug use or heavy smoking. Volunteers could not have used medication (including vitamins, herbal and/or mineral supplements) in the seven days preceding dosing, or during the course of the study, or opioids or opioid antagonists in the 90 days prior to dosing. Finally, participants had to fast for 6 hours prior to the administration of study medication.

5.2.2 STUDY DESIGN

Pilot study. The respiratory effects of 3 escalating doses of MR30365/07 and 1 infusion of placebo were tested on 4 separate days with at least 1 week for wash-out between test sessions. Three subjects received 0.025, 0.05 and 0.1 $\mu g.kg^{-1}$ MR30365/07 and placebo (cohort 1), three others 0.0125, 0.075 and 0.1 $\mu g.kg^{-1}$ MR30365/07 and placebo (cohort 2) and the last three subjects 0.05, 0.125 and 0.15 $\mu g.kg^{-1}$ MR30365/07 and placebo (cohort 3). From the results of this study the doses of the main study were determined. After infusion of the drug was completed, ventilation was continuously measured breath-to-breath for 1 h under iso-hypercapnic conditions (see below).

Main study. In this double-blind randomized study 92 volunteers participated. None of them had been part of the pilot study and all were dosed only once. 46 subjects participated in the respiratory part of the study, 46 others in the analgesia part. In both parts, placebo (n = 6), 0.0125 $\mu g.kg^{-1}$ MR30365/07 (n = 4), 0.075 $\mu g.kg^{-1}$ MR30365/07 (n = 6), 0.125 $\mu g.kg^{-1}$ MR30365/07 (n = 6), 0.15 $\mu g.kg^{-1}$ MR30365/07 (n = 4), 0.5 $\mu g.kg^{-1}$ fentanyl (n = 4), 1 $\mu g.kg^{-1}$ fentanyl (n = 6), 2 $\mu g.kg^{-1}$ fentanyl (n = 6) and 3 $\mu g.kg^{-1}$ fentanyl (n = 4) were administered by intravenous infusion over 10 min. The randomization list was prepared by the sponsor of the study and sent to the local pharmacy where blinded syringes were prepared based on the weight of the subject. Each syringe was identical in size, drug volume and color and was unmarked. The randomization list was available to the sponsor, the pharmacy and an independent data safety monitoring committee.

Study medications. Citrate buffer, fentanyl and MR30365/07 were obtained from Mundipharma Research Limited (Cambridge, UK). All drugs were infused intravenously

(in the arm or hand) using a syringe pump (Beckton Dickinson, St. Etienne, France).

5.2.3 MEASUREMENTS

Respiratory measurements. Following infusion, ventilation was continuously measured on a breath-to-breath basis for 1 hour under iso-hypercapnic conditions. End-tidal gas forcing and data acquisition were performed using the dynamic end-tidal forcing technique (see Dahan *et al*.[9,10] for an explanation of the technique). In brief: Subjects breathed through a facemask connected to a pneumotachograph and differential pressure transducer (#4813, Hans Rudolph, Myandotta, MI). The pneumotachograph was connected to a custom-made gas mixing system attached to three mass-flow controllers (Bronkhorst, Veenendaal, The Netherlands). A computer delivered signal to the mass flow controllers so that the composition of the inspired gas could be adapted to steer the end-tidal oxygen and carbon dioxide concentrations according to a pre-set pattern over time. The inspired and expired oxygen and carbon dioxide concentrations and the arterial hemoglobin–oxygen saturation were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter, respectively (Datex-Engstrom, Helsinki, Finland). End-tidal concentrations of oxygen and carbon dioxide, inspired minute ventilation (Vi), and oxygen saturation were collected for further analysis. Ventilation levels and end-tidal concentrations were observed in real time on a breath-to-breath basis on a computer screen.
In the current study the end-tidal oxygen level was clamped to a value of 110 mmHg, while the end-tidal carbon dioxide concentration was slowly increased to a value that caused ventilation levels of 20 ± 2 $L.min^{-1}$. This end-tidal carbon dioxide value was maintained throughout the study. Respiratory measurements started when the inspired minute ventilation had reached a steady state; 4-5 min later drug infusion started. Respiratory measurements ended 60 min after the end of drug infusion (t = 70 min).

Pain measurements. Pain was induced using a transcutaneous electrical stimulus to the skin over the left tibial bone (10 cm above the ankle).[11] A 20 Hz (pulse duration 0.1 ms) stimulus train was delivered to the subject causing activation of cutaneous nociceptors. The stimulus train starts at 0 mA and was increased at a rate of 0.5 mA per 2 s (with a cutoff value of 128 mA). The delivery of the current is controlled by a computer via a current stimulator, which is connected to a control box with two buttons. The subject was instructed to press the first button when pain was felt (*i.e.* pain threshold) and the second button when the subject wanted the stimulus train to stop (*i.e.* pain tolerance). These respective currents were collected on disc for further analysis. The subject was familiarized with the system prior to the study to obtain reliable baseline values. In this study, the pain threshold values were used in the analysis. Four pain threshold values were obtained in the 30 minutes prior to drug infusion. These values were averaged and served as a baseline estimate. Following drug infusion, pain measurements were obtained

at the following time points (t = 0 is the start of drug infusion): 10 (end of infusion), 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 420 and 480 min.

5.2.4 SAMPLE SIZE AND STATISTICAL ANALYSIS

The pilot study was designed to determine which doses of MR30365/07 were to be tested in the main study. Four doses were chosen for the main study: 0.0125, 0.125, 0.075 and 0.15 $\mu g.kg^{-1}$.

For the main study, sample size selection was achieved by performing a power analysis in NONMEM,[12] using estimated data on the effect of opioids on respiratory depression.[8] We assumed inter-subject variability in effect of 50% ($\omega^2 = 0.25$) and a 10% residual error for effect ($\sigma^2 = 0.01$) and aimed to detect a value of $\rho < 0.5$ or > 2.0 (where $C_{50A,\ MR30365/07}/C_{50R,MR30365/07}$) $= \rho \times C_{50A,FENTANYL}/C_{50R,FENTANYL}$ and C_{50A} and C_{50R} are the concentrations causing 50% analgesia and respiratory depression for drugs MR30365/07 and fentanyl, respectively), with $\alpha < 0.05$ and $\beta = 0.8$. In the analysis we assumed that $C_{50A,MR30365/07} = C_{50A,FENTANYL}$ (i.e. concentrations are equianalgesic). Values of $\rho < 0.5$ indicate that fentanyl produces respiratory depression at concentrations at least twice as low as MR30365/07 and vice versa for $\rho > 2$. It was assumed that the logarithm of the C_{50} ratio has a normal distribution with variance = 1. The sample size selection was next verified by simulations in NONMEM with 1,000 simulated data sets. The analysis resulted in a sample size of 34, which was rounded upwards to 40 (20 subjects per opioid treatment). Six additional subjects were added to receive placebo. The subject number chosen for the analgesia part of the study was identical to that calculated for the respiratory part of the study, as we, somewhat arbitrarily, assumed similar drug effects and variability.

Average respiratory drug effect. The breath-to-breath data were averaged over 1-min episodes. In order to get an impression of the average drug effect on respiration, we calculated the area below the respiration curve (AUC) from t = 0 to t = 70.[5,13] The AUC was subtracted from the area obtained by taking baseline ventilation forward (BASELINE AREA, from t = 0 to t = 70 min, see Fig. 1). Next the data were normalized by the baseline area giving an average % of respiratory depression (average drug effect = [baseline area AUC – AUC]/baseline area AUC). An average drug effect of 40 indicates an average of 40% respiratory depression over the measured time period (0-70 min). The average drug effect and time to peak effect were analyzed using a one-way analysis of variance (factor dose). The MR30365/07 and fentanyl data were analyzed separately in Sigmaplot v12.3 (Systat Software GmbH, Ekrath, Germany). *P*-values < 0.05 were considered significant. Values given are mean ± SD.

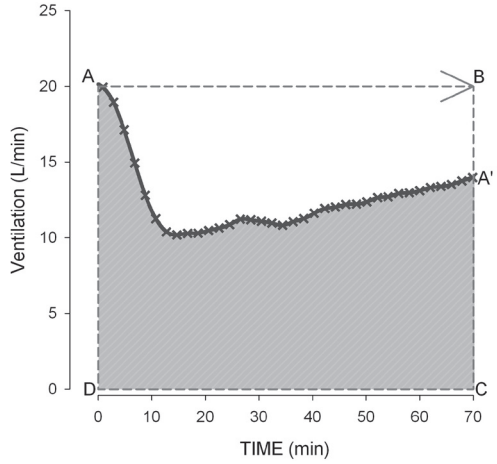

Figure 1. Calculation of the average respiratory drug effect. First the area-under-the curve (AUC) is calculated for the respiration curve (line from A to A'). This AUC (grey field) was subtracted from the area obtained by taking baseline ventilation (A) forward (the arrow from point A to B; the baseline area AUC is the box depicted by the red broken lines ABCD). Next the data were normalized by the baseline area giving an average % of respiratory depression (average drug effect = [baseline area AUC – AUC]/baseline area AUC).

Peak respiratory depression. For each subject peak respiratory depression was calculated as the nadir in ventilation and presented as ratio relative to baseline (*e.g.* a value of 0.5 indicates a nadir in ventilation in magnitude 50% of baseline ventilation). Using the statistical package *R* (version 8.2; www.r-project.org), a sigmoid EMAX function was fitted through the MR30365/07 and fentanyl dose-effect data (effect = peak respiratory depression) using a model of the form:

$$\text{Peak effect(dose)} = 100 + [E_{MIN} - 100] \times [\text{dose}^{\gamma} + ED50^{\gamma}] \qquad \text{eqn. (1)}$$

where ED_{50} is the dose causing a 50% effect (ventilation in the middle of baseline ventilation and E_{MIN}), E_{MIN} the asymptotic minimum in ventilation, and γ a shape parameter. *P*-values < 0.01 were considered significant. The data analysis was performed on the complete data set (fentanyl data and MR30365/07 data from pilot and main studies). The data are presented as mean ± SD.

Analgesic effect. Two measures of analgesic effect were calculated in each experiment: peak analgesia (defined as the highest value of pain threshold in mA) and average analgesic effect (as defined as the area under the pain threshold curve from t = 0 to t = 8 h normalized by the baseline area, see above). Peak and average analgesic effects were analyzed using a one-way analysis of variance (factor 'dose'). The MR30365/07 and fentanyl data were analyzed separately using SigmaPlot v. 12.3. *P*-values < 0.05 were considered significant. Values given are mean ± SD.

5.3 RESULTS

Pilot study. Nine volunteers completed the study without unexpected side effects. One subject developed ECG changes that, although not clinically relevant, precluded proper assessment of the effect of the study medication on the ECG. As a precautionary measure,

another subject replaced this subject after having completed a placebo and 0.05 μg.kg^{-1} MR30365/07 experiment. The clamped end-tidal PCO_2 was 6.6 ± 0.5 kPa (49.5 ± 4.5 mmHg) and baseline (pre-drug) ventilation was 21.5 ± 1.7 L.min^{-1}. The mean respiratory responses to MR30365/07 are given in Fig. 2A. At all dosages, the drug displayed a nadir in ventilation, which occurred at t = 17.1 ± 3.8 min following the start of drug infusion. The respiratory responses to MR30365/07 dosages of 0.075, 0.10, 0.125 and 0.15 μg.kg^{-1} overlap. The dose-response curves (peak respiratory depression and average drug effect) are given in Figs. 2B and C showing that the dose-response levels off at dosages of 0.075 μg.kg^{-1} and greater (MR30365/07 at 0.075, 0.125 and 0.15 μg.kg^{-1}: $P > 0.05$).

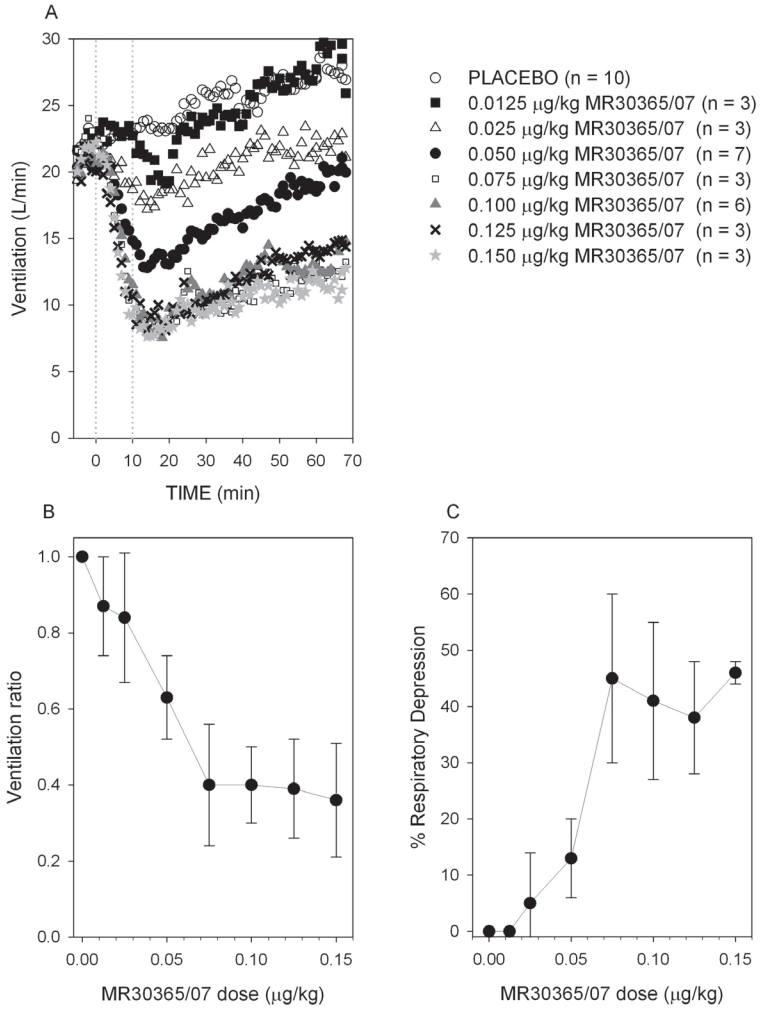

Figure 2. Results of the pilot study. **A**. Mean respiratory responses to placebo and MR30365/07. **B.** Dose-response data: Peak respiratory depression. **C**. Dose-response data: Average drug effect. The data in panels B and C are mean ± SD.

A small positive trend was observed in the ventilation data as was best observed in the placebo responses (Figs. 2-4). The magnitude of the trend ranged from 30-60 ml.min^{-2} (about 1.5-3% of total ventilation) and corresponds with the presence of a slow component (time constant about 1 h) in the ventilatory response to CO_2.[9,14]

Main study: Respiration. All 46 subjects completed the study without unexpected side effects. In the MR30365/07 experiments, the end-tidal PCO_2 was clamped at 6.8 ± 0.2 kPa (51.0 ± 1.5 mmHg) and baseline (pre-drug) ventilation was 19.3 ± 1.4 L.min^{-1}. The

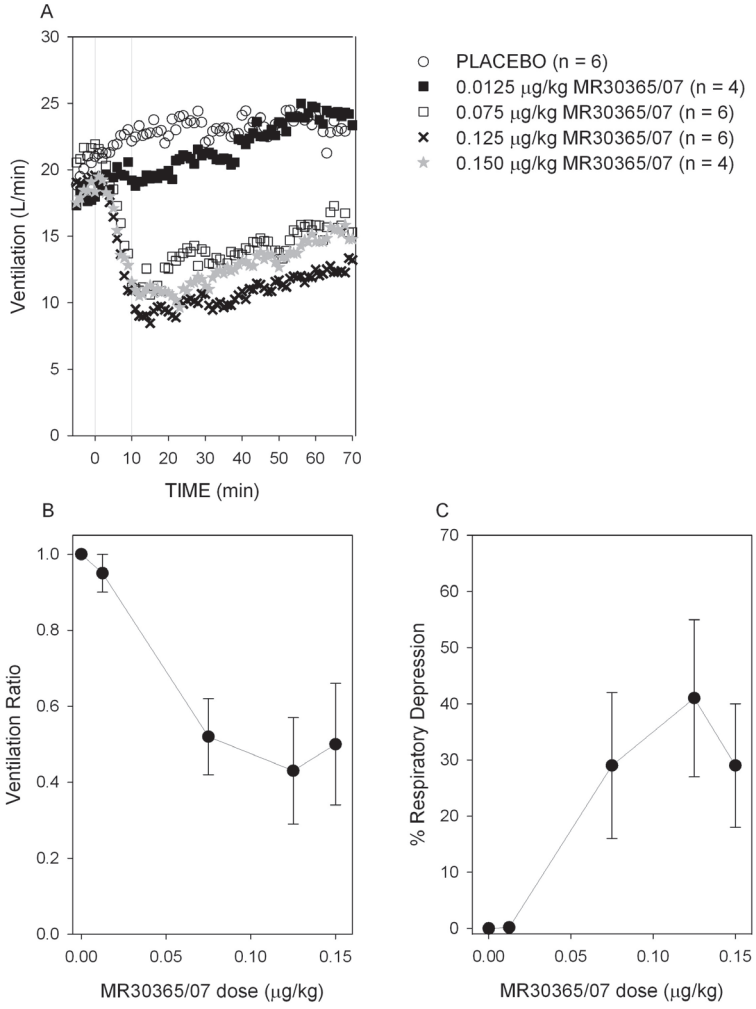

Figure 3. Results of the main study: Effect of MR30365/07 on respiration. **A.** Mean respiratory responses to placebo and MR30365/07. **B.** Dose-response data: Peak respiratory depression. **C.** Dose-response data: Average drug effect. The data in panels B and C are mean ± SD.

mean respiratory responses to MR30365/07 are given in Fig. 3A. No nadir in ventilation was observed in the placebo data and the lowest MR30365/07 dose tested. The time to peak effect was dose-independent and occurred at 17.3 ± 5.5 min. The dose-response curves for peak respiratory depression and average drug effect are given in Figs. 3B and C, respectively, showing that the dose-response levels off at a ventilation level of approximately 50% of baseline. None of the subjects that received MR30365/07 developed irregular breathing or apnea.

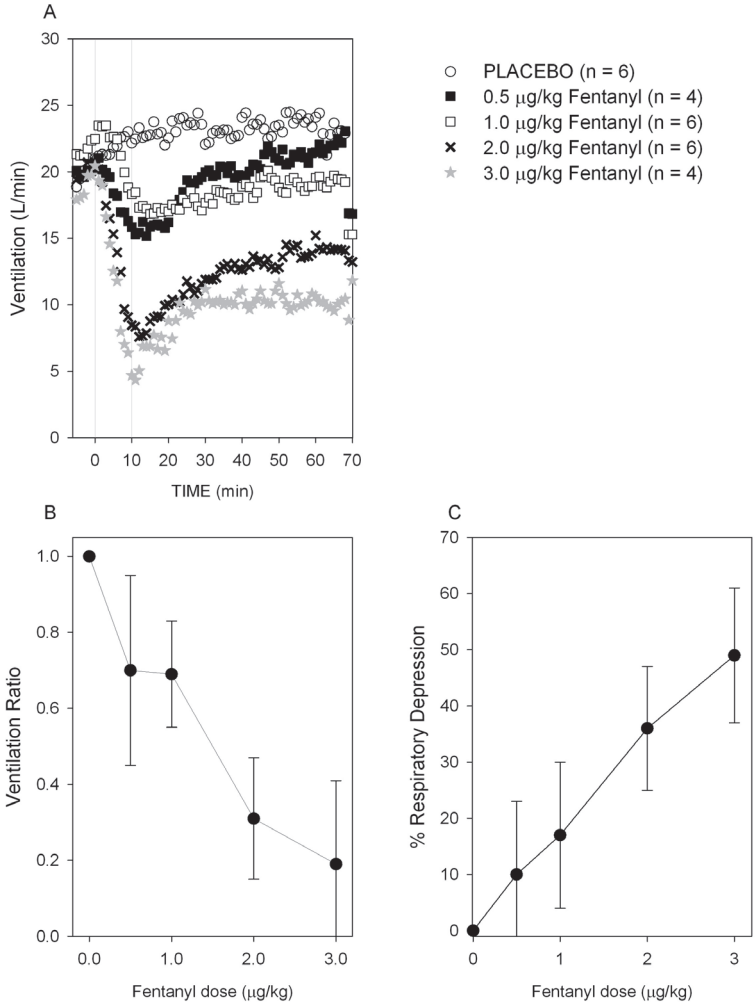

Figure 4. Results of the main study: Effect of fentanyl on respiration. **A**. Mean respiratory responses to placebo and the four fentanyl dosages. **B.** Dose-response data: Peak respiratory depression. **C**. Dose-response data: Average drug effect. The data in panels B and C are mean ± SD.

In the fentanyl experiments, the end-tidal PCO_2 was clamped at 6.6 ± 0.1 kPa (49.5 ± 0.8 mmHg) and baseline (pre-drug) ventilation was 20.2 ± 0.9 $L.min^{-1}$. A nadir in respiratory response was observed for all doses tested (Fig. 4A). The time to peak effect was dose-independent and occurred on average at 12.8 ± 2.1 min. The dose-response curves for peak respiratory depression and average drug effect are given in Figs. 4B and C, respectively. Dose-dependent respiratory depression was apparent in peak ventilation ($P < 0.001$) and average drug effect ($P < 0.001$). The maximum observed respiratory depression was observed at the highest fentanyl dose tested (3 $\mu g.kg^{-1}$; peak effect = 19% of baseline). Two subjects developed irregular breathing after the highest dose of fentanyl, one of which developed apnea (defined by the absence of breathing activity > 20 s), just after ending the 10-min fentanyl infusion.

The parameter estimates of the model analysis of peak respiratory depression are given in Table 1. The model fits are given in Figs. 5A (MR30365/07) and B (fentanyl). Two parameters were significantly different between treatments ($P < 0.01$): ED_{50} and E_{MIN}. An apparent 30-fold difference in potency was observed with ED_{50} values of 0.04 $\mu g.kg^{-1}$ for MR30365/07 and 1.27 $\mu g.kg^{-1}$ for fentanyl. For fentanyl the value of E_{MIN} or the asymptotic minimum ventilation was not different from zero, but greater than zero for MR30365/07: 32.8% of baseline ventilation or 6.6 $L.min^{-1}$ ($P < 0.01$). The shape parameter γ and residual error variance (σ^2) did not differ between treatments.

Main study: Analgesia. All 46 subjects completed the study without unexpected side effects. Baseline pain thresholds were 11.8 ± 0.9 mA (MR30365/07), 12.7 ± 0.4 mA (fentanyl) and 11.0 ± 0.6 mA (placebo). The effect of placebo was limited with an effect no greater than 10% of baseline. Both MR30365/07 and fentanyl produced dose-dependent effects in terms of peak analgesic effect and average drug effect (Figs. 6 A and B; drug-effect: $P < 0.01$) with no indication of reaching a ceiling.

5.4 DISCUSSION

Development of novel opioid analgesics with limited respiratory depression at high doses is highly relevant as OIRD is a major cause of opioid morbidity and mortality.[1-3] In this experimental phase 1 study we showed that in contrast to fentanyl, MR30365/07 displayed a ceiling in respiratory depression in the tested dose range starting at 0.075 $\mu g.kg^{-1}$. The respiratory effects of MR30365/07 0.075, 0.10, 0.125 and 0.15 $\mu g.kg^{-1}$ overlap with peak respiratory depression at about 40% of baseline ventilation. Fentanyl showed dose-dependent respiratory depression with, at high dose, irregular breathing and apnea. Neither fentanyl, nor MR30365/07, produced a ceiling in analgesia over the dose range tested.

We used the computer-steered 'dynamic end-tidal forcing' or DEF technique to clamp end-tidal PCO_2 to a fixed ventilation level of 20 ± 2 $L.min^{-1}$.[9,10] On average this was achieved by increasing end-tidal PCO_2 to 6.65 kPa (50 mmHg). The advantages of the DEF technique

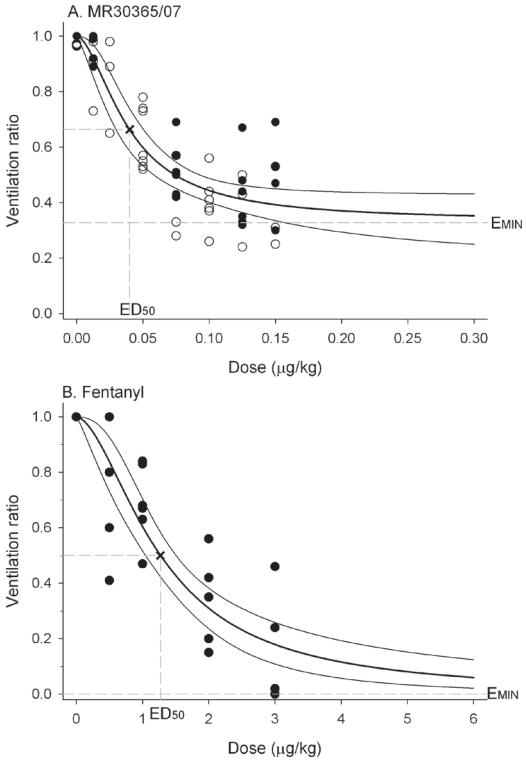

Figure 5. Model fits of peak respiratory depression versus dose for MR30365/07 **(A)** and fentanyl **(B)**. On the y-axis, ventilation relative to pre-drug baseline ventilation. The continuous thick lines are the model fits and the thin lines are the 2.5% and 97.5% percentiles. The curves are extrapolated to 0.3 $\mu g.kg^{-1}$ MR30365/07 and 6 $\mu g.kg^{-1}$ fentanyl. In panel **A,** the closed circles are data from the main study, the open circles are data from the pilot study. In panels **A** and **B,** the respective ED_{50} and E_{MIN} values are depicted by the symbol × and broken grey lines. For both drugs the ED_{50} is the dose half-way between baseline ventilation and E_{MIN}; for fentanyl this is at 50% respiratory depression, for MR30365 at 33.6%.

Table 1. Parameter estimates of the model analysis of peak respiratory depression

Parameter	Mean	SD	2.5% percentile	97.5% percentile
ED_{50} MR30365/07 ($\mu g.kg^{-1}$)	0.04	0.009	0.026	0.06
ED_{50} Fentanyl ($\mu g.kg^{-1}$)	1.27	0.116	1.04	1.50
γ	1.80	0.32	1.23	2.51
E_{MIN} MR30365/07 (% of baseline)*	32.8	0.06	16.7	42.6
E_{MIN} Fentanyl (% of baseline)*	0	-	-	-
σ^2	0.014	0.002	0.010	0.019

ED_{50} is the dose causing a 50% reduction in ventilation, E_{MIN} the asymptotic minimum in ventilation, γ a shape parameter, and σ^2 the variance of the residual error.

* baseline ventilation = 100%.

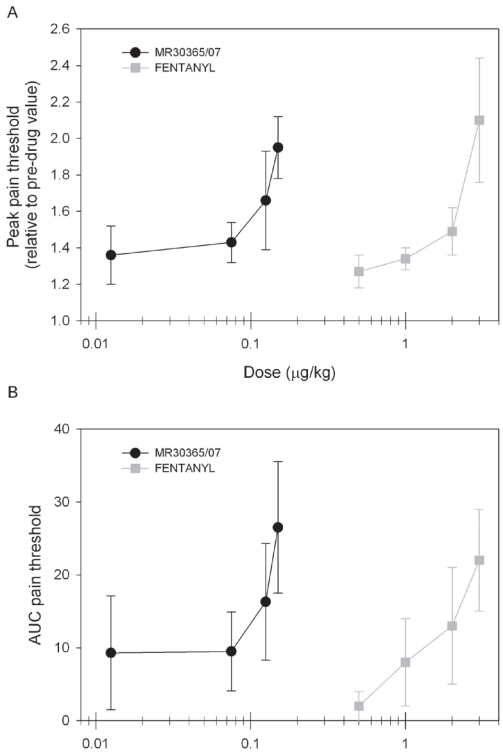

Figure 6. Results of the main study: Effect of MR30365/07 and fentanyl on pain threshold. **A.** Peak analgesia, defined as the highest value of pain threshold in mA). **B.** Average analgesic effect, defined as the area under the pain threshold curve (AUC) from t = 0 to t = 8 h normalized by the baseline area. Note that the logarithmic x-axis scales.

over more conventional techniques are that respiratory response of the test drug is (i) independent of the confounding effects of changes in arterial CO_2 and (ii) independent of the speed of administration of the drug.[5,6,15] These two items are interconnected. For example, we showed previously that administration of remifentanil aimed at a target plasma concentration of 5 ng.ml^{-1} will cause severe respiratory depression when the drug target is reached after 5 min.[14] This is due to the rapid transport of the drug to the brain causing immediate depression of brainstem respiratory neurons (no CO_2 having accumulated at this time). Respiratory depression is less likely when the remifentanil target is reached after 30 min. Part of the respiratory depression is offset by the accumulation of CO_2 in the brain compartment. Performing studies under poikilocapnic conditions would therefore lead to an underestimation of respiratory potency of a drug as is exemplified by the study of Mildh *et al*. for fentanyl.[16] They estimated the fentanyl plasma concentration that caused a 50% depression in respiration at 6.1 ng.ml^{-1}. Considering the changes in arterial CO_2 (for example, by modeling the stimulatory effects of CO_2 on the respiratory control system)

or by using the DEF technique, a value of 1.1 $ng.ml^{-1}$ is estimated;[8] 6-fold lower than estimated from poikilocapnic studies and in agreement with clinical observations. The DEF technique allows reliable comparison of drug effect on the respiratory control system. In our studies, the differences observed for MR30365/07 and fentanyl in dose-response relationship (ceiling in respiratory depression for MR30365/07 but not fentanyl), the more rapid onset of effect for fentanyl (peak effect occurred at 2.8 min after the end of the 10 min infusion versus 7.3 min for MR30365/07) and the longer-lasting respiratory depression are due to CO_2-independent differences in pharmacokinetics and pharmacodynamics.

From a clinical perspective, the existence of ceiling in respiratory depression is advantageous only when no ceiling in analgesic efficacy exists or when ceiling occurs at much higher (supra clinical) drug concentrations. Indeed, in our study we observed that over the dose range tested, both fentanyl and MR30365/07 displayed a dose-dependent increase in peak pain response and average analgesic effect (Figs. 6A and B). These data give reasonable evidence that for MR30365/07, in contrast to respiration, pain relief does not display ceiling over the dose range tested. At the highest dose tested both drugs produced an increase in pain threshold of about 100% (MR30365/07 0.15 $\mu g.kg^{-1}$ response = 1.95 × pre-drug response; fentanyl 3.0 $\mu g.kg^{-1}$ response = 2.1 × pre-drug response). This indicates a MR30365/07-fentanyl difference in potency of 18.5. This difference is smaller than the apparent potency difference observed for respiratory depression (factor = 30). However, since the ED_{50} is an estimation of ventilation in the middle of baseline ventilation and E_{MIN}, a better comparison than ED_{50} would be the dose causing 50% depression of ventilation (in absolute values). For fentanyl this is identical to ED_{50} (1.27 $\mu g.kg^{-1}$), and for MR30365/07 this is 0.075 $\mu g.kg^{-1}$. This then suggests a potency difference of 17 very similar to the value observed for antinociception. Taking all data together, our data suggests that MR30365/07 has a better therapeutic window than fentanyl.

Three issues remain unresolved by our studies. The first is the cause of the ceiling in MR30365/07 respiratory effect. To the best of our knowledge, ceiling in respiratory depression has been demonstrated in humans for only one other opioid, buprenorphine.[5,6] The partial agonism (*i.e.* partial effect despite full receptor occupancy) of buprenorphine at the MOR is considered responsible for its ceiling effect. This mechanism is not relevant for our studies as MR30365/07 is a full agonist at the MOR. Lutfy *et al.*,[7] however, propose a different mechanism for development of ceiling. They showed lack of buprenorphine-induced antinociception in MOR knockout mice but enhancement of antinociception in ORL1 receptor knockout mice with loss of ceiling in analgesia. In that study, it was concluded that the concurrent activation of ORL1-receptors severely compromised the MOR-mediated antinociceptive effect of buprenorphine in mice.[7] In contrast to these data, a recent study in primates shows that ORL1 receptor agonists enhance buprenorphine-induced antinociception, however, without causing additional respiratory depression.[17] These data suggest that ORL1 activation may be implicated in the mechanism of ceiling in respiratory effect as observed in our studies. However, although MR30365/07 has affinity

for the ORL1 receptor, its Ki is several orders of magnitude higher than for the MOR (confidential data, Mundipharma Research Limited). Whether such low affinity for the ORL1 receptor is sufficient to cause the profound ceiling we observed is questionable. Another possible mechanism may be related to MR30365/07's high affinity for the KOR, which is approximately 1 order of magnitude lower than for the MOR (confidential data, Mundipharma Research Limited). There is evidence that KOR agonists may selectively antagonize MOR agonist effects, including respiratory depression.[18,19] For example, the KOR agonist U50,488H antagonized MOR agonist-induced respiratory depression in the rat, a KOR mediated effect.[18] It may well be that at high doses the MR30365/07-induced and MOR-mediated respiratory depression is antagonized by the effect of MR30365/07 at the KOR. KOR agonists have been associated with dysphoria.[20] Interestingly, in our study, over a 24-h observation period no significant differences in dysphoria were observed between MR30365/07 and fentanyl.

A final proposed mechanism involves the intra neuronal regulatory protein β-arrestin.[6] Opioid receptors belong to the 7-transmembrane G-protein-coupled receptors that, upon activation, bind to intracellular G-proteins and β-arrestin 1 and/or β-arrestin 2 proteins. Recent studies show that absence of β-arrestin 2 protein causes the attenuation of morphine-induced respiratory depression with maintained antinociception.[21,22] It was hypothesized that (G-protein independent) activation of β-arrestin 2 is involved in MOR signal transduction of respiratory neurons but not in neurons involved in modulation of pain pathways.[21] It may well be that extent of G protein and of β-arrestin 2 activation is ligand specific. Such ligand-specific, *i.e.* biased differences in G protein and β-arrestin activation, has been first described for the angiotensin1A receptor.[23] Extrapolating these findings to our study would suggest that our results are then well explained by a lesser ability of MR30365/07 to activate β-arrestin 2. Further work is required to elucidate the mechanism by which ceiling of respiratory effect is observed at the high MR30365/07 doses as observed in our study.

A second issue is the mechanism of the differential MR30365/07 effect on respiration and analgesia. We previously proposed and discussed that this may be due to a difference in receptor density at brain sites involved in analgesia versus brain sites involved in respiratory depression.[6] This has for example been demonstrated experimentally by performing a progressive MOR knockdown by administration of the MOR antagonist β-funaltrexamine.[24] Reduction of opioid bindings sites transformed the MOR agonist alfentanil into a partial agonist. Another mechanism involved may be the lesser ability of MR30365/07 to engage the transduction protein β-arrestin 2, as discussed above. This latter mechanism explains both the observed ceiling effect in MR30365/07-mediated respiratory depression and the selectivity of the ceiling effect.

A third issue is whether the experimental observation of ceiling in the respiratory effects of MR30365/07 may be extrapolated to the clinical setting, and whether such a phenomenon

indeed leads to less respiratory events in patients. This is a highly relevant topic as there has been a recent increase in the number of fatalities from misuse or abuse of legally prescribed opioids.[1-3,25] An apparent ceiling in respiratory depression at higher doses of MR30365/07 is certainly an advantage over other opioids that lack such a profile. However, whether this behavior persists in patients with their own complexities (co-medication, underlying disease, genetics, overdosing, etc.), needs to be further investigated.

In conclusion, we showed in this phase 1 study that in contrast to fentanyl, MR30365/07 shows ceiling in respiratory depression, but not analgesia over the dose range tested (0.0125-0.15 $\mu g.kg^{-1}$ MR30365/07).

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