Tolerance to Opioid-Induced Respiratory Depression in Chronic High-Dose Opioid Users: A Model-Based Comparison With Opioid-Naïve Individuals

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Chronic opioid consumption is associated with addiction, physical dependence, and tolerance. Tolerance results in dose escalation to maintain the desired opioid effect. Intake of high-dose or potent opioids may cause life-threatening respiratory depression, an effect that may be reduced by tolerance. We performed a pharmacokinetic-pharmacodynamic analysis of the respiratory effects of fentanyl in chronic opioid users and opioid-naïve subjects to quantify tolerance to respiratory depression. Fourteen opioid-naïve individuals and eight chronic opioid users received escalating doses of intravenous fentanyl (opioid-naïve subjects: 75–350 µg/70 kg; chronic users: 250–700 µg/70 kg). Isohypercapnic ventilation was measured and the fentanyl plasma concentration-ventilation data were analyzed using nonlinear mixed-effects modeling. Apneic events occurred in opioid-naïve subjects after a cumulative fentanyl dose (per 70 kg) of 225 (n = 3) and 475 µg (n = 6), and in 7 chronic opioid users after a cumulative dose of 600 (n = 2), 1,100 (n = 2), and 1,800 µg (n = 3). The time course of fentanyl's respiratory depressant effect was characterized using a biophase equilibration model in combination with an inhibitory maximum effect (E_{max}) model. Differences in tolerance between populations were successfully modeled. The effect-site concentration causing 50% ventilatory depression, was 0.42 ± 0.07 ng/mL in opioid-naïve subjects and 1.82 ± 0.39 ng/mL in chronic opioid users, indicative of a 4.3-fold sensitivity difference. Despite higher tolerance to fentanyl-induced respiratory depression, apnea still occurred in the opioid-tolerant population indicative of the potential danger of high-dose opioids in causing life-threatening respiratory depression in all individuals, opioid-naïve and opioid-tolerant.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Consumption of high-dose or potent opioids may cause life-threatening respiratory depression. Although opioid tolerance may reduce opioid respiratory effects, there are ample animal data that show lower and slower development of tolerance to opioid-induced respiratory depression.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ To address opioid respiratory depression tolerance in humans, the pharmacokinetics and respiratory behavior of escalating intravenous fentanyl doses were tested in opioid-naïve individuals and chronic opioid users. Fentanyl respiratory depression potency was estimated using a modeling approach.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Fentanyl was 4.3-fold less potent in chronic opioid users compared with opioid-naïve individuals. Despite the evident tolerance to respiratory depression, apneic events did occur in opioid-tolerant individuals albeit at higher opioid doses than in opioid-naïve individuals.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ In the current opioid epidemic, many chronic opioid users die of a fatal opioid overdose. Our current data demonstrate that despite opioid tolerance, fatal apneas may occur, especially upon dose escalation.

Prolonged use of opioids, such as morphine, oxycodone, or fentanyl, is associated with addiction, physical dependence, and tolerance.[1–3] Tolerance occurs due to adaptive changes at the neuronal level and results in the need for dose escalation to maintain the desired intensity of response.[1,3–5] Importantly, the consumption of high-dose or potent opioids is potentially

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Received April 25, 2020; accepted August 11, 2020. doi:10.1002/cpt.2027

life-threatening, as it may cause opioid-induced respiratory depression (OIRD) and ultimately death from silencing of neurons in brainstem respiratory networks.[6] When tolerance to analgesic and euphoric opioid effects coincides with tolerance to opioid respiratory effects, tolerance may reduce the respiratory effects of opioids. However, several animal studies indicate that tolerance to the analgesic and respiratory effects are dissociated with lower and slower development of tolerance to OIRD than of other opioid effects.[7–9]

Although there is indirect proof for the development of tolerance to the respiratory effects of chronic opioid use in humans,[10,11] no studies systematically addressed chronic tolerance to respiratory opioid effects. To examine this issue, we conducted a secondary analysis of data from a clinical study on the ability of buprenorphine, a partial agonist at the mu-opioid receptor, to prevent fentanyl-induced respiratory depression.[12] The descriptive analysis of that study is reported elsewhere[12,13]; here, we report on a population pharmacokinetic/pharmacodynamic (PK/PD) analysis of the control (placebo) arm of that study in which escalating doses of fentanyl were administered to opioid-naïve individuals and chronic opioid users while measuring isohypercapnic ventilation. The aim of the present analysis was to compare the respiratory depression potency of fentanyl in a population that chronically uses high-dose opioids and an opioid-naïve population.

METHODS

This study was part of a larger project aimed at assessing the effect of buprenorphine vs. placebo on fentanyl-induced respiratory depression and was performed from March 2018 through January 2019.[12,13] We here report on the part in which participants received placebo and fentanyl (in the other part of the study, the same subjects received buprenorphine and fentanyl). The protocol was approved by the Medical Review and Ethics Committee of the BEBO Foundation (Assen, The Netherlands) and registered at European Union Drug Regulating Authorities Clinical Trials Database under identifier 2017-004858-42. Before enrollment, all participants gave written informed consent. Study procedures were conducted according to good clinical practice guidelines and the Declaration of Helsinki.

Participants

Healthy volunteers of either sex, aged 18–45 years, body mass index 18–30 kg/m^2, without a history of any substance use disorder, were eligible to participate. Exclusion criteria included inability to give informed consent; pregnancy/lactation; positive pregnancy test on the morning of the experiment; current substance use disorder according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders 5th edition; a history of any medical, psychiatric, or neurologic condition; smoking (including e-cigarettes) or having smoked in the last 6 months; use of nicotine replacement products; alcohol consumption > 20 units/week (men) or > 13 units/week (women); use of any medication within 14 days or 5 half-lives before dosing; opioid use (including opioid antagonists) within 30 days before dosing; use of medication that induces/inhibits relevant cytochrome P450 (CYP450) enzymes; history of suicidal ideation or suicide attempt within 1 or 6 months prior to informed consent, respectively; abnormal blood chemistry values; or any other condition that, in the opinion of the investigators, could interfere with the ability to participate in the study.

Chronic opioid users of either sex, 18–55 years, body mass index 18–32 kg/m^2, using daily doses of opioids ≥ 90 mg oral morphine equivalents, and in stable condition as defined by the investigator and based on their medical history, vital signs, electrocardiogram, and blood and urine analysis were eligible to participate. Exclusion criteria were inability to give informed consent; pregnancy/lactation; positive pregnancy test on the morning of the experiment; inability to abstain from smoking during experimental sessions; alcohol consumption > 27 units/week (men) or > 20 units/week (women); use of medication that induces/inhibits relevant CYP450 enzymes; history of suicidal ideation or suicide attempt within 1 or 6 months prior to informed consent, respectively; abnormal blood chemistry values; or any other condition that, in the opinion of the investigators, could interfere with the ability to participate in the study.

Study design

Opioid-naïve volunteers were admitted on the day before dosing with fentanyl; chronic opioid users were admitted 2–5 days before dosing, and those not taking oxycodone were transitioned to oral oxycodone at least 2 days before dosing. This procedure ensured washout of the regularly used opioids and titration to an adequate individualized bridging schedule. Only when stable dosing with oxycodone was reached and the subject was doing well (as judged by the investigators), the subject was admitted into the fentanyl dosing part of the study. The last dose of oxycodone was administered no less than 15 hours before the expected study drug administration.

After arrival in the laboratory at 8 AM, all participants received an intravenous line for administration of study medication and an arterial line for blood sampling. The arterial line was placed in the radial artery opposite to the arm through which the study drug was infused. Isohypercapnic ventilation was measured for ~ 6 hours. Isohypercapnia was such that minute ventilation before any drug administration was stable at 20 ± 2 L/minute. To maintain isohypercapnia, we used the dynamic end-tidal forcing technique, which is described elsewhere.[14,15] In brief, subjects breathed through a facemask, attached to a pneumotachograph/pressure transducer system (Hans Rudolph Inc.) and three mass flow controllers (Bronkhorst High-Tech, Ruurlo, The Netherlands) for the delivery of O_2, CO_2, and N_2. The mass flow controllers were controlled by a computer running custom-made software RESREG/ACQ (Leiden University Medical Center, Leiden, The Netherlands) that controlled end-tidal gas concentrations by varying inspired concentrations and captured ventilation data. The gas concentrations were measured at the mouth (Datex Capnomac, Helsinki, Finland), and arterial oxygen saturation was measured by pulse oximetry (Masimo, Irvine, CA). One-minute breath-to-breath ventilation averages were used in the analyses.

The study was performed at isohypercapnia with constant target end-tidal oxygen concentrations of 13.5 volume%.[14,15] After hypercapnic ventilation had stabalized, a continuous intravenous infusion with normal saline was started (i.e., placebo treatment, part of the larger trial of buprenorphine vs. placebo) and continued until the end of the experiment. The start of the saline infusion was $t = 0$ minute. At $t = 120$, 180, 240, and 300 minutes, escalating intravenous fentanyl doses were administered over 90 seconds. In opioid-naïve subjects, the intravenous fentanyl (Hameln Pharmaceuticals , Gloucester, UK) doses (per 70 kg) were 75 μg at 120 minutes, 150 μg at 180 minutes, 250 μg at 240 minutes, and 350 μg at 300 minutes. In chronic opioid users, the intravenous fentanyl doses (per 70 kg) were 250 μg at 120 minutes, 350 μg at 180 minutes, 500 μg at 240 minutes, and 700 μg at 300 minutes. In case of an apneic episode (defined as a 20-second pause in respiration) or drop in SpO_2, the participants were stimulated to breathe. In all cases, this was sufficient to restore ventilation and return oxygen saturation above 94%. Subsequent fentanyl dosing was not performed if the participant experienced prolonged apnea or required stimulation for breathing following a previous dose or for any other reason as determined by the investigators (e.g., overt sedation, nausea, restlessness, and subject discomfort). Stimulated, nonspontaneous breathing data were set at zero (apnea) for data analyses.

Blood sampling and fentanyl assay. Blood samples were drawn from the arterial line for measurement of fentanyl plasma concentrations. Sampling times for determination of fentanyl plasma concentrations were t = 120, 122, 125, 130, 135, 140, 150, 180, 182, 185, 190, 195, 200, 210, 240, 242, 245, 250, 255, 260, 270, 300, 302, 305, 310, 315, 320, 330, 360, 375, 420, 480, and 540 minutes. In case a fentanyl bolus was not administered, sampling continued at hourly intervals and again according to schedule from 360 minutes on. Plasma was separated within 30 minutes of blood collection and stored at −20°C until analysis.

Fentanyl plasma concentrations were determined using a liquid chromatography with tandem mass spectrometry assay. Human K_2EDTA plasma containing fentanyl and the internal standard, fentanyl-d5, were extracted with methyl tert-butyl ether after the addition of sodium carbonate (liquid-liquid extraction). After extraction, a small portion of the organic phase was transferred to an autosampler vial that contained formic acid in water. An aliquot was injected on a Sciex API 5500 liquid chromatography with tandem mass spectrometry equipped with a high-performance liquid chromatography column. The peak area of the m/z 337 → 188 fentanyl product ion was measured against the peak area of the m/z 342 → 188 fentanyl-d5 internal standard product ion. Quantitation was performed using weighted ($1/x^2$) linear least squares regression analyses generated from calibration standards prepared immediately prior to each run. The assay was fully validated for linearity, selectivity, recovery, matrix effect, accuracy, precision, and stability before application to the sample analysis. The calibration range was 0.100–50.0 ng/mL. The overall accuracy and precision for quality control samples during the sample analyses were all within 5.3%. All the plasma samples were analyzed within the established stability window.

Population pharmacokinetic/pharmacodynamic analyses

As previously mentioned, the study was part of a larger project comparing the effect of buprenorphine vs. placebo on fentanyl-induced respiratory depression. The effect of buprenorphine vs. placebo were evaluated at 2 occasions separated by 10–17 days in opioid-naïve volunteers and a minimum of 40 hours in chronic opioid users. A two-sequence, single-blind crossover design was used for opioid-naïve volunteers, whereas in chronic opioid users, an open-label design was used with the placebo session preceding the buprenorphine session. The present PD analysis focuses on the placebo arm only; the PK analysis includes data from both placebo and buprenorphine arms, allowing interoccasion variability to be assessed. PK and PD data were analyzed with nonlinear mixed-effects modeling software version VII (NONMEM; ICON Development Solutions, San Antonio, TX). Empirical Bayesian estimates obtained from the population PK analysis was used in the population PK/PD analysis (sequential PK/PD approach). For the population PK analysis, the first-order conditional estimation method was used; concentration data were log-transformed for analysis. For the population PK/PD analysis, the stochastic approximation expectation maximization with interaction algorithm was used, followed by importance sampling for objective function evaluation. The number of stochastic approximation expectation maximization iterations was determined automatically by NONMEM and convergence was confirmed by visual inspection. For both PD and log-transformed PK data, residual variability was assumed to be additive and normally distributed. Between-subject variability and when applicable, interoccasion variability, were estimated assuming log-normal distributions. Differences between opioid-naïve participants and chronic opioid users were evaluated. The covariate analysis was performed using forward selection and backward elimination, with nominal alpha levels of 0.05 (difference in minimum objective function value (ΔMOFV): 3.84) and 0.001 (ΔMOFV: 10.83), respectively.

Two-compartment and three-compartment models were tested for the population PK analysis. Based on prior knowledge and mechanistic reasons,[16] clearances (CLs) were allometrically scaled to liters per hour at 70 kg body weight by $CL = CL_{TV}(\text{weight}/70)^{0.75}$ and compartment volumes were scaled to 70 kg body weight by $V = V_{TV}(\text{weight}/70)$ (typical value (TV)). For the population PK/PD analysis, an effect compartment was postulated to account for the hysteresis between the fentanyl plasma concentrations and effect on ventilation.[17] This effect compartment equilibrates with the plasma compartment with plasma-effect-site equilibration half-life ($t_{½k_{e0}}$).

The 1-minute ventilation averages were then modeled using an inhibitory sigmoid maximum effect (E_{max}) model[17,18]

$$RF(t) = (C_E(t)/C_{50})/(1+C_E(t)/C_{50}) \quad (1)$$

and

$$V(t) = V_B \times [1-\alpha \times RF(t)] \quad (2)$$

where RF is the fraction of the opioid receptors bound by fentanyl, C_E the fentanyl concentration at the postulated effect-site, C_{50} the fentanyl concentration at the postulated effect-site causing 50% depression of ventilation, $V(t)$ minute ventilation, V_B baseline ventilation, and α a parameter that combines receptor reserve and intrinsic ligand activity. Apnea data were censored at zero using M3 methodology in NONMEM. The $\alpha > 1$ allowed for the possibility that apnea occurred at finite fentanyl concentrations.

Model selection was based on MOFV (applying the likelihood ratio test for nested models), goodness-of-fit plots (observed vs. predicted data, and weighted residuals vs. time), prediction-corrected and variability-corrected visual predictive checks,[19] and normalized prediction discrepancies.[20] It was visually checked that the normalized prediction discrepancies vs. time showed no trends, heteroscedasticity, or both. Data are reported as mean ± SD or estimate ± standard error of the estimate, unless otherwise stated.

RESULTS

Twenty-two persons were included in the study, 14 opioid-naïve subjects (7 men/7 women, mean age: 24 years (range 20–35 years), weight: 72 ± 4 kg, body mass index: 23.0 ± 4.0 kg/m^2) and 8 chronic opioid users (3 men/5 women, mean age: 42 (range 31–53 years), weight: 77 ± 11 kg, body mass index: 26 ± 4 kg/m^2) that used high-dose opioids for at least 3 months (range 0.25–29 years; **Table 1**). Two opioid-naïve individuals withdrew consent after their first fentanyl dosing session but their data were included in the analysis (PK/PD data for one subject and only PK data for the other). Two chronic opioid users were in a medically supervised substitution setting. One used intranasal heroin obtained from the harm reduction clinic, and the other was on stable substitution treatment with buprenorphine. Both had concurrent use of other drugs of abuse, including cocaine and cannabis. Other chronic opioid users were taking prescription opioid medications (predominantly oral oxycodone or fentanyl transdermal patch) for a variety of chronic pain conditions. The mean morphine-equivalent dose was 220 ± 134 mg per day. All chronic opioid users were successfully transitioned to oxycodone and, on average, 114 mg oxycodone (range 50–315 mg) was consumed daily on bridging days. On the study day, no oxycodone or any opioid other than fentanyl was consumed.

All participants completed the session without unexpected side effects. The median number of fentanyl doses was two (range 2–3) in opioid-naïve individuals and four (range 2–4) in chronic

Table 1 Description of the individual participants with chronic opioid use

Age, years	Sex	Weight, kg	BMI, kg/m^2	Underlying condition	Substance use (since)[a]	Bridging
44	F	68	24	Anal fissures	Oxycodone 60 mg/day (2017)	60 mg oxycodone/day
52	M	79	25	Substance abuse	Cannabis (1986), heroin 250 mg/day (1989), cocaine (1989)	60 mg oxycodone/day
53	F	89	32	Polyneuropathy of the lower left extremity	Fentanyl transdermal patch 50 mg/hour (2012)	50 mg oxycodone/day
34	F	64	30	Ehlers-Danlos syndrome	Oxycodone 60 mg/day (2007), fentanyl transdermal patch 75 mg/hour (2014)	100 mg oxycodone/day
46	M	93	31	Lower back pain due to disc herniation	Cannabis (2005), oxycodone 60 mg/day (2012), fentanyl transdermal patch 25 mg/hour (2017)	50 mg oxycodone two times/day
33	F	86	23	Pelvic and back pain	Oxycodone 90 mg/day (2015), fentanyl transdermal patch 75 mg/hour (2017), tapentadol 50 mg/day (2018)	80 mg oxycodone three times/day
31	F	70	23	Persistent pain following humerus fracture	Cannabis (2009), oxycodone 60 mg/day (2018)	60 mg oxycodone/day
48	M	71	22	Substance abuse	Cocaine (1990), heroin (1995–2013), cannabis (2000), buprenorphine 16 mg/day (2013)	Oxycodone 40 mg six times/day
42 ± 9		77 ± 11	26 ± 4			

BMI, body mass index.
[a]Substance use at screening.

opioid users. The reason for dose restrictions was unstable breathing with episodes of apnea or drops in SpO_2 < 85% after the previous dose, or persistent nausea. Apneic events occurred in 9 opioid-naïve subjects after a cumulative fentanyl dose (per 70 kg) of 225 μg (n = 3) and 475 μg (n = 6), and in 7 chronic opioid users after a cumulative dose of 600 μg (n = 2), 1,100 μg (n = 2), and 1,800 μg (n = 3). The cumulative intravenous fentanyl dose administered was four-times higher in chronic opioid users (1,455 ± 595 μg) than opioid-naïve subjects (349 ± 124 μg).

An example of an experiment in an opioid-naïve individual is given in **Figure 1**. The first two intravenous fentanyl doses (70 and 140 μg at t = 120 and 180 minutes) caused a rapid decline in ventilation and small drops in SpO_2; the subsequent dose of 240 μg at 240 minutes caused the rapid onset of apnea and was accompanied by a short drop in SpO_2 to 86%. The subject was verbally stimulated to breathe to increase SpO_2 to values > 94%. Thereafter, the subject resumed spontaneous breathing, no further stimulation was needed, and no subsequent fentanyl injections were given.

Population pharmacokinetic-pharmacodynamic data analyses

Pharmacokinetics. The final population PK model was a three-compartment model that was superior to a two-compartment model based on the likelihood ratio test (ΔMOFV > 500, P < 0.001). Best, median, and worst fits are given in **Figure S1**, with corresponding goodness-of-fit plots, including normalized predicted discrepancies, in **Figure S2**. Inspection of the data fits and the diagnostic plots indicate that the model adequately described the data. The PK parameter estimates are given in **Table 2**. The opioid state of the participants was initially identified as a significant covariate with respect to peripheral compartment V_2 and intercompartmental clearance Q_2, but was not retained in the final model following backward elimination.

Pharmacodynamics. The 1-minute ventilation averages were well-described by the inhibitory E_{max} model as observed by the best, median, and worst data fits (**Figure 2**), and diagnostic plots (**Figure 3** and **Figure S2**). As observed in **Figure 2b**, apneic episodes were adequately described by the model. The PK/PD parameter estimates are given in **Table 2**. Initially, the variance-covariance matrix for individual random effects (Ω) allowed for the estimation of covariances between all individual PK/PD parameters, except between V_B and σ (additive error term with associated interindividual variability). This model had a MOFV of 20,974.15. Including a separate value for the potency parameter C_{50} between the populations reduced the MOFV by 17.25 points (P < 0.001). Setting covariances to zero, except for the covariance between C_{50} and α, had no significant effect on the MOFV (ΔMOFV = 0.38). Finally, fixing the population mean of α to 1 (interindividual variability still estimated) had no significant impact on the MOFV (ΔMOFV = 3.56 < 3.84).

C_{50} estimates were 0.42 ± 0.07 ng/mL and 1.82 ± 0.39 ng/mL in opioid-naïve subjects and chronic opioid users, respectively, indicating a 4.3-fold lower sensitivity to fentanyl in chronic opioid users. The delay between fentanyl plasma concentration and effect ($t½k_{e0}$) was similar between populations: 17.2 ± 3.0 minutes. As stated above, the covariance between C_{50} and α was 0.17 ± 0.06 (ρ = 0.74, ΔMOFV = 12.51). The population mean of α was fixed to 1, and although this would suggest that apnea occurs at high (infinite) fentanyl concentrations, the fact that interindividual

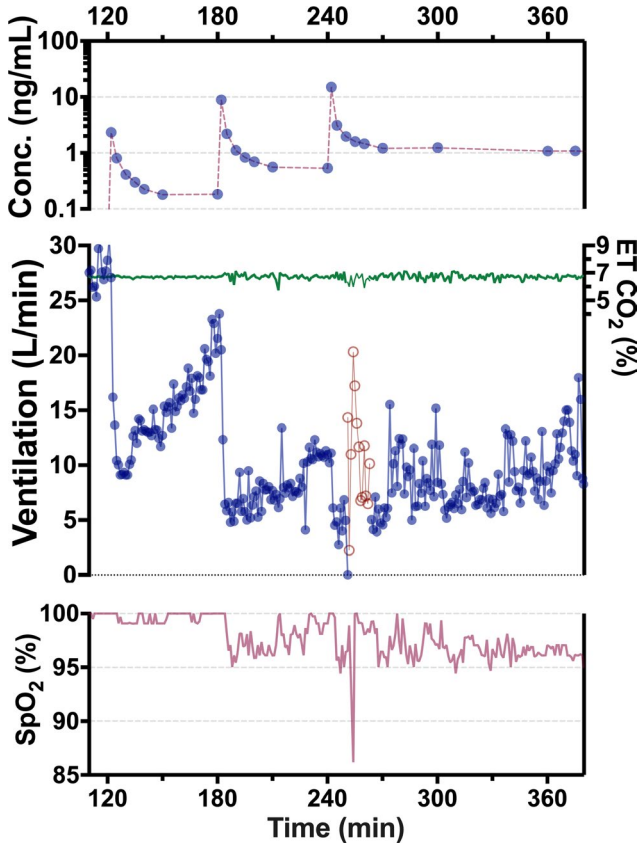

Figure 1 Example of effect of three fentanyl administrations (75 μg/70 kg, 150 μg/70 kg, and 250 μg/70 kg) on isohypercapnic ventilation in an opioid-naïve individual (subject #110). Top panel: The measured fentanyl plasma concentrations (Conc.). Middle panel: 1-minute ventilation averages (blue symbols = spontaneous breathing, red symbols = stimulated breathing during a 12-minute apneic period following the third fentanyl administration) and end-tidal CO_2 concentration (green line); bottom panel: oxygen saturation (SpO_2).

variability parameter was estimated indicates that α was greater than 1 in some subjects, and consequently that apnea had a certain probability to occur at each of the fentanyl doses administered (**Figures 3–5**). The probability of apnea was predicted for nine intravenous fentanyl doses (75–2,000 μg, all per 70 kg; **Figure 4**). Because we analyzed 1-minute ventilation averages per definition, apnea is now defined as a pause in spontaneous respiration of at least 60 seconds. Probabilities of apnea in chronic opioid users were many-fold lower than in the opioid-naïve population for a similar fentanyl dose. For example, after an intravenous administration of 500 μg fentanyl, the maximum probabilities of apnea were 2.6% and 23% in chronic opioid users and opioid-naïve individuals, respectively. In chronic opioid users, the probability of apnea at fentanyl doses < 500 μg was < 1% and 0% at a dose of 75 μg.

The steady-state relationships between fentanyl plasma concentration and isohypercapnic ventilation are shown in **Figure 5a,b**, covering the 0–3 ng/mL range, which corresponds to the lower dose tested in opioid-naïve individuals (75 μg). **Figure 5c,d** depicts the simulated effect of an intravenous fentanyl administration of 250 μg in opioid-naïve individuals and 1,000 μg in chronic opioid users on isohypercapnic ventilation. Opioid-naïve individuals had a higher probability of apnea that lasted longer after 250 μg fentanyl (probability = 11%; **Figure 5**) than chronic opioid users after 1,000 μg fentanyl (probability = 9.7%).

DISCUSSION

We studied the PKs and respiratory effects of fentanyl in chronic opioid tolerance. The data came from a clinical study where escalating intravenous fentanyl doses were administered to opioid-naïve volunteers and chronic opioid users while measuring isohypercapnic ventilation. The fourfold higher fentanyl doses administered in chronic opioid users indicate that these individuals are best described as opioid-tolerant. Still, data from studies in animal and humans indicate that tolerance to the antinociceptive, analgesic, and hedonistic effects of chronic opioid consumption is not directly translated into tolerance to respiratory depression.[5,7–10] Tolerance to opioid analgesia and euphoria develops faster and more extensively than tolerance to respiratory depression. Here, we show that the fentanyl effect-site concentration to decrease isohypercapnic ventilation by 50% in a small population of chronic opioid users (average timespan of opioid use: 10 years, average daily dose of oral morphine equivalents: 220 mg) is 4.3-fold higher than in a population of opioid-naïve individuals (**Table 2**). However, despite the evident tolerance to fentanyl-induced respiratory depression, apneic episodes did occur in seven opioid-tolerant individuals. These data indicate that these individuals remain in danger of life-threatening respiratory depression upon dose escalation or upon use of opioids of increasing potency. This is further corroborated in simulations (**Figures 4 and 5**): the probability of apnea increased from 10% after 1,000 μg to 21% after 2,000 μg fentanyl (doses per 70 kg) in chronic opioid users.

Opioid tolerance has PK and/or PD components.[5] PK components include metabolic enzyme induction (which causes enhanced opioid clearance) or upregulation of drug transporters in brain endothelial cells, such as P-glycoprotein, that enhance opioid efflux from the brain compartment.[5,21–24] Fentanyl is metabolized in the liver by CYP3A4 enzyme and is a P-glycoprotein substrate.[21,22,24] No significant differences in fentanyl PK parameters, including clearance, were observed between opioid-naïve individuals and chronic opioid users. Although greater estimates for V_2 and Q_2 were obtained in chronic opioid users during model development, those were not sufficiently significant to be retained in the final model. In addition, based on simulations, they would not have contributed to PD differences between the two populations. Although we cannot exclude a potential P-glycoprotein upregulation, the similar $t½k_{e0}$ values in the two populations suggest that this phenomenon would be of minor clinical relevance.

In our model, the larger fentanyl C_{50} value in chronic opioid users relative to opioid-naïve individuals reflects the PD component of opioid tolerance. Various cellular and molecular adaptations during chronic opioid exposure are associated with tolerance development.[1,4,5] One such adaptation that is particularly of importance to our results is receptor desensitization (related to

Table 2 Parameter estimates for fentanyl population pharmacokinetic and PK/PD models

	Estimate ± SEE (%RSE)	ω^2 ± SEE (%CV)	ν^2 ± SEE (%CV)
Pharmacokinetics			
V_1, L at 70 kg	10.5 ± 1.47 (14)	0.430 ± 0.113 (73)	0.047 ± 0.020 (22)
V_2, L at 70 kg	14.4 ± 2.65 (18)	0.518 ± 0.115 (82)	—
V_3, L at 70 kg	166 ± 9.63 (5.8)	0.060 ± 0.018 (25)	—
CL, L/minute at 70 kg	1.26 ± 0.08 (6.3)	0.087 ± 0.019 (30)	0.009 ± 0.003 (9.4)
Q_2, L/minute at 70 kg	2.03 ± 0.29 (14)	0.388 ± 0.088 (69)	0.022 ± 0.018 (15)
Q_3, L/minute at 70 kg	2.29 ± 0.17 (7.6)	0.098 ± 0.038 (32)	0.018 ± 0.007 (14)
σ	0.15 ± 0.009 (6.0)		
Pharmacodynamics			
V_B, L/minute	22.9 ± 0.60 (2.6)	0.014 ± 0.006 (12)	—
C_{50}, ng/mL			
Opioid-naïve individuals	0.42 ± 0.07 (16)	0.620 ± 0.174 (93)	—
Chronic opioid users	1.82 ± 0.39 (21)		—
α	1 (FIX)	0.080 ± 0.029 (29)	—
$t½k_{e0}$, minute	17.2 ± 3.03 (18)	0.636 ± 0.199 (94)	—
σ	2.54 ± 0.14 (5.7)	0.066 ± 0.065 (26)	—

Covariance between C_{50} and α was 0.17 ± 0.06.
σ, residual error component; C_{50}, the fentanyl effect-site concentration causing a 50% decrease in ventilation; α, parameter that combines receptor reserve and intrinsic ligand activity; CL, clearance; CV, coefficient of variation for interindividual or interoccasion variability (calculated as $\sqrt{\exp(\omega^2)-1}$ multiplied by 100); PD, pharmacodynamic; PK, pharmacokinetic; Q_{2-3}, intercompartmental clearances; RSE, relative standard error; SEE, standard error of estimate; $t½k_{e0}$, effect-site equilibration half-life (delay between plasma concentration and effect); ν^2, interoccasion variability; V_B, isohypercapnic baseline ventilation; V_{1-3}, volumes of pharmacokinetic compartments 1–3; ω^2, interindividual variability.

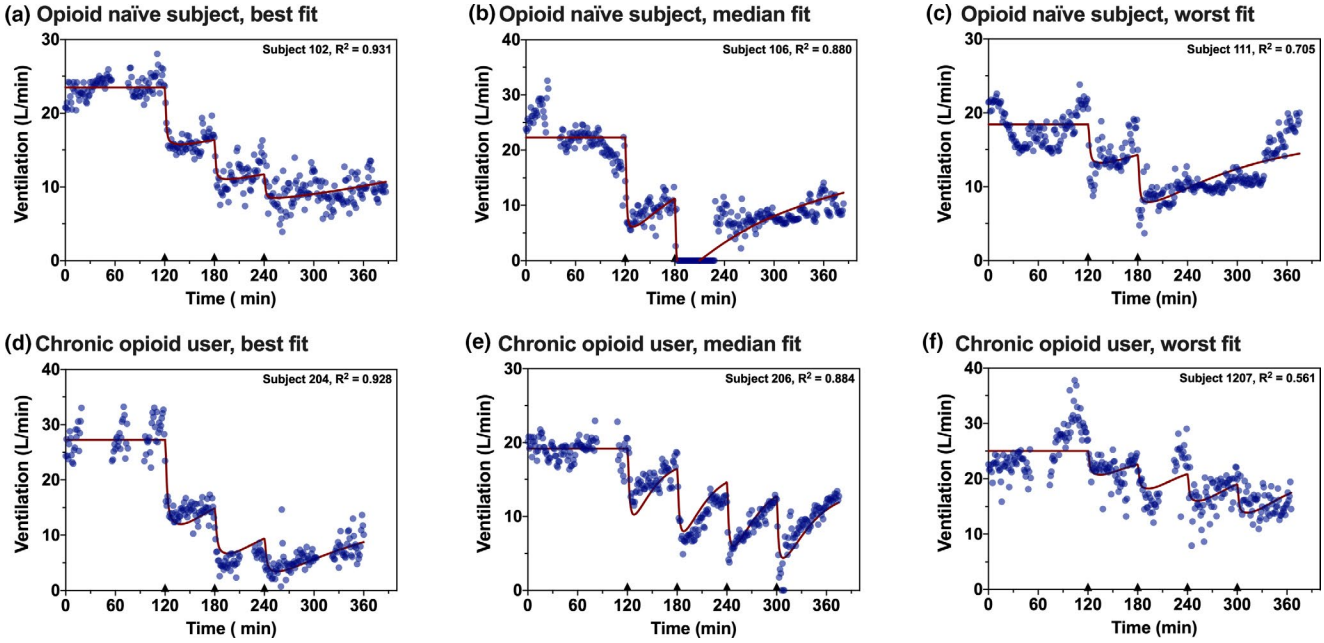

Figure 2 Best, median, and worst pharmacodynamic data fits (based on the coefficient of determination, R^2) in opioid-naïve subjects (**a**–**c**) and chronic opioid users (**d**–**f**). Blues dots are the 1-minute averages of the measured breath-to-breath ventilation data; the red line is the data fit. The black triangles indicate the time of the intravenous fentanyl administrations.

receptor endocytosis, degradation, and downregulation) mediated by neuronal upregulation of protein kinase C.[1,24] It has been speculated that the observation of lower and slower development of tolerance to OIRD relative to analgesia is related to lower levels of protein kinase C in brainstem respiratory neurons.[25] Interestingly, the observation that ethanol reverses tolerance to morphine-induced respiratory depression in mice may possibly be related to the inhibition of protein kinase C activity by ethanol.[25] Other mechanisms of opioid tolerance development include β-arrestin-2 recruitment, increased adenylate cyclase activity, activation of

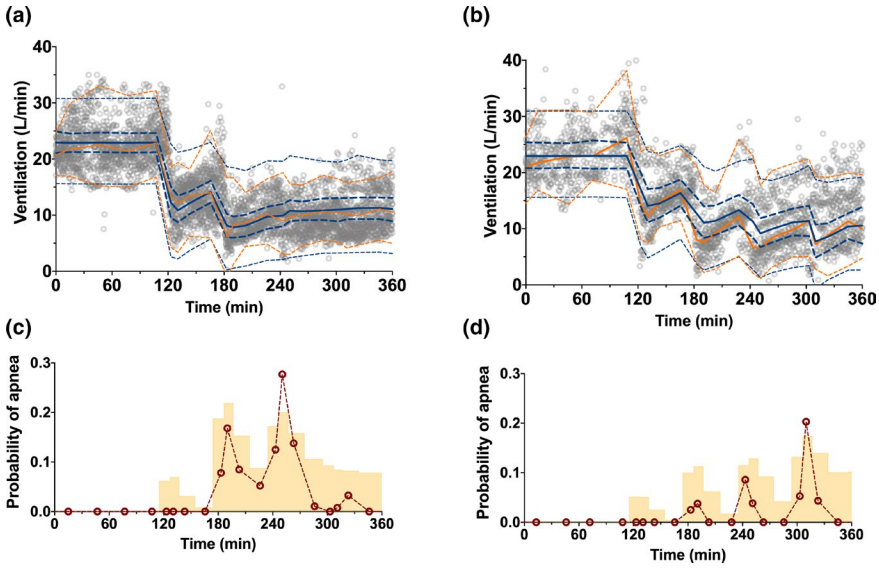

Figure 3 Prediction-corrected and variability-corrected visual predictive checks of the pharmacodynamic model in opioid-naïve subjects (**a**) and chronic opioid users (**b**). The dots are the 1-minute ventilation averages. The continuous blue line is the simulated median, the thick broken blue line is the 95% confidence interval of the simulated median, and the thin dotted blue line is the simulated 2.5th and 97.5th percentiles. The continuous orange line is the measured median ventilation and the thin broken orange line is the measured 2.5th and 97.5th percentiles. Probability of apnea in opioid-naïve subjects (**c**) and chronic opioid users (**d**). The red symbols are the probabilities of the observed apneic episodes; the yellow areas are the simulated 95% confidence intervals of the probability of apnea.

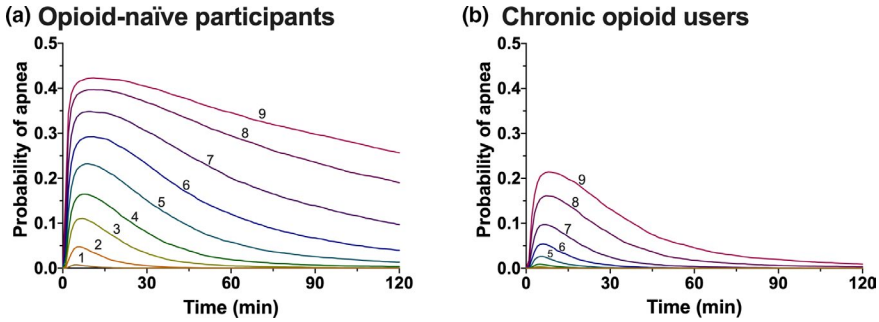

Figure 4 The probability of apnea in opioid-naïve subjects (**a**) and chronic opioid users (**b**) for nine intravenous fentanyl doses: 1 = 75 μg/70 kg, 2 = 150 μg/70 kg, 3 = 250 μg/70 kg, 4 = 350 μg/70 kg, 5 = 500 μg/70 kg, 6 = 700 μg/70 kg, 7 = 1,000 μg/70 kg, 8 = 1,500 μg/70 kg, and 9 = 2,000 μg/70 kg. In chronic opioid users, the probability of apnea at doses < 500 μg/70 kg is < 1%.

N-methyl-D-aspartate receptors, and glia cell activation.[1,4,5] Their role in chronic tolerance to OIRD is unknown.

In our population of chronic opioid users, the majority of participants were polydrug users (**Table 1**). The effect of polydrug use on the development of tolerance to OIRD is unknown. Many opioid overdose victims have multiple substances in their system,[26,27] and additional substance use possibly reduces tolerance to chronic opioids. As stated above, ethanol reduces tolerance to morphine in mice.[25] Further studies in larger populations are required to improve our understanding of the interactive effect of centrally acting legal substances (ethanol), as well as illicit and prescription drugs on the development of tolerance to OIRD. Additionally, it is important to realize that tolerance is a dynamic phenomenon and tolerance to OIRD may decline after a period of abstinence, contributing to an increased risk of fatalities related to opioid overdose following detoxification or incarceration.[25,28,29]

Ventilation was described by an inhibitory E_{max} model whereby ventilation is a function of $\alpha \times RF$ (Eq. 2), where RF is the fraction of opioid receptors activated by fentanyl and α, a parameter that incorporates receptor reserve and intrinsic ligand activity. In cases where $\alpha < 1$, ventilation displays an apparent maximum in ventilatory depression. In such cases, apnea is not reached, even at very high concentrations with full receptor occupancy; $\alpha < 1$ occurs with mu-opioid receptor partial agonists, such as buprenorphine.[17] In our analysis, the population mean for α was not different from 1, but the estimated interindividual variability in α indicated that apnea could occur with $\alpha > 1$ at the individual level (**Table 2**). We earlier modeled ventilation using a different (power) function that predicts that apnea occurs at a finite opioid plasma concentration in all subjects.[30,31] We preferred the current approach, as the E_{max} model is more versatile and apnea did not occur in every subject. Our modeling results in opioid-naïve subjects are in agreement with earlier findings, with, for example, similar values for α (0.91)

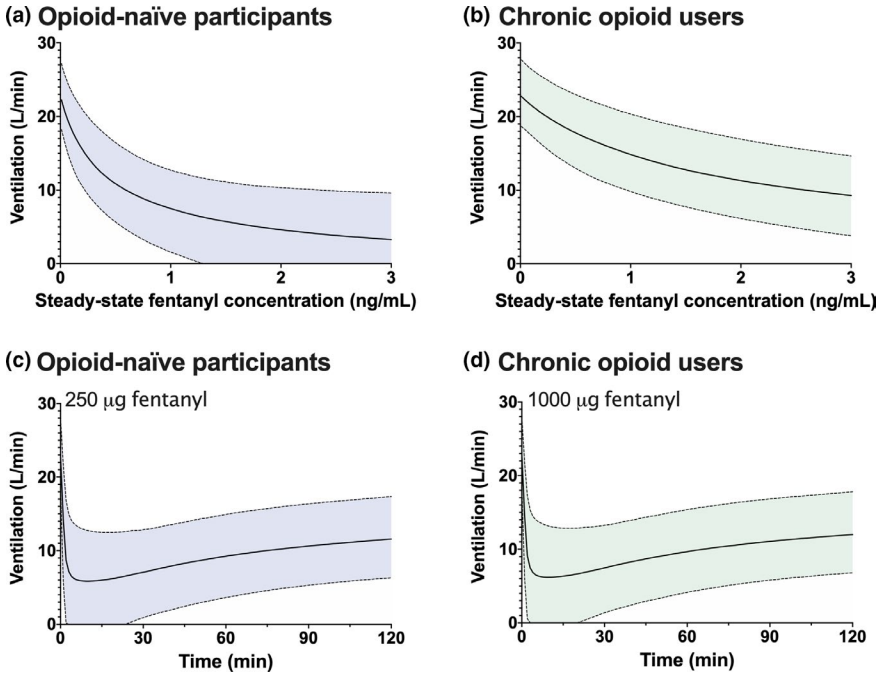

Figure 5 Steady-state plasma concentration-isohypercapnic ventilation relationships in opioid-naïve subjects (**a**) and chronic opioid users (**b**) with 90% prediction intervals indicating that there is a probability of apnea in opioid-naïve subjects but not in chronic opioid users over the steady-state concentration range of 0–3 ng/mL. Simulations of the effect of an intravenous fentanyl injection of 250 µg (**c**) and 1,000 µg (**d**) in a population of opioid-naïve individuals and chronic opioid users, respectively (at a reference weight of 70 kg), on isohypercapnic ventilation. The band around the median values represents 90% prediction intervals. Opioid-naïve individuals have a higher probability of apnea that lasts longer after 250 µg fentanyl (probability = 13%) than chronic opioid users after 1,000 µg (probability = 7%).

and $t½k_{e0}$ (16 minutes).[17,32] PK modeling results are also consistent with previous analyses, with similar clearance estimate (0.98 L/minute) and volumes of distribution of the same order of magnitude.[17]

Finally, the two study populations were not age-matched. Hence, it may be reasoned that some of the observed differences were related to age (opioid-naïve subjects: 20–35 years, vs. opioid-tolerant subjects: 31–52 years). We are unaware of major differences in opioid PKs and (respiratory) PDs between these relatively close age ranges. In fact, we earlier observed that elderly opioid-naïve subjects (range 66–77 years) display an enhanced sensitivity to the respiratory effects of oxycodone compared with younger opioid-naïve subjects (range 21–28 years).[33] This suggests that any impact of using age-matched controls would be to favor a greater C_{50} ratio.

In conclusion, we compared the respiratory effects of fentanyl in chronic opioid users and opioid-naïve individuals. Differences in tolerance between populations were successfully characterized by PK/PD modeling. Although we observed that the fentanyl effect-site concentration required to decrease isohypercapnic ventilation by 50% in chronic opioid users was 4.3-fold higher than that in opioid-naïve individuals, apneic events still occurred, albeit at higher fentanyl doses than in opioid-naïve individuals. These data, supported by model simulations, exemplify the potential danger of fentanyl in causing life-threatening respiratory depression in both opioid-naïve and opioid-tolerant individuals.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

ACKNOWLEDGMENTS

Editorial assistance was provided by Peloton Advantage, an OPEN Health company (Parsippany, NJ) with support from Indivior, Inc. (Richmond, VA).

FUNDING

This study was supported by Indivior Inc., North Chesterfield, VA.

CONFLICT OF INTEREST

R.L.D. and C.M.L. are employees of Indivior and declare no other competing interests. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.H.A., E.O., L.M., R.L.D., M.N., M.v.V., G.J.G., J.H., C.M.L., and A.D. wrote the manuscript. R.L.D., G.J.G., and A.D. designed the research. M.H.A., L.M., M.N., M.v.V., J.H., A.D., and G.J.G. performed the research. E.O., C.M.L., and A.D. analyzed the data.

1. Williams, J.T. *et al*. Regulation of mu-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. *Pharmacol. Rev.* **65**, 223–254 (2013).
2. Kreek, M.J., Reed, B. & Butelman, E.R. Current status of opioid addiction treatment and related preclinical research. *Sci. Adv.* **5**, eaax9140 (2019).

3. Freye, E. & Latasch, L. Development of opioid tolerance – molecular mechanisms and clinical consequences. *Anasthesiol. Intensivmed. Notfallmed. Schmerzther.* **38**, 14–26 (2003).
4. Colvin, L.A., Bull, F. & Hales, T.G. Perioperative opioid analgesia-when is enough too much? A review of opioid-induced tolerance and hyperalgesia. *Lancet* **393**, 1558–1568 (2019).
5. Martyn, J.A.J., Mao, J. & Bittner, E.A. Opioid tolerance in critical illness. *N. Engl. J. Med.* **380**, 365–378 (2019).
6. Dahan, A., Aarts, L. & Smith, T.W. Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* **112**, 226–238 (2010).
7. Emery, M.J., Groves, C.C., Kruse, T.N., Shi, C. & Terman, G.W. Ventilation and the response to hypercapnia after morphine in opioid-naive and opioid-tolerant rats. *Anesthesiology* **124**, 945–957 (2016).
8. Mohammed, W. *et al*. Comparison of tolerance to morphine-induced respiratory and analgesic effects in mice. *Toxicol. Lett.* **217**, 251–259 (2013).
9. Greenwald, M.K. Effects of opioid dependence and tobacco use on ventilatory response to progressive hypercapnia. *Pharmacol. Biochem. Behav.* **77**, 39–47 (2004).
10. Walsh, T.D., Rivera, N.I. & Kaiko, R. Oral morphine and respiratory function amongst hospice inpatients with advanced cancer. *Support. Care Cancer* **11**, 780–784 (2003).
11. Santiago, T.V., Pugliese, A.C. & Edelman, N.H. Control of breathing during methadone addiction. *Am. J. Med.* **62**, 347–354 (1977).
12. Moss, L.*et al*.Sustained high plasma buprenorphine concentrations inhibit respiratory effects of intravenous fentanyl in opioid-tolerant individuals. Submitted.
13. Wiest, K., Algera, M.H., Moss, L., van Velzen, M. & Dobbins, R.High plasma buprenorphine concentrations decrease respiratory effects of intravenous fentanyl [poster]. Presented at the Annual Meeting of the American Society of Addiction Medicine; April 4–7, 2019; Orlando, FL.
14. Dahan, A., Nieuwenhuijs, D. & Teppema, L. Plasticity of central chemoreceptors: effect of bilateral carotid body resection on central CO2 sensitivity. *PLoS Med* **4**, e239 (2007).
15. Dahan, A., DeGoede, J., Berkenbosch, A. & Olievier, I.C. The influence of oxygen on the ventilatory response to carbon dioxide in man. *J. Physiol.* **428**, 485–499 (1990).
16. Anderson, B.J. & Holford, N.H. Mechanistic basis of using body size and maturation to predict clearance in humans. *Drug Metab. Pharmacokinet.* **24**, 25–36 (2009).
17. Yassen, A. *et al*. Mechanism-based PK/PD modeling of the respiratory depressant effect of buprenorphine and fentanyl in healthy volunteers. *Clin. Pharmacol. Ther.* **81**, 50–58 (2007).
18. Olofsen, E. *et al*. Naloxone reversal of morphine- and morphine-6-glucuronide-induced respiratory depression in healthy volunteers: a mechanism-based pharmacokinetic-pharmacodynamic modeling study. *Anesthesiology* **112**, 1417–1427 (2010).
19. Bergstrand, M., Hooker, A.C., Wallin, J.E. & Karlsson, M.O. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *AAPS J.* **13**, 143–151 (2011).
20. Comets, E., Brendel, K. & Mentre, F. Model evaluation in nonlinear mixed effect models, with applications to pharmacokinetics. *J. Soc. Fr. Statistique.* **151**, 106–128 (2010).
21. Mercer, S.L. & Coop, A. Opioid analgesics and P-glycoprotein efflux transporters: a potential systems-level contribution to analgesic tolerance. *Curr. Top Med. Chem.* **11**, 1157–1164 (2011).
22. Holmquist, G.L. Opioid metabolism and effects of cytochrome P450. *Pain Med.* **10** (suppl. 1), S20–S29 (2009).
23. Labroo, R.B., Paine, M.F., Thummel, K.E. & Kharasch, E.D. Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions. *Drug Metab. Dispos.* **25**, 1072–1080 (1997).
24. Yu, C., Yuan, M., Yang, H., Zhuang, X. & Li, H. P-Glycoprotein on blood-brain barrier plays a vital role in fentanyl brain exposure and respiratory toxicity in rats. *Toxicol. Sci.* **164**, 353–362 (2018).
25. Hill, R. *et al*. Ethanol reversal of tolerance to the respiratory depressant effects of morphine. *Neuropsychopharmacology* **41**, 762–773 (2016).
26. Hickman, M., Lingford-Hughes, A., Bailey, C., Macleod, J., Nutt, D. & Henderson, G. Does alcohol increase the risk of overdose death: the need for a translational approach. *Addiction* **103**, 1060–1062 (2008).
27. Levine, B., Green, D. & Smialek, J.E. The role of ethanol in heroin deaths. *J. Forensic Sci.* **40**, 808–810 (1995).
28. White, J.M. & Irvine, R.J. Mechanisms of fatal opioid overdose. *Addiction* **94**, 961–972 (1999).
29. Bukten, A., Riksheim Stavesth, M., Skurtveit, S., Tverdal, A., Strang, J. & Clausen, T. High risk of overdose death following release from prison: variations in mortality during a 15-year observation period. *Addiction* **112**, 1432–1439 (2017).
30. Dahan, A., Nieuwenhuijs, D., Olofsen, E., Sarton, E., Romberg, R. & Teppema, L. Response surface modeling of alfentanil-sevoflurane interaction on cardiorespiratory control and bispectral index. *Anesthesiology* **94**, 982–991 (2001).
31. Romberg, R., Olofsen, E., Sarton, E., Teppema, L. & Dahan, A. Pharmacodynamic effect of morphine-6-glucuronide versus morphine on hypoxic and hypercapnic breathing in healthy volunteers. *Anesthesiology* **99**, 788–798 (2003).
32. Boom, M. *et al*. Fentanyl utility function: a risk-benefit composite of pain relief and breathing responses. *Anesthesiology* **119**, 663–674 (2013).
33. van der Schrier, R. *et al*. Influence of ethanol on oxycodone-induced respiratory depression: a dose-escalating study in young and elderly individuals. *Anesthesiology* **126**, 534–542 (2017).