British Journal of Anaesthesia **113** (5): 875–83 (2014)
Advance Access publication 2 July 2014 · doi:10.1093/bja/aeu182

BJA

GAL-021, a new intravenous BK_{Ca}-channel blocker, is well tolerated and stimulates ventilation in healthy volunteers

J. F. McLeod[1]*, J. M. Leempoels[2], S. X. Peng[1], S. L. Dax[1], L. J. Myers[3] and F. J. Golder[1]

[1] Galleon Pharmaceuticals Corporation, 213 Witmer Road, Horsham, PA 19044, USA
[2] SGS Life Science Services, Lange Beeldekensstraat 267, Antwerpen B-2060, Belgium
[3] Vivonoetics, 27 Dorian, Newport Coast, CA, 92657, USA

* Corresponding author. E-mail: jmcleod@galleonpharma.com; james@mcleod.bz

Editor's key points

- Activated BK_{Ca} (voltage and calcium sensitive K^+ channels) allow K^+ efflux, causing cell hyperpolarization.
- Blockage of these receptors in the carotid bodies increases respiratory drive.
- The authors performed a first-in-human study of a BK_{Ca}-channel blocker.
- At the highest doses tested, the agent stimulated ventilation but also caused a burning sensation at the administration site.

Background. Potassium-channels in the carotid body and the brainstem are important regulators of ventilation. The BK_{Ca}-channel contains response elements for CO, O_2, and CO_2. Its block increases carotid body signalling, phrenic nerve activity, and respiratory drive. GAL-021, a new BK_{Ca}-channel blocker, increases minute ventilation in rats and non-human primates. This study assessed the single-dose safety, tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of GAL-021 in healthy volunteers.

Methods. Thirty subjects participated in a nine-period, randomized, double-blinded, placebo-controlled, crossover, ascending dose, first-in-human study with i.v. infusions of 0.1–0.96 mg kg^{-1} h^{-1} for 1 h and intermediate doses up to 4 h.

Results. Adverse event rates were generally similar among dose levels and between placebo- and GAL-021-treated subjects. At higher GAL-021 doses, a mild/moderate burning sensation at the infusion site occurred during the infusion. No clinically significant changes in vital signs or clinical chemistries were noted. Minute ventilation increased ($AUE_{0-1\,h} \approx 16\%$, $P<0.05$) and end-tidal carbon dioxide (F'_{CO_2}) decreased ($AUE_{0-1\,h} \approx 6\%$, $P<0.05$) during the first hour at 0.96 mg kg^{-1} h^{-1} with 1/2-maximal $\dot{V}_E$ and F'_{CO_2}-change occurring by 7.5 min. Drug concentration rose rapidly during the infusion and decreased rapidly initially (distribution $t_{1/2}$ of 30 min) and then more slowly (terminal $t_{1/2}$ of 5.6 h).

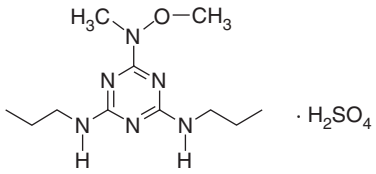
Conclusions. GAL-021 was safe and generally well tolerated with adverse events comparable with placebo except for an infusion site burning sensation. GAL-021 stimulated ventilation at the highest doses suggesting that greater infusion rates may be required for maximum PD effects. GAL-021 had PK characteristics consistent with an acute care medication.

Keywords: BK_{Ca} channel; breathing; carotid body; EudraCT: 2011-003371-11; KCNMA1; pharmacokinetics; potassium channel; respiratory stimulant; ventilatory function

Accepted for publication: 22 January 2014

Respiratory insufficiency in the hospital setting is a common, yet difficult-to-address, medical issue particularly in postoperative patients and those in the intensive care unit.[1] After surgical procedures, severe perioperative and post-procedure respiratory depression remain prominent concerns and occur in 0.5–2% and ≈5% of hospitalized patients, respectively (L. Brookes, 2012, Personal communication, Premier Research: Incidence of acute respiratory insufficiency post surgery in community hospitals).[2–4] These respiratory events are often associated with opioid analgesia and with significant additional morbidity and hospital costs. Prospective studies conducted to continuously monitor a patient's ventilatory and cardiovascular responses while receiving opioids report an incidence rate of 12% for O_2 desaturation <90% and an incidence of 41% for bradypnea rates in general surgical patients.[1 5] Certain surgical populations, such as bariatric patients, have oxyhaemoglobin desaturation events reported for nearly 100% of the patients studied.[6 7]

Respiration is controlled largely in the brainstem with input from higher brain structures and peripheral nerves. Chemoreceptors exist both centrally in the nucleus tractus solitarius (NTS) and peripherally (e.g. carotid body) and are sensitive to oxygen tension, carbon dioxide tension, pH, and other chemical stimuli.[8 9] The NTS is the principal site of termination for ventilatory-related sensory afferents arising from the lungs, airways, and peripheral chemoreceptors. The NTS connects to the pre-Botzinger complex forming the primary pacemaker for normal respiration.[10–12] Inhibition of several ion channels (e.g. BK_{Ca}, TASK-1, and TASK-3) in the carotid body glomus cells has been associated with an increase in respiratory drive and minute ventilation.[13 14]



GAL-021 hydrogen sulphate salt

Fig 1 Chemical structure of GAL-021 (2-*N*,*O*-dimethylhydroxylamino-4,6-bispropylamino-*S*-triazine). The drug product is prepared as a H_2SO_4 salt.

GAL-021 (Fig. 1) inhibits large-conductance Ca^{2+}/voltage-activated K^+ channels, BK_{Ca} (also known as Maxi-K, KCNMA1, Slo1) primarily working through the carotid body.[15] Upon i.v. administration by bolus or infusion to rats[16] or cynomolgus monkeys,[17] GAL-021 elicits dose-dependent increases in minute ventilation and shows robust, dose-dependent, reversal of opioid (morphine/fentanyl), benzodiazepine (midazolam), and anaesthetic (isoflurane/propofol)-induced respiratory depression. Unlike opioid receptor antagonists, GAL-021 does not reverse or compromise opioid analgesia in rats.[18]

GAL-021 is being developed as an i.v. therapeutic agent for short-term use to stimulate ventilation in patients with acute respiratory insufficiency, including such conditions as opioid-induced respiratory depression and post-anaesthetic atelectasis. GAL-021 is intended to increase minute ventilation by increasing tidal volume and secondarily through increasing respiratory rate (RR). It is in these postoperative care settings that GAL-021 will likely provide benefit by stimulating ventilatory drive without antagonizing μ-opioid receptors, reversing analgesia, or acting as a generalized CNS stimulant. The current study was the initial, first-in-human (FIH), GAL-021 clinical study with the primary objectives of determining safety, tolerability, pharmacokinetics (PKs), and ventilatory pharmacodynamics (PDs) of the compound.

Methods

The study was conducted at a single centre (SGS Life Science Services, Antwerpen, Belgium) after approval by the ethics committee (Commisie Voor Medische Ethiek—Ziekenhuisnetwerk Antwerpen: 001-4047409-63) and the Belgium Federal Agency for Medicines and Health Products (EudraCT clinical trial registry: 2011-003371-11). This was a randomized, double-blinded, placebo-controlled, nine-period, single-rising dose study in three alternating cohorts of healthy subjects. The inclusion criteria were: 18–45 yr of age, BMI of 18–30 kg m^{-2}, body weight of ≥60 to ≤90 kg with normal screening clinical laboratory/haematology/urinalysis, pulmonary function tests (>80% of predicted), and oxyhaemoglobin saturation (Sp_{O_2}). Subjects were excluded for concurrent psychiatric disease, drug use/alcohol abuse within the past 2 yr, tobacco product use, recent blood donation, pulmonary disease history, clinical trial participation within the past 8 weeks, or daily caffeine consumption >750 mg. Females had to be postmenopausal or surgically sterilized. The rotating panel study design was chosen to better assess safety and tolerability and to provide assessment of intrasubject PK dose proportionality and potentially PD dose response. Three cohorts of 10 subjects each (8 treated, 2 placebo) received 3 treatments each (Table 1). The subjects randomized to placebo during the first period also received placebo during the second and third periods. Similarly, drug-treated subjects received GAL-021 in all the three periods. Safety and PK were evaluated between dose levels. The infusion rate was reduced for longer infusion periods to maintain similar C_{max} concentrations.

Table 1 Study design with treatment period, subject panel/cohort, and GAL-021 infusion rate and duration. During the first dosing period, all panels had 10 subjects

Treatment period	Cohort	Infusion rate and duration (mg kg^{-1} h^{-1} × h)
1	A	0.1 × 1
2	B	0.3 × 1
3	C	0.3 × 1
4	A	0.6 × 1
5	B	0.96 × 1
6	C	0.72 × 2
7	A	0.72 × 3
8	B	0.54 × 4
9	C	0.72 × 1, then 0.36 × 3

Subjects were screened up to 28 days before the treatment period. On the evening before dosing, subjects were admitted and baseline safety evaluations conducted. After an overnight fast, subjects received an i.v. infusion of GAL-021 or placebo. PD, PK, and safety evaluations were conducted during the next 24 h. Laboratory tests (haematology, clinical chemistry, and urinalysis), vital signs, ECG parameters (telemetry and 12-lead ECG), physical examinations, blood pressure (BP), heart rate, adverse events, pulmonary function tests, and Ramsay Sedation Scale were collected throughout the dosing periods to assess safety and tolerability. After their last treatment, subjects returned 1 week later for final safety assessments.

Adverse events were classified by body system/organ class and severity. For vital signs, change and percentage change from predose was integrated during the infusion, the next 2 h thereafter, and from then to the 9-h timepoint. PD endpoints were assessed by two independent systems: a Dräger Infinity Delta Series patient monitor (Drägerwerk AG, Lübeck, Germany, software VF8) and a portable respiratory monitor (NOX-T3, NOX Medical, Reykjavik, IS). The Dräger system assessed minute ventilation ($\dot{V}_E$), RR, tidal volume (V_T) via pneumotachometer, and oxyhaemoglobin saturation (Sp_{O_2}), and end-tidal carbon dioxide (F'_{CO_2}) partial pressure by Capnostat sensor. The NOX-T3 device monitored RR, V_T via respiratory inductance plethysmography (RIP), Sp_{O_2} (Nonin), actigraphy, and nasal air flow. Relative gains in rib cage and abdominal RIP signals were determined from 5 min of regular natural

breathing during the baseline period using the qualitative diagnostic calibration method.[19] The weighted RIP signal was then calibrated to each subject's average baseline V_T from the pneumotachometer.

Ventilatory PD data ($\dot{V}_E$, RR, V_T, F'_{CO_2}) were collected with subjects lying in a partially upright position and evaluated over a 60-min baseline interval before dosing; 5-min intervals centred at 7.5, 22.5, 37.5, and 52.5; and 10 min intervals centred at 75 and 105 min post-infusion initiation. For longer infusions (Periods 6–9), evaluations were performed for 10-min intervals centred at 135 and 165 min (2+ h), 195 and 225 min (3+ h), and 265 and 285 min (4 h). Before unblinding, PD data were evaluated by polysomnographers to identify high-quality recording periods from which the respiratory parameters were assessed (Vivonoetics, Newport Coast, CA, USA). Before the study, the Dräger pneumotachometer was designated as the primary device for ventilatory PD assessments. The NOX-T3 device was included for comparison with pneumotachometers across several studies (cross-study data not presented). Individual change and percentage change from predose were calculated and integrated over the 0–1 and 0–2-h periods (2+-h infusions). The values at 1 and 2 h were estimated using the time weight average of the two flanking timepoints or last value carried forward when no subsequent timepoint was available. Group mean, median, standard deviation (SD), and standard error (SE) were calculated by dose level. Data from treatment Periods 6 and 7 were pooled for analysis during the first 2 h after infusion initiation. PD responses in placebo and active treated subjects were compared using unpaired *t*-test assuming non-similar distributions (Microsoft Excel 2007). The *P*-values reported are all in comparison with placebo. The integrated change in $\dot{V}_E$ during the first hour was declared as the primary PD variable before study start. If the primary PD endpoint result was statistically significant, other inferential comparisons were made without corrections for multiple comparisons.

GAL-021 (Fig. 1) was prepared as a sterile product for injection ready for dilution in normal saline (colourless, pH 3.1). GAL-021 or placebo (normal saline) was diluted in normal saline (final volume ≈250 ml) and administrated intravenously by infusion pump.

Venous blood samples for plasma GAL-021 concentrations were collected in K_2EDTA pre-infusion and at 0.25, 0.5, 0.75 (1-h infusion only), 1, 2, 3, and 24 h thereafter from the contralateral arm from the drug infusion. Additional PK samples were collected at infusion termination and 0.25, 0.5, 1, 2, 4, and 8 h thereafter. All GAL-021-treated subjects were included in the PK analysis. Plasma PK samples were analysed using a validated liquid chromatography with the tandem mass spectrometry method. The method precision (%CV) and accuracy (%RE) were <15% for all of the quality control sample concentrations (0.25, 0.75, 50, and 300 ng ml^{-1}). PK evaluations including compartmental analyses were performed using WinNonlin Professional Edition Version 5.3 (Pharsight Corporation, Mountain View, CA, USA) and Microsoft Excel (Version 2010). Concentration values below the lower limit of quantitation (<0.250 ng ml^{-1}) were treated as a zero for descriptive statistics. The concentration–time profile for each subject at each treatment was analysed individually in both the non-compartmental and compartmental PK analysis.

All PK parameters (C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, CL_p, V_z, and V_{ss}), except for t_{max} were transformed logarithmically for analysis, means, SDs, and coefficients of variation computed. The power model was used to evaluate dose proportionality of a PK parameter ($Y=\alpha\cdot\text{Dose}^{\beta}$). Dose proportionality was declared when β is 1 and ideal dose-independency is established when β is zero. *Y* is dose proportional when the 90% confidence interval (CI) for β falls within the critical region $[1+\ln(\theta_L)/\ln(r), 1+\ln(\theta_H)/\ln(r)]$, where β is the slope of the plot of log *Y* *vs* log Dose, $\theta_L=0.80$, $\theta_H=1.25$, and *r* is the ratio of the highest to the lowest dose.[20] *Y* is dose-independent when the 90% CI for β includes zero.

For compartmental modelling, individual plasma concentration–time profiles were visually inspected to determine which compartment models best fit the characteristics of the dataset. Compartmental modelling was performed using two- and three-compartment open models without lag time. In these multi-compartment models, a constant infusion input into the central compartment, first-order transfer between compartments, and first-order elimination from the central compartment were assumed. The following modelling parameters were considered to be log-normally distributed and included in the analysis. For the two-compartment model, V_1 [central compartment volume of distribution (V_D)], V_2 [peripheral compartment (V_D)], CL_1 (central compartment elimination clearance), and CL_2 (inter-compartment distribution clearance). For the three-compartment model, V_3 (deep peripheral compartment V_D) and CL_3 (slow inter-compartment distribution clearance) were added. Initial parameter values were generated by WinNonlin. A weighting factor of 1 yr^{-2} was utilized along with Gauss–Newton minimization algorithm for non-linear least squares regression.

Results

In total, 28 male and 2 female healthy volunteers (29 Caucasian, 1 Black) participated in the study with a mean age of 33.0 (7.6) yr [mean (SD)] (range: 20–44 yr), a weight of 77.5 (7.7 kg); a height of 1.77 (0.07 m), and a BMI of 24.8 (2.5 kg m^{-2}). The study was completed over a 9-week period with the interval between repeat dosing of the same subject ranging from 9 to 20 days. Three subjects discontinued the study early: one because of a non-serious adverse experience and because of protocol violations before their second treatment period. Eight dose levels were administered to three cohorts (Table 1) with the first seven dose levels infused at fixed rates (0.1–0.96 mg kg^{-1} h^{-1}) for 1, 2, 3, or 4 h and the last dose infused by a loading dose (0.72 mg kg^{-1} h^{-1} for 1 h) followed by a maintenance dose (0.36 mg kg^{-1} h^{-1} for 3 h).

Twenty-five subjects (83%) reported at least one adverse event (AE) during the study. All AEs were mild to moderate in severity. The most common AEs across all doses were venous pain at the infusion site (60%), headache (23%), nasopharyngitis (16.7%), nausea (10%), and catheter site pain in the

contralateral arm to the drug infusion site (10%). AEs that occurred more than once are presented in Table 2. Overall, the AE rate was similar across all dose levels, during the first, second, and third exposures, and comparable with placebo, except for the mild/moderate burning sensation at the infusion site. The burning sensation at the infusion site occurred only in GAL-021-treated subjects at higher dose levels. The infusion site sensation started usually soon after infusion initiation (≈15 min), generally declining in intensity during the infusion and ended soon after the infusion. There was no evidence of local or venous injury post-infusion. One subject experienced burning sensation shortly after the initiation of the infusion (0.96 mg kg^{-1} h^{-1}). Vasovagal syncope occurred and infusion was stopped at the fourth minute of infusion after which the AE rapidly resolved. No cardiac arrhythmias were noted and BP promptly returned to predose levels. Plasma drug concentrations at that timepoint were less than those during the previous dosing period which was well tolerated by the subject.

No clinically significant changes in blood chemistry, haematological parameters, PFT, or ECG occurred in any treatment group. Heart rate was more variable and increased generally during the day in all treatments without clinically meaningful differences from placebo-treatment. No significant changes in diastolic and systolic BP were observed across dose levels. During Periods 1–3, no mean arterial pressure changes were noted. During Periods 4 and 5 (second exposure), small mean arterial BP increased by 3.3–4.2%. The same subjects returned for a third treatment (Periods 7–9) with larger total exposures and had no significant change in mean arterial pressure (0.2–0.6%).

No clinical hyperventilation was noted during the study. RR varied during the 2-h assessment period (baseline: 12.0 min^{-1}) without a consistent post-dose pattern and a small mean integrated change usually <1 min^{-1}. Tidal volume (V_T) had an initial upward deflection at 7.5 min in all dose groups. Thereafter, V_T declined towards baseline in most groups. Over the 2-h assessment period, V_T changes were small with only the 0.96 mg kg^{-1} h^{-1} dose having a generally sustained increase of >10%. At the highest infusion rate, $\dot{V}_E$ increased rapidly, exceeding 1/2-maximal effect (14.5%) at the first point assessed at 7.5 min (Fig. 2A), had a sustained effect (14.5–18.9%) until infusion cessation, and gradually declined thereafter. F'_{CO_2} decreased in all groups after infusion initiation and remained below baseline during the first hour. The 0.96 mg kg^{-1} h^{-1} infusion produces a far greater decrease of F'_{CO_2}, which was statistically significant ($P<0.05$) at several timepoints, and then returned towards baseline after infusion cessation (Fig. 2B). The 0.72 mg kg^{-1} h^{-1} infusion (2 and 3 h duration) had a sustained F'_{CO_2} decrease which was statistically different ($P<0.05$) from the 37.5-min through the 2-h timepoints. Haemoglobin oxygen saturation (Sp_{O_2}) was unchanged in lower dose groups. At the highest infusion rate, mean Sp_{O_2} increased by 0.74–1.13% from a baseline of 95.5% and was statistically different at all timepoints ($P<0.05$) during the first hour.

To further analyse the responses, the area under the effect curve (AUE) was determined for 0–1 and 0–2-h intervals. Minute ventilation did not change significantly except at the highest infusion rate, at which the integrated $\dot{V}_E$ over the first hour (AUE_{0-1h}) was increased compared with placebo (16.1 *vs* 1.1%, $P<0.05$) (Table 3). In the pooled 0.72 mg kg^{-1} h^{-1} group, $\dot{V}_E$ AUE_{0-1h} increased to a lesser extent (6.3%, NS). During the first hour in Period 9, the same 0.72 mg kg^{-1} h^{-1} infusion rate was administered and similar PD effects observed (5.4%). When Period 9 data were pooled with Periods 6 and 7, the $\dot{V}_E$ AUE_{0-1h} was statistically significant ($P<0.05$) suggesting the lack of significance at this infusion rate was sample size-related. Greater minute ventilation changes were detected with the NOX-T3 device at the highest infusion rate [23.9 *vs* −2.9% (placebo); $P<0.05$] confirming the PD effect. The patterns of PD effects of $\dot{V}_E$, V_T, and RR were similar between the two devices with similar statistically significant differences among treatments. At 0.96 mg kg^{-1} h^{-1}, integrated F'_{CO_2} decreased (−5.6%) during the first hour and returned towards baseline during the second hour (−4.3%) ($P<0.05$, both intervals). At 0.72 mg kg^{-1} h^{-1}, F'_{CO_2} decreased

Table 2 Summary of adverse events with greater than a single occurrence. *Total number of individual subjects with specific adverse event. The total subjects with any adverse events include adverse events that occurred only once during the study and are not included in the table

Period	**1**	**2/3**	**4**	**5**	**6**	**7**	**8**	**9**	**All**	
Rate (mg kg^{-1} h^{-1})	**0.1**	**0.3**	**0.6**	**0.96**	**0.72**	**0.72**	**0.54**	**0.72/0.36**	**Placebo**	**Total subjects**
Duration (h)	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**1/3**	**various**	**reporting AE-**
# Subjects	**8**	**16**	**7**	**7**	**8**	**7**	**6**	**8**	**6×3 periods**	
Abdominal pain		1						1		2*
Common cold rhinitis				1	1				4	5*
Cough						1	1			2*
Headache	1	2	2	1		1	1	1	4	7*
Loose stools	1		1		1					2*
Nausea	1	2		1			1			3*
Venous pain contralateral (PK sampling site)			1			1			1	3*
Venous pain infusion site			2	5	4	6	5	6		18*
# Subjects with any AE	2	6	5	5	5	6	6	7	5	25

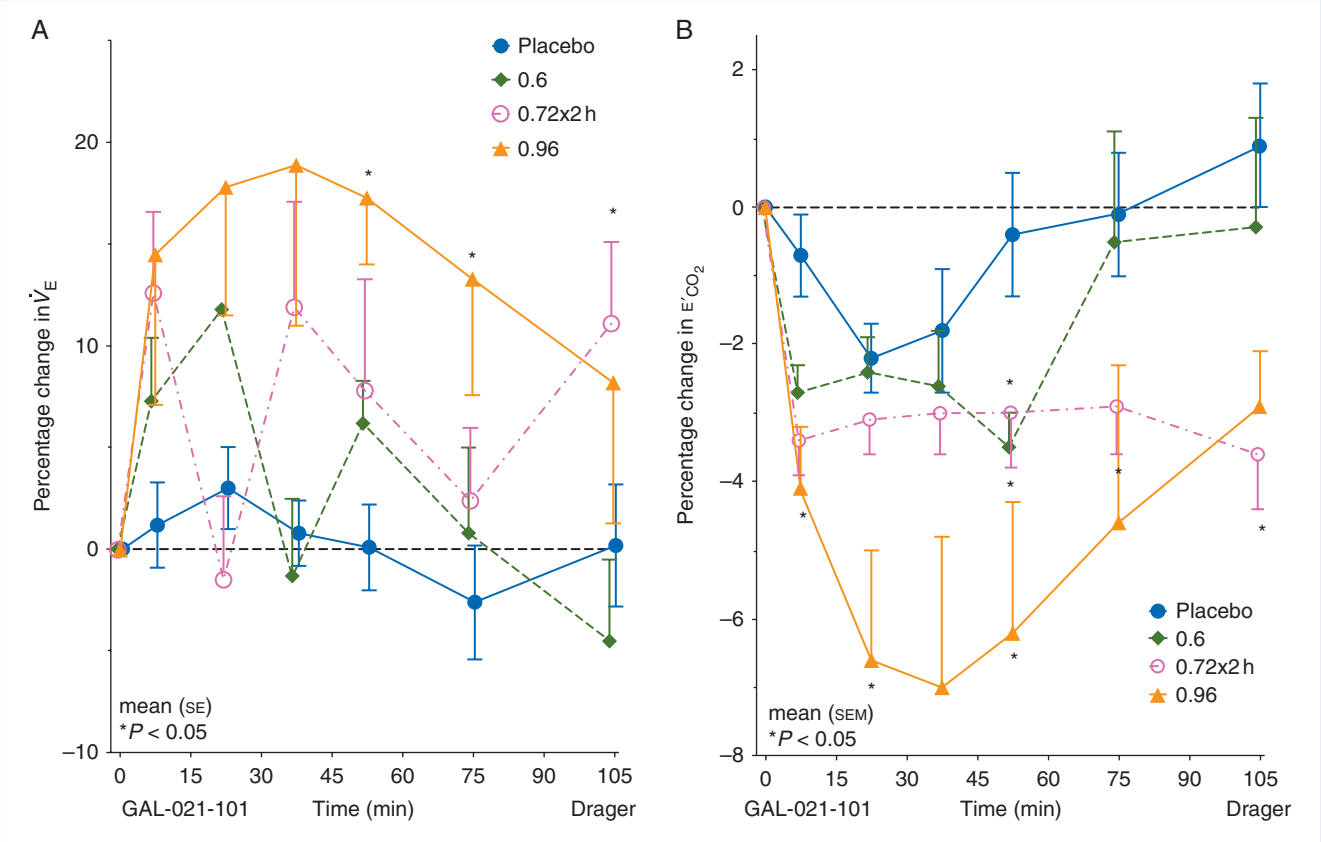


Fig 2 Percentage change in minute ventilation (A) and E'_{CO_2} (B) after initiation of GAL-021 infusion (mg kg^{-1} h^{-1}). Only the three highest dose infusion rates are displayed for clarity. Periods 6 and 7 with identical infusion 0.72 mg kg^{-1} h^{-1} rates were pooled for time course analysis as described in the Methods section [mean (SEM), *P<0.05].

Table 3 Integrated percentage change from baseline period during infusion during the interval from the start of infusion to first hour (0–1) and through the first 2 h (0–2) for infusions ≥2 h. Mean (SEM); *P<0.05 *vs* placebo group; †P<0.01 *vs* placebo group, All infusion times are for 1 h, except for 0.54 and 0.72 mg kg^{-1} h^{-1} which were two or more hours

Ventilatory parameters	Baseline	Duration (h)	Infusion rate (mg kg^{-1} h^{-1})						
			Placebo	0.1	0.3	0.54	0.6	0.72	0.96
Tidal volume	565 (31 ml)	0–1	−0.9 (1.7)	4.4 (4.5)	3.8 (3.8)	2.8 (3.9)	3.0 (2.5)	1.5 (1.9)	15.0 (10.4)
		0–2	−1.5 (1.5)			0.6 (2.9)		0.9 (1.9)	
RR	12.0 (0.7 min^{-1})	0–1	2.2 (2.0)	0.1 (2.7)	−1.1 (2.8)	1.9 (3.7)	3.1 (2.1)	5.7 (2.5)	3.5 (5.7)
		0–2	2.0 (2.0)			3.4 (3.0)		5.8 (2.4)	
Minute ventilation	7.1 (0.3 litre min^{-1})	0–1	1.1 (1.2)	1.3 (1.5)	3.0 (1.8)	4.3 (3.3)	5.4 (2.9)	6.8 (2.6)	16.1 (5.2*)
		0–2	0.2 (1.7)			3.6 (3.2)		6.5 (2.8)	
E'_{CO_2}	5.46 (0.01 kPa)	0–1	−1.2 (0.5)	−1.1 (1.1)	−1.4 (0.4)	−1.8 (0.5)	−2.6 (0.4)	−2.9 (0.4*)	−5.6 (1.2*)
		0–2	−0.4 (0.5)			0.0 (0.9)		−2.6 (0.5†)	

(−2.9%) during the first hour, which was unchanged during the second hour of continued infusion (−2.5%) (P<0.05, both intervals). At 0.96 mg kg^{-1} h^{-1}, integrated Sp_{O_2} during the first hour increased 0.85% compared with placebo (−0.05%, P<0.01).

At all infusion rates, the shape of plasma concentration–time profiles was similar. Plasma concentrations increased rapidly during the infusion, and decreased sharply initially and then gradually after the infusion (Fig. 3). The mean C_{max} values were generally reached at the end of infusion or the end of loading dose infusion in the case of the biphasic infusion (Period 9). After the end of infusion, all plasma concentration–time profiles displayed apparent bi-exponential decay with a steep distributional phase and a gradual terminal phase with a mean terminal $t_{1/2}$ of 5.6 h (Table 4). The PK analysis of GAL-021 in healthy volunteers was performed using both the

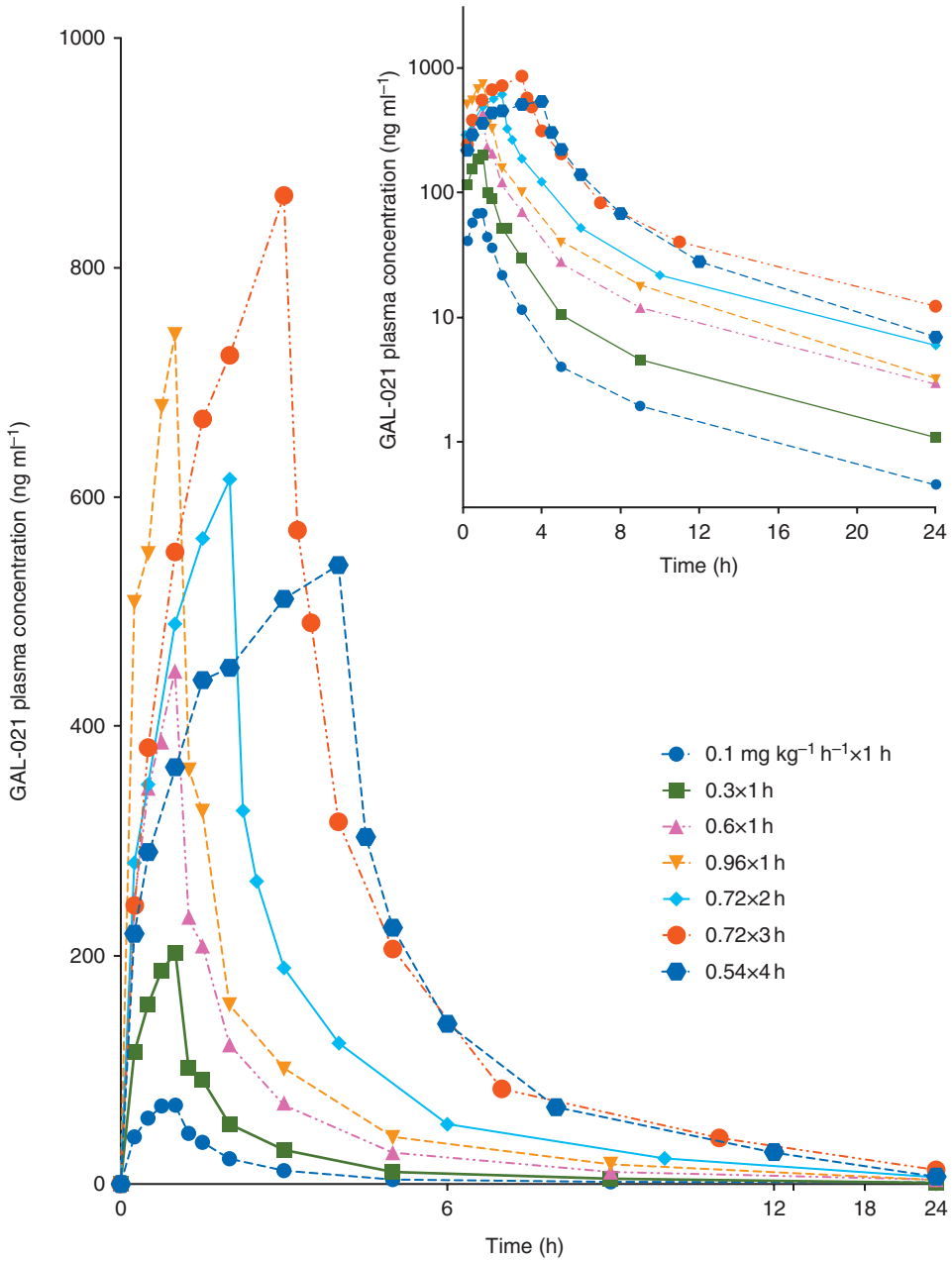


Fig 3 Mean plasma concentration–time profiles of GAL-021 after i.v. infusion to healthy volunteer. Inset panel contains the same data plotted in log–linear graph. The mean concentration is displayed. For clarity, error bar is not included. The mean and SDs for key PK parameters may be found in Table 4.

non-compartmental and compartmental approaches. Based on the non-compartmental analysis, the mean plasma clearance (CL_p) was 11.9 ml min^{-1} kg^{-1}, approximately one-half of hepatic blood flow rate in humans. The mean steady-state volume of distribution (V_{ss}) was 2.17 litre kg^{-1}, which is 2–3 times the total body water in humans, suggesting that GAL-021 was highly distributed to tissues. Compartmental analysis was evaluated with two- and three-compartment models with resultant similar Akaike information criteria values. Using a two-compartment model, mean central (V_1) and peripheral (V_2) volume of distribution values were 0.75 litre kg^{-1} (range: 0.6–0.9) and 1.74 litre kg^{-1} (range: 1.4–2.0), respectively. The mean inter-compartment clearance (CL_2) was 5.3 ml min^{-1} kg^{-1} (range: 4.2–6.1) with a mean central compartment elimination clearance (CL_1) of 12.7 ml min^{-1} kg^{-1} (range: 11–14). The mean distribution half-life ($t_{1/2,\alpha}$) was 0.45 h (range: 0.4–0.6) with a mean terminal disposition half-life ($t_{1/2,\beta}$) of 5.6 h (range: 4.5–7.2).

The plasma concentration at 1-h of infusion (C_{1h}) was common to all doses and was dose proportional using the

Table 4 Mean PK parameters of GAL-021 in human plasma. Data are geometric mean values (% coefficient of variation) for each parameter. The $t_{1/2}$ was logarithmically transformed and then geometric mean estimated. For T_{max}, median values (range) are reported. [a]From six of the eight subjects because of two subjects having below LOQ concentrations at 24 h. [b]Subject with AE is not included in analysis because of premature termination of infusion after 4 min

Period	Cohort	N	Infusion rate (mg kg^{-1} h^{-1})	Duration (h)	Dose (mg kg^{-1})	T_{max} (h)	C_{max} (ng ml^{-1})	$C_{(1h)}$ (ng ml^{-1})	AUC_{0-24} (ng h ml^{-1})	$AUC_{0-\infty}$ (ng h ml^{-1})	$AUC_{0-\infty}/D$ (h kg l^{-1})	$t_{1/2}$ (h)	CL_p (ml min^{-1} kg^{-1})	V_z (l kg^{-1})	V_{ss} (l kg^{-1})
1	1	8	0.1	1	0.102	0.86 (0.75–0.97)	71.4 (17)	160 (16)[a]	144 (27)	1.41 (27)	4.29 (43)	11.8 (39)	4.37 (36)	1.72 (33)	
2 and 3	2 and 3	16	0.3	1	0.300	0.97 (0.75–0.97)	203 (22)	388 (14)	397 (14)	1.32 (14)	5.87 (22)	12.6 (15)	6.40 (19)	2.34 (24)	
4	1	7	0.6	1	0.600	0.97 (0.97–0.97)	449 (15)	901 (7.2)	923 (7.3)	1.54 (7.5)	6.01 (11)	10.9 (7)	5.63 (9.4)	2.20 (14)	
5	2	6[b]	0.96	1	0.960	0.97 (0.75–0.97)	728 (21)	1395 (15)	1418 (16)	1.48 (16)	5.36 (14)	11.3 (15)	5.24 (14)	1.91 (32)	
6	3	8	0.72	2	1.44	1.97 (1.5–1.97)	611 (18)	482 (18)	1841 (13)	1890 (13)	1.31 (13)	5.94 (22)	12.7 (13)	6.53 (22)	2.52 (36)
7	1	7	0.72	3	2.16	2.97 (2.97–2.97)	847 (8)	543 (20)	3353 (11)	3464 (11)	1.61 (11)	6.47 (18)	10.4 (11)	5.82 (17)	2.29 (19)
8	2	6	0.54	4	2.16	4.00 (3.00–4.00)	539 (11)	361 (13)	2812 (6.5)	2862 (7.2)	1.32 (7.2)	5.12 (19)	12.6 (7)	5.58 (14)	2.18 (12)
9	3	8	0.72/0.36	1/3	1.80	0.97 (0.97–0.97)	508 (13)		2445 (11)	2516 (12)	1.40 (12)	5.86 (31)	11.9 (11)	6.05 (29)	2.06 (32)
1–9	1–3	24	All	1–4	All							5.61 (24)	11.9 (18)	5.76 (23)	2.17 (28)

power model (Fig. 4A). The dose proportionality β value was 1.05 (90% CI, 0.992, 1.098) and completely contained within the critical region (0.900, 1.100). The total systemic exposure ($AUC_{0-\infty}$) was also dose proportional (Fig. 4B). The dose proportionality β-value was 1.01 (90%CI, 0.980, 1.046) entirely within the critical region (0.927, 1.073). Intra-subject variability is generally less than inter-subject variability for $t_{1/2}$, CL_p, and V_{ss}, suggesting that effects from other factors may exist.

Discussion

During the FIH study, GAL-021 was a generally well tolerated potential therapeutic agent. The overall AE profile was similar to placebo (Table 2). The burning sensation, the only notable difference from placebo, has occurred with previous i.v. medications and may be a result of either the local concentration of GAL-021 or low infusate pH (3.1).[21] There were no post-infusion clinical findings suggestive of venous injury. Additional evaluations will be required to determine the aetiology of the burning sensation. Small BP increases were observed at mid dose, but not reproduced at greater and longer duration exposures and greater plasma GAL-021 concentrations in the same subjects. Any pressor effects, if present, will likely be limited and unlikely to be a significant concern in the post-anaesthesia setting where hypotensive findings are common.[22]

The presence of respiratory stimulation under ambient air conditions was evaluated throughout the study. No stimulation was observed at lower infusion rates. At the two highest infusion rates, $\dot{V}_E$ increased as measured by both the Dräger and NOX-T3 systems; thereby confirming the PD effects despite using differing measurement approaches—pneumotachometer with direct breath-by-breath integration *vs* inductance plethysmography. The rapid $\dot{V}_E$ increase correlated with an equally rapid reciprocal decrease in F'_{CO_2}. The $\dot{V}_E$ and F'_{CO_2} changes at the highest infusion rates suggest that greater infusion rates may be required to establish maximum stimulatory effects and confirm the current observation. The magnitude of the $\dot{V}_E$ changes is similar to those observed by Okubo[23] at doxapram concentrations greater than attained with approved doses for the treatment of postoperative respiratory depression.[24] The rapid increase in $\dot{V}_E$ with $\frac{1}{2}$ maximum effect attained at 7.5 min would support GAL-021 use in treating acute respiratory depression with further refinement of the loading/maintenance dosing approach explored in Period 9. The rapid increase in $\dot{V}_E$ and slow decline after the infusion cessation suggests a non-linear relationship between PK and PD. Under more stimulatory conditions (e.g. hypercapnia and hypoxia), the dose response may be altered with lower or greater infusion rates required for stimulating minute ventilation.

GAL-021 demonstrated low variability and tightly dose-proportional PKs over the dose range studied. Plasma concentration–time profiles in humans are similar to those in dog and rat with plasma concentrations increasing rapidly during the infusion and falling sharply at first and gradually thereafter after cessation of infusion. In general, all plasma concentration–time profiles fit well to a two-compartmental model with a short distribution half-life of $<$30 min. With

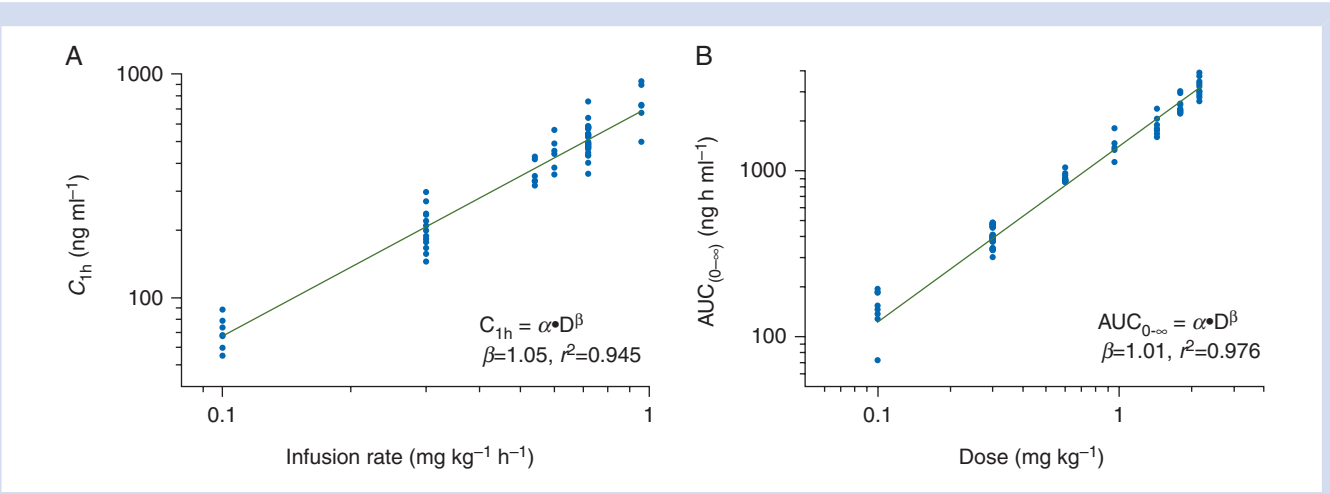


Fig 4 Plasma concentration at 1-h infusion timepoint as a function of infusion rate for GAL-021 (A). Total systemic exposure ($AUC_{0-\infty}$) as a function of infusion dose for GAL-021 (B). The solid line denotes the regression line derived from the power model. The results of the power analysis are included in each graph.

C_{max} and $AUC_{0-\infty}$ being dose-proportional and $t_{1/2}$, CL_p, and V_{ss} being dose-independent, the dosage regimen design and adjustment in the future could be simplified potentially as a result of the linear PK.

Overall, GAL-021 was safe, generally well tolerated during this study and demonstrated potentially clinically useful respiratory stimulatory effects. The respiratory effects had a rapid onset and slower offset which should allow effective up titration and prevent a rapid loss of effect that has been associated with some anaesthetic/analgesic reversal agents. The drug concentration profile of rapid increase/decrease is consistent with drugs used in an acute care setting. Very rapid onset of near maximal $\dot{V}_E$ (<1 min) was observed in primates and rodents with bolus/maintenance dosing of GAL-021.[16 17] It is likely that the rapid stimulatory effect will translate to humans with further refinement of the infusion regimen. The results of this study support continued clinical development of GAL-021 and suggest that the BK_{Ca}-channel may be a therapeutic target for the treatment of respiratory depression.

Authors' contributions

J.F.M. was the medical monitor for the clinical study, managed the regulatory submission and responses, and contributed to study design, data analysis, study report, and manuscript. J.M.L conducted the clinical study and reviewed the data, study report, and manuscript. S.X.P. analysed the PK samples and reported the PK results in the study report and manuscript. S.L.D. synthesized numerous similar chemical analogues, identified GAL-021 for clinical testing, and managed production of the drug supplies used in the study. L.M. performed the analysis of the primary and derived ventilatory PD data. F.J.G. contributed to study design and analysed secondary ventilatory and cardiovascular PD responses.

Acknowledgements

The authors thank Steven Ramael, Liesbet Poels, and Annelies Donckers, of SGS for advice and facilitating conduct of the study; Janet Boyle, John Connor, and Michele Hyman of BCH Research Solutions (West Chester, PA, USA) for clinical data review; Lucy Shneyer (Denville, NJ, USA) for thoughtful guidance on the statistical analyses; Paul Hoskins of Galleon for preparation for the study conduct, monitoring of the study conduct, and preparation of the study report and contribution and review of the manuscript; Euan MacIntyre and James Mannion of Galleon and Dudley Tabakin of Vivonoetics for thoughtful comments and guidance.

Declaration of interest

The authors are employees of Galleon Pharmaceuticals or received grants from Galleon Pharmaceuticals for performing components of the study.

Funding

The study was funded by Galleon Pharmaceuticals Corporation.

References

1 Overdyk FJ, Carter R, Maddox RR, Callura J, Herrin AE, Henriquez C. Continuous oximetry/capnometry monitoring reveals frequent desaturation and bradypnea during patient-controlled analgesia. *Anesth Analg* 2007; **105**: 412–8

2 Masoomi H, Reavis KM, Smith BR, Kim H, Stamos MJ, Nguyen NT. Risk factors for acute respiratory failure in bariatric surgery: data from the Nationwide Inpatient Sample, 2006–2008. *Surg Obes Relat Dis* 2013; **9**: 277–81

3 Overdyk FJ. Postoperative opioids remain a serious patient safety threat. *Anesthesiology* 2010; **113**: 259–60

4 Boom M, Niesters M, Sarton E, Aarts L, Smith TW, Dahan A. Non-analgesic effects of opioids: opioid-induced respiratory depression. *Curr Pharm Des* 2012; **18**: 5994–6004

5 Shapiro A, Zohar E, Zaslansky R, Hoppenstein D, Shabat S, Fredman B. The frequency and timing of respiratory depression in 1524 postoperative patients treated with systemic or neuraxial morphine. *J Clin Anesth* 2005; **17**: 537–42

6 Ahmad S, Nagle A, McCarthy RJ, Fitzgerald PC, Sullivan JT, Prystowsky J. Postoperative hypoxemia in morbidly obese patients with and without obstructive sleep apnea undergoing laparoscopic bariatric surgery. *J Anesth Analg* 2008; **107**: 138–43

7 Gallagher SF, Haines KL, Osterlund LG, Mullen M, Downs JB. Postoperative hypoxemia: common, undetected, and unsuspected after bariatric surgery. *J Surg Res* 2010; **159**: 622–6

8 Ward SA. Ventilatory control in humans: constraints and limitations. *Exp Physiol* 2007; **92**: 357–66

9 Nurse CA. Neurotransmitter and neuromodulatory mechanisms at peripheral arterial chemoreceptors. *Exp Physiol* 2010; **95**: 657–567

10 Smith JC, Abdala AP, Koizumi H, Rybak IA, Paton JF. Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. *J Neurophysiol* 2007; **98**: 3370–87

11 Koizumi H, Smith JC. Persistent Na^+ and K^+-dominated leak currents contribute to respiratory rhythm generation in the pre-Bötzinger complex in vitro. *J Neurosci* 2008; **28**: 1773–85

12 St-John WM, Stornetta RL, Guyenet PG, Paton JF. Location and properties of respiratory neurones with putative intrinsic bursting properties in the rat in situ. *J Physiol* 2009; **587**: 3175–88

13 Peers C, Wyatt CN, Evans AM. Mechanisms for acute oxygen sensing in the carotid body. *Resp Physiol Neurobiol* 2010; **174**: 294–8

14 Lahiri S, Roy A, Baby SM, Hoshi T, Semenza GL, Prabhakar NR. Oxygen sensing in the body. *Prog Biophys Mol Biol* 2006; **91**: 249–86

15 McCartney CE, McClafferty H, Huibant JM, Rowan EG, Shipston MJ, Rowe IC. A cysteine-rich motif confers hypoxia sensitivity to mammalian large conductance voltage- and Ca-activated K (BK) channel alpha-subunits. *Proc Natl Acad Sci USA* 2005; **102**: 17870–6

16 Baby SM, Gruber RB, Puskovic V, *et al.* GAL-021, a novel respiratory stimulant, attenuates opioid-induced respiratory depression without compromising analgesia. *FASEB J* 2012; **26**: 704–28 (abstract)

17 Golder FJ, Wardle RL, Van Scott MR, *et al.* GAL-021 acts as a novel respiratory stimulant in non-human primates. *FASEB J* 2012; **26**: 704–27 (abstract)

18 Baby SM, Golder FJ, Gruber RJ, *et al.* Reversal of opioid-induced respiratory depression by GAL-021, a novel respiratory stimulant. *Am J Respir Crit Care Med* 2012; **185**: A24–42 (abstract)

19 Sackner MA, Watson H, Belsito AS, *et al.* Calibration of respiratory inductive plethysmograph during natural breathing. *J Appl Physiol* 1989; **66**: 410–20

20 Smith BP, Vandenhende FR, DeSante KA, *et al.* Confidence interval criteria for assessment of dose proportionality. *Pharm Res* 2000; **17**: 1278–83

21 Klement W, Arndt JO. Pain on i.v. injection of some anaesthetic agents is evoked by the unphysiological osmolality or pH of their formulations. *Br J Anaesth* 1991; **66**: 189–95

22 Cashman JN, Dolin SJ. Respiratory and haemodynamic effects of acute postoperative pain management: evidence from published data. *Br J Anaesth* 2004; **93**: 212–23

23 Okubo S, Konno K, Ishizaki T, *et al.* Serum doxapram and respiratory neuromuscular drive in normal man. *Eur J Clin Pharmacol* 1988; **34**: 55–9

24 Yost CS. A new look at the respiratory stimulant doxapram. *CNS Drug Rev* 2006; **12**: 236–49

Handling editor: A. R. Absalom