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Higher naloxone dosing in a quantitative systems pharmacology model that predicts naloxone-fentanyl competition at the opioid mu receptor level

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Abstract

Rapid resuscitation of an opioid overdose with naloxone, an opioid antagonist, is critical. We developed an opioid receptor quantitative systems pharmacology (QSP) model for evaluation of naloxone dosing. In this model we examined three opioid exposure levels that have been reported in the literature (25 ng/ml, 50 ng/ml, and 75 ng/ml of fentanyl). The model predicted naloxone-fentanyl interaction at the mu opioid receptor over a range of three naloxone doses. For a 2 mg intramuscular (IM) dose of naloxone at lower fentanyl exposure levels (25 ng/ml and 50 ng/ml), the time to decreasing mu receptor occupancy by fentanyl to 50% was 3 and 10 minutes, respectively. However, at a higher fentanyl exposure level (75 ng/ml), a dose of 2 mg IM of the naloxone failed to reduce mu receptor occupancy by fentanyl to 50%. In contrast, naloxone doses of 5 mg and 10 mg IM reduced mu receptor occupancy by fentanyl to 50% in 5.5 and 4 minutes respectively. These results suggest that the current doses of naloxone (2 mg IM or 4 mg intranasal (IN)) may be inadequate for rapid reversal of toxicity due to fentanyl exposure and that increasing the dose of naloxone is likely to improve outcomes.

Background

Data from the Centers for Disease Control (CDC) has identified a rise of almost 10% in deaths due to drug overdoses killing approximately 71,000 Americans in 2017 [1]. New provisional data from the CDC suggests a drop in overall deaths (68,000) due to drug overdose in 2018 [2]. However, the number of deaths due to illicitly manufactured synthetic opioids, such as fentanyl, continues to rise [2]. Fentanyl is a synthetic opiate that is considered 50 to 100-fold more potent than morphine [3] and like other opiates, binds to the mu opiate receptors in the central nervous system (CNS). Fentanyl is known to cause respiratory depression within minutes of exposure [4, 5]. Rapid brain hypoxia and ultimately death can occur within minutes after fentanyl exposure [6]. The potency of fentanyl is thought to be due to rapid binding and high

occupancy of the opiate mu receptors in the brainstem respiratory centers of the CNS [7, 8]. Recent reported overdose systemic blood levels of fentanyl vary widely from to 0.5 ng/ml to 162 ng/ml [9–12]. The mean level of fentanyl exposure was 52.9 ng/ml in one series of overdose patients [13].

Naloxone is a synthetic derivative of oxymorphone that antagonizes opioids and is used as a countermeasure in acute opioid overdose. It has been postulated to antagonize the three opiate receptors in the brain (mu, kappa, alpha) [14]. The current approved doses of naloxone for home or self-administration are 4 mg IN and 2 mg IM. These two different dosage forms approximate similar systemic exposure as the IN administration route results in approximately 45% bioavailability compared to IM [15]. Prior to the new synthetic opioid era, community programs reported nearly 100% naloxone post administration survival rates with approved doses of naloxone [16]. However, the continuing rise of the more potent illicitly manufactured fentanyl and other synthetic opioids has created new challenges for the adequate treatment of overdoses. Naloxone is considered the recommended treatment for acute opioid toxicity [17] but an assessment of adequacy of the current doses has yet to be examined because an assessment in clinical trials would be logistically and ethically problematic.

We developed an opioid receptor QSP model and conducted simulation experiments to evaluate the effects of naloxone dosing. The model examined reported ranges of fentanyl systemic exposure in overdoses and predicted naloxone-fentanyl competition at the mu opioid receptor over a range of naloxone doses. Outcompeting fentanyl with naloxone at the mu opioid receptor in the CNS is critical to reversing opioid toxicity. The simulations examined the change in % mu receptor occupancy by fentanyl after different doses of naloxone administration. In addition, we examined time to a reduction to 50% mu receptor occupancy by fentanyl, as this endpoint has been associated with clinical reversal of opiate toxicity [18].

The results of the simulations suggest that the current approved doses of naloxone (2 mg IM or 4 mg IN) may be inadequate for rapid reversal of toxicity at fentanyl levels observed in overdose victims and that outcomes could be improved with increased doses.

Methods

A PhysioPD Research Platform (model) was developed using SimBiology (The MathWorks, MATLAB 2018b). The model includes non-linear ordinary differential equations (ODEs) to represent plasma and brain pharmacokinetics (PK) for fentanyl and naloxone, and mu receptor dynamics. The mu receptor submodel includes standard competitive binding dynamics and accounts for receptor synthesis, internalization, and recycling. (Fig 1 and S1 Table) Parameters to characterize these dynamics were taken from literature and are summarized in Table 1. Plasma PK for IM naloxone was based on a published model [19]. Various routes of administration are possible for fentanyl. Model simulations do not specify a particular route of fentanyl administration, but instead start with a high plasma concentration relevant for overdose scenarios and capture the clearance phase of fentanyl PK. Because of non-specific binding, concentrations in the brain of fentanyl available for binding to mu receptor are difficult to measure experimentally. Published data for the relationship between naloxone dose and mu receptor occupancy [20], and between fentanyl dose, receptor occupancy, and pain relief [21–23] were used to infer brain exposures. For example, a fentanyl plasma level of 5 ng/ml is associated with pain relief [24], and pain relief is evident at mu receptor occupancy above 10% [25]. The model assumes 15% mu receptor occupancy at a plasma concentration of 5 ng/ml. Using a Ki of 1.35 nM (Table 1), this suggests a fentanyl concentration at the mu receptor of ~0.24 nM for pain relief. The model contains a single subject representing a habitual opioid
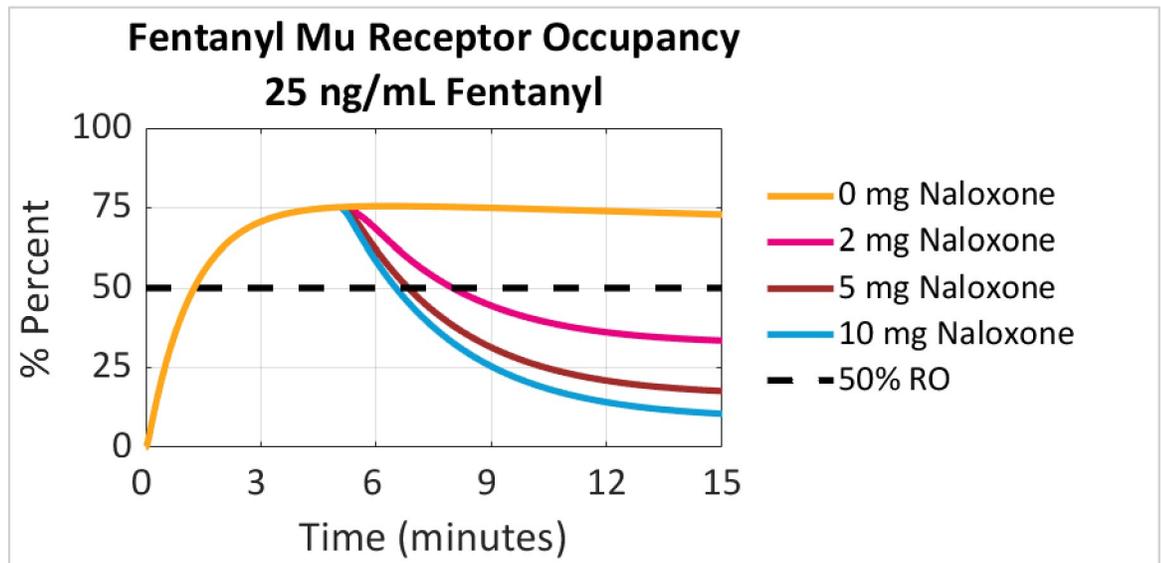
Fig 1. Graphical depiction of the mu receptor submodel. The model accounts for mu receptor synthesis and degradation, competitive binding to the receptor, internalization, recycling, and clearance.

user. The amount of mu receptor and rate of receptor internalization have been set to represent a subject with opioid tolerance [26, 27].

Model simulations illustrate the time course of fentanyl concentration in plasma and brain, naloxone concentration in plasma and brain, and fentanyl and naloxone mu receptor

Table 1. Data from the literature on receptor dynamics used in the model *.

Species	Parameter	Model Value	Range in literature	References
Naloxone	Mu receptor binding Ki	1.115 nM	0.16–6.6 nM	[29, 35–50]
Fentanyl	Mu receptor binding Ki	1.35 nM	0.76–3 nM	[41, 43, 49, 51–53]
Mu Receptor	Number of receptors in brain	2.37×10^7 fmol/brain	1.9×10^7–3×10^7 fmol/brain	[45, 54–57]
Mu Receptor	Unbound receptor half-life	12 hours	12 hours	[58]
Mu Receptor	Bound receptor half-life	7 hours	7 hours	[58]
Mu Receptor	Naloxone-bound receptor internalized after 30 minutes	15%	Little to none	[59, 60]
Mu Receptor	Fentanyl-bound receptor internalized after 30 minutes	80%	50–88%	[61]
Naloxone	Plasma PK	Based on published model		[19]
Fentanyl	Plasma PK	Based on published models		[10, 11, 62–70]
Fentanyl	Plasma concentration, surgery	10	1–20 ng/mL	[71–82]
Fentanyl	Plasma concentration, loss of consciousness	25	3–34	[68, 83–86]
Fentanyl	Plasma concentration in overdose	50	1–102	[10, 11, 62–69]
Fentanyl	Appearance in the brain	<1 minute post dose	<1 minute post dose	Based on appearance rates [42, 87–89]
Naloxone	Appearance in the brain	<1 minute post dose	<1 minute post dose	[20, 28, 90, 91]
Fentanyl	Brain concentration	Based on dose	Based on dose	[87, 92–95]
Naloxone	Brain concentration	Based on dose	Based on dose	[20]

occupancy. We assumed plasma exposure level of fentanyl at 50 ng/ml as mid-range exposure based on plasma concentrations in overdose victims (Table 1) and also examined fentanyl exposure levels of 25 ng/ml and 75 ng/ml. Naloxone doses examined were 2 mg, 5 mg, and 10 mg IM. Naloxone administration was assumed to occur as a single dose at 5 minutes after peak fentanyl concentration in plasma, which may reasonably reflect response time following initial symptoms of overdose.

Results

A single dose of IM naloxone was administered at dose levels of 2 mg, 5 mg, and 10 mg IM five minutes after peak fentanyl plasma concentration of 25 ng/mL, 50 ng/mL or 75 ng/mL.

As noted in Fig 2, model simulations predicted that a fentanyl overdose exposure level of 25 ng/ml leads to 73% mu receptor occupancy by fentanyl without competition from naloxone. Administration of naloxone leads to rapid reduction in mu receptor occupancy by fentanyl due to competition by naloxone, as would be expected based on naloxone's fast appearance in brain [20, 28], and the rapid dissociation rates of fentanyl and naloxone [29]. After administering a 2 mg IM of naloxone, mu receptor occupancy by fentanyl was predicted to decrease to 33% within ten minutes of naloxone being administered. The model also predicted that giving a 5 mg IM dose of naloxone would decrease mu receptor occupancy by fentanyl to 17% and giving a 10 mg IM dose of naloxone would decrease of mu receptor occupancy by fentanyl to 9% within ten minutes.

The time to decreasing mu receptor occupancy by fentanyl to below 50% with exposure level of fentanyl at 25 ng/ml with various naloxone doses was also examined. While the
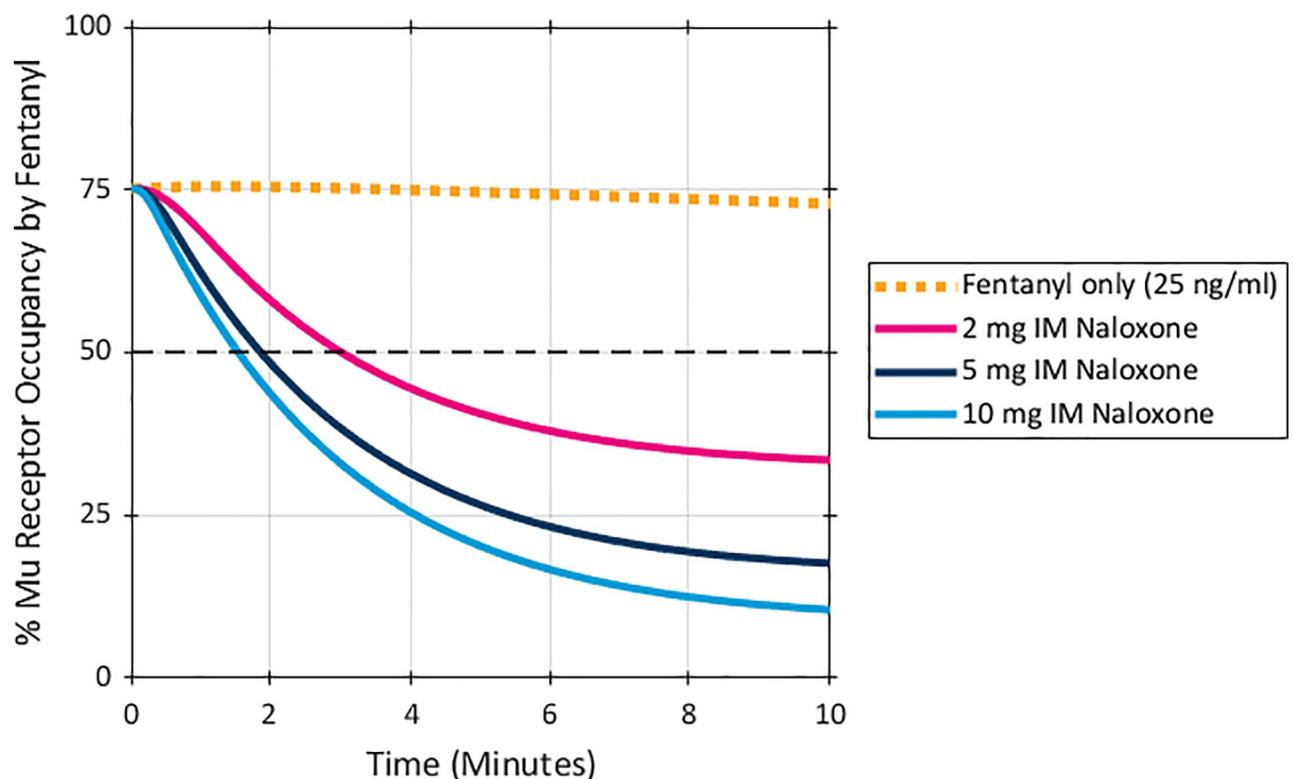

Fig 2. Predicted % mu receptor occupancy by fentanyl after a peak fentanyl plasma concentration of 25 ng/ml. Naloxone was given at time 0 and simulated based on an IM dose PK. Dashed black line marked at 50% receptor occupancy.
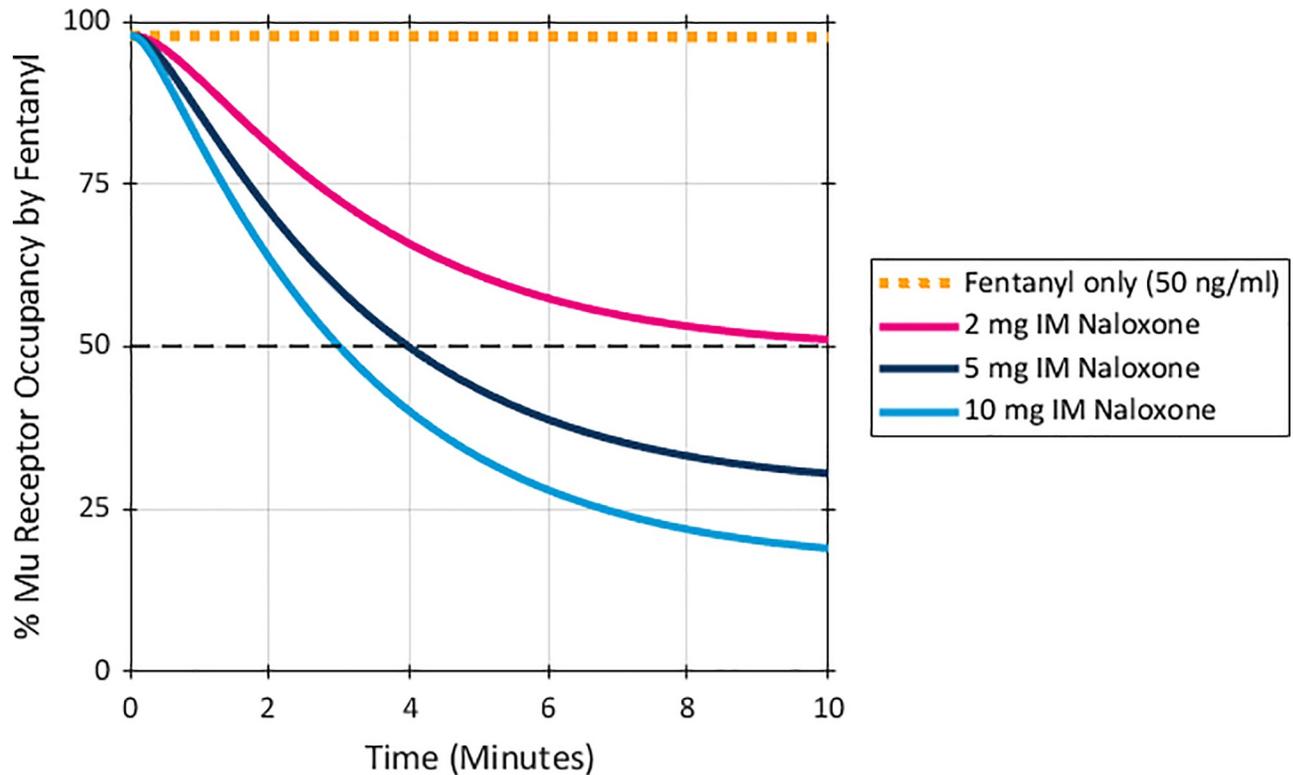
Fig 3. Predicted %mu receptor occupancy by fentanyl after a peak fentanyl plasma concentration of 50 ng/ml. Naloxone was given at time 0 and simulated based on an IM dose PK. Dashed black line marked at 50% receptor occupancy.

receptor occupancy associated with respiratory arrest may vary across patients, it is nonetheless instructive to observe the effect of dose escalation on the time to cross a given level of receptor occupancy. As shown in Fig 2, increasing dose is associated with a faster time to cross the threshold. A dose of 2 mg IM reduced mu receptor occupancy by fentanyl to 50% in 3 minutes, while doses of 5 mg IM and 10 mg IM of naloxone resulted in a decrease to 50% mu receptor occupancy by fentanyl in 2 and 1.5 minutes, respectively.

The next set of simulations examined a higher fentanyl plasma exposure of 50 ng/ml which was predicted to result in 97% mu receptor occupancy by fentanyl without naloxone administration, as shown in Fig 3. At this fentanyl exposure level, a dose of 2 mg IM naloxone was predicted to decrease mu receptor occupancy by fentanyl to 50%, while naloxone doses of 5 mg and 10 mg IM reduced mu receptor occupancy by fentanyl to 29% and 17%, respectively.

The time to 50% mu receptor occupancy by fentanyl with an exposure to fentanyl level of 50 ng/ml again showed a significant dose effect. As shown in Fig 3, a dose of 2 mg IM of naloxone reduced mu receptor occupancy by fentanyl to 50% in 10 minutes, while the 5 mg and 10 mg IM doses of naloxone shortened the time to a 50% decrease in mu receptor occupancy by fentanyl to 4 minutes and 3 minutes, respectively.

Results for the even higher fentanyl exposure levels (75 ng/ml), as shown in Fig 4, were consistent with the patterns observed at the lower exposures. The model predicted a 98.9% mu receptor occupancy by fentanyl without naloxone administration. A dose of 2 mg IM, naloxone reduced mu receptor occupancy by fentanyl to 62% at 10 minutes after naloxone administration, while naloxone doses of 5 mg and 10 mg IM decreased receptor occupancy by fentanyl to 40% and 26%, respectively, ten minutes post administration.

Fig 4. Predicted % mu receptor occupancy by fentanyl after a peak fentanyl plasma concentration of 75ng/ml. Naloxone was given at time 0 and simulated based on an IM dose PK. Dashed black line marked at 50% receptor occupancy.
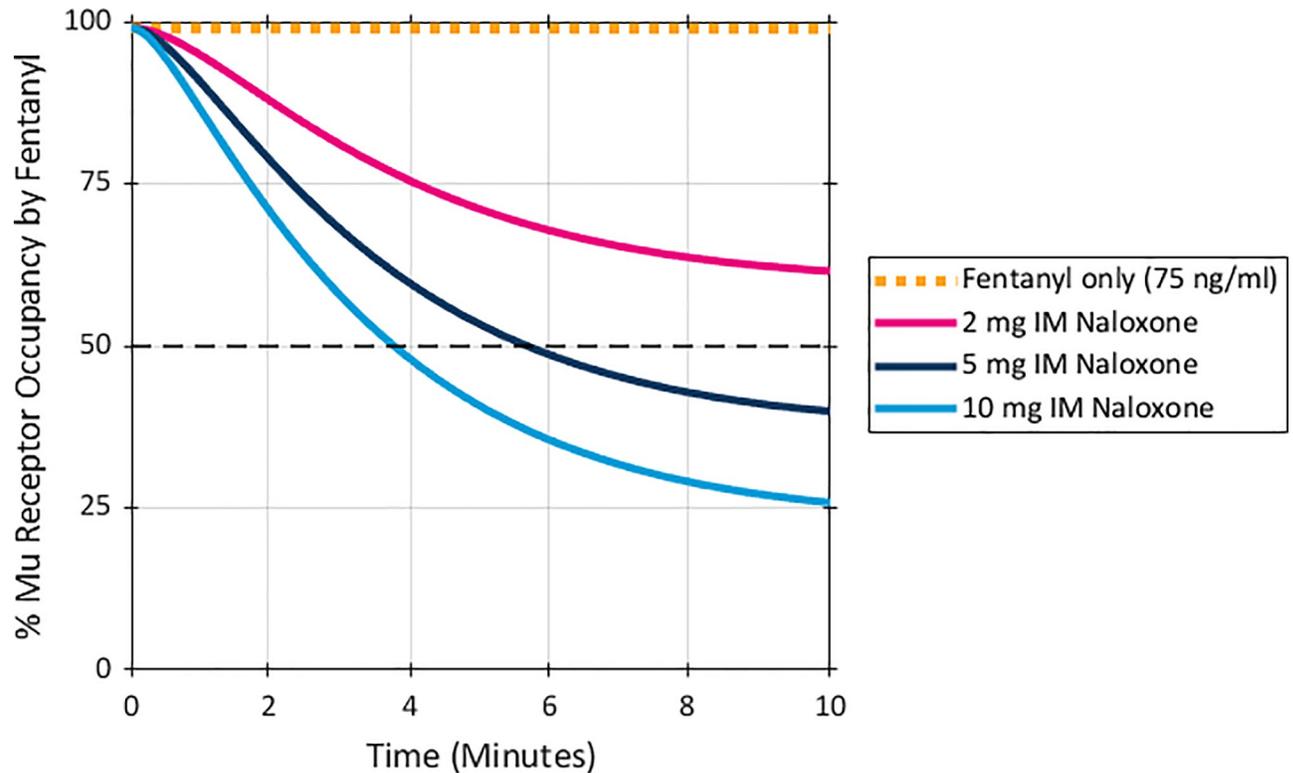
As was the case at the lower fentanyl exposures, the time to decreasing to 50% mu receptor occupancy by fentanyl after an exposure level of 75 ng/ml was responsive to dose. As shown in Fig 3, a dose of 2 mg IM did not reduce mu receptor occupancy by fentanyl to 50% within 10 minutes at this exposure level, while doses of 5 mg and 10 mg IM of naloxone reduced mu receptor occupancy by fentanyl below 50% in 5.5 minutes and 4 minutes, respectively.

Discussion

Rapidly decreasing opioid mu receptor occupancy seems imperative for successful clinical reversal, particularly for the more potent synthetic opioids. Sommerville, et al., reported [30] a study where 83% of patients required greater than 2 mg naloxone doses prior to a clinical response. Of particular concern is that 36% of fatal deaths had a drug overdose within seconds to minutes after drug use. Upon Emergency Medical Service (EMS) arrival, 90% were pulse-less. This report and others support the notion that acutely administered higher doses of naloxone are needed for rapid and adequate clinical reversal, particularly when the more potent synthetic opioids are abused.

Using an opioid receptor QSP model we predicted naloxone-fentanyl interaction at the mu opioid receptor over a range of naloxone doses. We examined three levels of fentanyl exposure in the plasma found in overdose patients. The high receptor mu occupancy of fentanyl at the doses observed in this model without naloxone administration is consistent with reports of the potency of this synthetic opioid and its relation to a recent spike in overdoses and deaths. Fentanyl is known to be persistently lipophilic and has been described as having a rapid transport in and out of the CNS [31].

The results of simulations in our model suggest that at exposure levels of fentanyl capable of resulting in respiratory depression and death (50 ng/ml and 75 ng/ml), the current doses of naloxone (2 mg IM and 4 mg IN) may be inadequate for a rapid successful reversal. Respiratory depression occurs within minutes of fentanyl exposure [4] and brain damage and death occur within 6 minutes of anoxia [32]. With a fentanyl exposure level of 50 ng/ml, the model results suggested that the time to 50% fentanyl receptor occupancy for 2 mg IM of naloxone was 10 minutes. Reversing fentanyl toxicity in 10 minutes is likely too long for a successful reversal. In contrast, the time to 50% fentanyl occupancy was 4 and 3 minutes, for 5 mg and 10 mg of naloxone IM administrations, respectively. Even more concerning was the finding from the model that at higher fentanyl exposure levels (75 ng/ml), a 2 mg IM of naloxone did not result in decreasing mu receptor occupancy by fentanyl below 50% within ten minutes after administration. Higher doses of naloxone, 5 mg and 10 mg IM, however, resulted in time to decreasing to 50% mu receptor occupancy by fentanyl in 5.5 and 4 minutes, respectively. These response times are still within a window for a potential successful resuscitation.

The model results also suggested that at lower levels of fentanyl exposure (25 ng/ml), current doses of naloxone (2 mg IM or 4 mg IN) may be adequate, with a relatively short time to 50% occupancy displacement of fentanyl (3 minutes). However, the assumption of this model is that naloxone would be given after 5 minutes of fentanyl exposure. Giving a higher dose of naloxone would still be advantageous if naloxone administration was delayed for longer than 5 minutes after drug exposure. In addition, it is impractical to ascertain the level of fentanyl exposure in an overdosed patient. Therefore, undertreating the patient, with the current doses of naloxone, based on model predictions, may have dire consequences.

The model uses a number of assumptions but is consistent with other studies suggesting that the mu receptor occupancy by fentanyl increases with an increase in systemic fentanyl exposure [21], and that naloxone receptor occupancy increases as naloxone dose increases [23].

Our model has a number of limitations. It represents plasma and brain PK and mu receptor dynamics based on our current understanding of opioids and the brain, which is incomplete. It uses several plausible assumptions which may not always be valid in actual overdoses. It does not examine implications of different routes of fentanyl administration or of repeat fentanyl dosing. The current model also does not account for variability between patients and only examines one patient type, a habitual opioid user. Nonetheless, in the absence of definitive data and in light of the practical impossibility of a controlled clinical trial, the model vastly improves upon qualitative statements and mental interpolations by utilizing and integrating available clinical and mechanistic evidence and known receptor competition dynamics. The results of the model simulations suggesting that at lower exposure levels of fentanyl, current doses of naloxone may be adequate for reversal of opioid toxicity are consistent with clinical observations. The simulation results suggesting that current doses of naloxone are inadequate for higher doses of fentanyl exposure (50 ng/ml and 75 ng/ml) are also consistent with clinical results. The model demonstrates that all of these observations are consistent with a receptor competition mechanism and helps quantify the additional benefit of the higher 5 mg and 10 mg IM doses. These analyses strongly support the idea that higher doses of naloxone (5 mg IM and 10 mg IM), as examined in this model would appear to be superior compared to the current naloxone doses (2 mg IM or 4 mg IN), in many overdose situations.

Conclusions

In summary, this study examined a simulation model of mu receptor occupancy and supports the notion that naloxone may be underdosed in many situations of fentanyl exposure. The risk

associated with underdosing of naloxone is an unsuccessful reversal of opioid toxicity leading to death, which outweighs the risk of opioid withdrawal [33]. This model supports the notion that higher doses of naloxone are required as a countermeasure to the new synthetic opioid epidemic [34].

Supporting information

S1 Fig. Simulated naloxone receptor occupancy vs. peak plasma concentration is consistent with published data from Johansson et al. (Johansson et al. 2019). Simulated naloxone receptor occupancy reflects binding affinity and interstitial fluid (ISF) concentration. Relevant IM and intranasal (IN) doses are indicated on the figure. IM administration has a higher bio-availability than intranasal (IN) administration, allowing for a higher plasma concentration at the same dose.
(TIF)

S2 Fig.
(TIFF)

S1 Table.
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Author Contributions

Conceptualization: Ronald B. Moss.

Data curation: Meghan McCabe Pryor.

Formal analysis: Rebecca Baillie, Katherine Kudrycki, Christina Friedrich, Mike Reed, Dennis J. Carlo.

References

1. CDC. Drug Overdose Deaths CDC.gov: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control; 2018 [updated March 19, 2020. Available from: https://www.cdc.gov/drugoverdose/data/statedeaths.html.

2. Ahmad FB, Rossen LM, Sutton P. Provisional Drug Overdose Death Counts CDC.gov: CDC/National Center for Health Statistics; 2020 [updated March 11, 2020. Available from: https://www.cdc.gov/nchs/nvss/vsrr/drug-overdose-data.htm.

3. Harper MH, Hickey RF, Cromwell TH, Linwood S. The magnitude and duration of respiratory depression produced by fentanyl and fentanyl plus droperidol in man. The Journal of pharmacology and experimental therapeutics. 1976; 199(2):464–8. PMID: 978496

4. Dahan A, Aarts L, Smith TW. Incidence, Reversal, and Prevention of Opioid-induced Respiratory Depression. Anesthesiology. 2010; 112(1):226–38. https://doi.org/10.1097/ALN.0b013e3181c38c25 PMID: 20010421

5. Vardanyan RS, Hruby VJ. Fentanyl-related compounds and derivatives: current status and future prospects for pharmaceutical applications. Future Med Chem. 2014; 6(4):385–412. https://doi.org/10.4155/fmc.13.215 PMID: 24635521

6. Solis E Jr., Cameron-Burr KT, Kiyatkin EA. Heroin Contaminated with Fentanyl Dramatically Enhances Brain Hypoxia and Induces Brain Hypothermia. eNeuro. 2017; 4(5).

7. Pattinson KT. Opioids and the control of respiration. British journal of anaesthesia. 2008; 100(6):747–58. https://doi.org/10.1093/bja/aen094 PMID: 18456641

8. White JM, Irvine RJ. Mechanisms of fatal opioid overdose. Addiction (Abingdon, England). 1999; 94 (7):961–72.

9. Tomassoni AJ, Hawk KF, Jubanyik K, Nogee DP, Durant T, Lynch KL, et al. Multiple Fentanyl Overdoses—New Haven, Connecticut, June 23, 2016. MMWR Morbidity and mortality weekly report. 2017; 66(4):107–11. https://doi.org/10.15585/mm6604a4 PMID: 28151928

10. Lee D, Chronister CW, Broussard WA, Utley-Bobak SR, Schultz DL, Vega RS, et al. Illicit Fentanyl-Related Fatalities in Florida: Toxicological Findings. Journal of analytical toxicology. 2016; 40(8):588–94. https://doi.org/10.1093/jat/bkw087 PMID: 27702938

11. Dwyer JB, Janssen J, Luckasevic TM, Williams KE. Report of Increasing Overdose Deaths that include Acetyl Fentanyl in Multiple Counties of the Southwestern Region of the Commonwealth of Pennsylvania in 2015–2016. Journal of forensic sciences. 2018; 63(1):195–200. https://doi.org/10.1111/1556-4029.13517 PMID: 28605020

12. Fogarty MF, Papsun DM, Logan BK. Analysis of Fentanyl and 18 Novel Fentanyl Analogs and Metabolites by LC-MS-MS, and report of Fatalities Associated with Methoxyacetylfentanyl and Cyclopropylfentanyl. Journal of analytical toxicology. 2018; 42(9):592–604. https://doi.org/10.1093/jat/bky035 PMID: 29750250

13. Sutter ME, Gerona RR, Davis MT, Roche BM, Colby DK, Chenoweth JA, et al. Fatal Fentanyl: One Pill Can Kill. Academic emergency medicine: official journal of the Society for Academic Emergency Medicine. 2017; 24(1):106–13.

14. Handal KA, Schauben JL, Salamone FR. Naloxone. Annals of emergency medicine. 1983; 12(7):438–45. https://doi.org/10.1016/s0196-0644(83)80343-6 PMID: 6309038

15. FDA. Highlights of Prescribing Information NARCAN® (naloxone hydrochloride) nasal spray Reference ID: 3848912: FDA; 2015 [Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208411lbl.pdf.

16. Fairbairn N, Coffin PO, Walley AY. Naloxone for heroin, prescription opioid, and illicitly made fentanyl overdoses: Challenges and innovations responding to a dynamic epidemic. The International journal on drug policy. 2017; 46:172–9. https://doi.org/10.1016/j.drugpo.2017.06.005 PMID: 28687187

17. General S. U.S. Surgeon General's Advisory on Naloxone and Opioid Overdose HHS.gov: Office of the Surgeon General; 2018 [updated April 5, 2018. Available from: https://www.hhs.gov/surgeongeneral/priorities/opioids-and-addiction/naloxone-advisory/index.html.

18. Melichar JK, Nutt DJ, Malizia AL. Naloxone displacement at opioid receptor sites measured in vivo in the human brain. European journal of pharmacology. 2003; 459(2–3):217–9. https://doi.org/10.1016/s0014-2999(02)02872-8 PMID: 12524149

19. Dowling J, Isbister GK, Kirkpatrick CM, Naidoo D, Graudins A. Population pharmacokinetics of intravenous, intramuscular, and intranasal naloxone in human volunteers. Therapeutic drug monitoring. 2008; 30(4):490–6. https://doi.org/10.1097/FTD.0b013e3181816214 PMID: 18641540

20. Johansson J, Hirvonen J, Lovro Z, Ekblad L, Kaasinen V, Rajasilta O, et al. Intranasal naloxone rapidly occupies brain mu-opioid receptors in human subjects. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 2019; 44(9):1667–73.

21. Yassen A, Olofsen E, van Dorp E, Sarton E, Teppema L, Danhof M, et al. Mechanism-based pharmacokinetic-pharmacodynamic modelling of the reversal of buprenorphine-induced respiratory depression by naloxone: a study in healthy volunteers. Clinical pharmacokinetics. 2007; 46(11):965–80. https://doi.org/10.2165/00003088-200746110-00004 PMID: 17922561

22. Stanski DR, Hug CC, Jr. Alfentanil—a kinetically predictable narcotic analgesic. Anesthesiology. 1982; 57(6):435–8. https://doi.org/10.1097/00000542-198212000-00001 PMID: 6128947

23. Fallows Z. Drug Chart MIT: Center for Health Promotion and Wellness at MIT Medical; 2012 [updated 2012-06-08. Available from: https://ocw.mit.edu/ans7870/ES/ES.S10/S13/studymaterials/drugchart.htm.

24. Scott JC, Ponganis KV, Stanski DR. EEG quantitation of narcotic effect: the comparative pharmacodynamics of fentanyl and alfentanil. Anesthesiology. 1985; 62(3):234–41. https://doi.org/10.1097/00000542-198503000-00005 PMID: 3919613

25. Yassen A, Olofsen E, Dahan A, Danhof M. Pharmacokinetic-pharmacodynamic modeling of the antinociceptive effect of buprenorphine and fentanyl in rats: role of receptor equilibration kinetics. The Journal of pharmacology and experimental therapeutics. 2005; 313(3):1136–49. https://doi.org/10.1124/jpet.104.082560 PMID: 15701707

26. Allouche S, Noble F, Marie N. Opioid receptor desensitization: mechanisms and its link to tolerance. Frontiers in pharmacology. 2014; 5:280. https://doi.org/10.3389/fphar.2014.00280 PMID: 25566076

27. Li F, Ma H, Wu N, Li J. IRAS Modulates Opioid Tolerance and Dependence by Regulating mu Opioid Receptor Trafficking. Mol Neurobiol. 2016; 53(7):4918–30. https://doi.org/10.1007/s12035-015-9417-6 PMID: 26363797

28. Eriksson O, Antoni G. [11C]Carfentanil Binds Preferentially to mu-Opioid Receptor Subtype 1 Compared to Subtype 2. Molecular imaging. 2015; 14:476–83. PMID: 26461068

29. Ilien B, Galzi JL, Mejean A, Goeldner M, Hirth C. A mu-opioid receptor-filter assay. Rapid estimation of binding affinity of ligands and reversibility of long-lasting ligand-receptor complexes. Biochemical pharmacology. 1988; 37(20):3843–51. https://doi.org/10.1016/0006-2952(88)90065-2 PMID: 2847746

30. Somerville NJ, O'Donnell J, Gladden RM, Zibbell JE, Green TC, Younkin M, et al. Characteristics of Fentanyl Overdose—Massachusetts, 2014–2016. MMWR Morbidity and mortality weekly report. 2017; 66(14):382–6. https://doi.org/10.15585/mmwr.mm6614a2 PMID: 28406883

31. Burns SM, Cunningham CW, Mercer SL. DARK Classics in Chemical Neuroscience: Fentanyl. ACS Chem Neurosci. 2018; 9(10):2428–37. https://doi.org/10.1021/acschemneuro.8b00174 PMID: 29894151

32. NIH. Cerebral Hypoxia Information Page. What research is being done? NIH.gov: National Institute of Neurological Disorders and Stroke; 2019 [updated 2019-03-27. Available from: https://www.ninds.nih.gov/Disorders/All-Disorders/Cerebral-Hypoxia-Information-Page.

33. Rzasa Lynn R, Galinkin JL. Naloxone dosage for opioid reversal: current evidence and clinical implications. Therapeutic advances in drug safety. 2018; 9(1):63–88. https://doi.org/10.1177/2042098617744161 PMID: 29318006

34. Moss RB, Carlo DJ. Higher doses of naloxone are needed in the synthetic opiod era. Substance abuse treatment, prevention, and policy. 2019; 14(1):6. https://doi.org/10.1186/s13011-019-0195-4 PMID: 30777088

35. Selley DE, Liu Q, Childers SR. Signal transduction correlates of mu opioid agonist intrinsic efficacy: receptor-stimulated [35S]GTP gamma S binding in mMOR-CHO cells and rat thalamus. The Journal of pharmacology and experimental therapeutics. 1998; 285(2):496–505. PMID: 9580589

36. Emmerson PJ, Clark MJ, Mansour A, Akil H, Woods JH, Medzihradsky F. Characterization of opioid agonist efficacy in a C6 glioma cell line expressing the mu opioid receptor. The Journal of pharmacology and experimental therapeutics. 1996; 278(3):1121–7. PMID: 8819494

37. Childers SR, Creese I, Snowman AM, Synder SH. Opiate receptor binding affected differentially by opiates and opioid peptides. European journal of pharmacology. 1979; 55(1):11–8. https://doi.org/10.1016/0014-2999(79)90142-0 PMID: 220062

38. Carroll JA, Shaw JS, Wickenden AD. The physiological relevance of low agonist affinity binding at opioid mu-receptors. British journal of pharmacology. 1988; 94(2):625–31. https://doi.org/10.1111/j.1476-5381.1988.tb11569.x PMID: 2840167

39. Maguire P, Tsai N, Kamal J, Cometta-Morini C, Upton C, Loew G. Pharmacological profiles of fentanyl analogs at mu, delta and kappa opiate receptors. European journal of pharmacology. 1992; 213 (2):219–25. https://doi.org/10.1016/0014-2999(92)90685-w PMID: 1355735

40. de Jong LA, Kramer K, Kroeze MP, Bischoff R, Uges DR, Franke JP. Development and validation of a radioreceptor assay for the determination of morphine and its active metabolites in serum. Journal of pharmaceutical and biomedical analysis. 2005; 39(5):964–71. https://doi.org/10.1016/j.jpba.2005.04.049 PMID: 15985356

41. Toll L, Berzetei-Gurske IP, Polgar WE, Brandt SR, Adapa ID, Rodriguez L, et al. Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. NIDA research monograph. 1998; 178:440–66. PMID: 9686407

42. Kalvass JC, Olson ER, Cassidy MP, Selley DE, Pollack GM. Pharmacokinetics and pharmacodynamics of seven opioids in P-glycoprotein-competent mice: assessment of unbound brain EC50,u and correlation of in vitro, preclinical, and clinical data. The Journal of pharmacology and experimental therapeutics. 2007; 323(1):346–55. https://doi.org/10.1124/jpet.107.119560 PMID: 17646430

43. Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI, et al. Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. Molecular pharmacology. 1994; 45(2):330–4. PMID: 8114680

44. Chen JC, Smith ER, Cahill M, Cohen R, Fishman JB. The opioid receptor binding of dezocine, morphine, fentanyl, butorphanol and nalbuphine. Life sciences. 1993; 52(4):389–96. https://doi.org/10.1016/0024-3205(93)90152-s PMID: 8093631

45. Yeadon M, Kitchen I. Comparative binding of mu and delta selective ligands in whole brain and pons/medulla homogenates from rat: affinity profiles of fentanyl derivatives. Neuropharmacology. 1988; 27 (4):345–8. https://doi.org/10.1016/0028-3908(88)90141-4 PMID: 2843777

46. Lipinski PFJ, Kosson P, Matalinska J, Roszkowski P, Czarnocki Z, Jaronczyk M, et al. Fentanyl Family at the Mu-Opioid Receptor: Uniform Assessment of Binding and Computational Analysis. Molecules (Basel, Switzerland). 2019; 24(4).

47. Alburges ME, Hanson GR, Gibb JW, Sakashita CO, Rollins DE. Fentanyl receptor assay. II. Utilization of a radioreceptor assay for the analysis of fentanyl analogs in urine. Journal of analytical toxicology. 1992; 16(1):36–41. https://doi.org/10.1093/jat/16.1.36 PMID: 1322477

48. Ellis CR, Kruhlak NL, Kim MT, Hawkins EG, Stavitskaya L. Predicting opioid receptor binding affinity of pharmacologically unclassified designer substances using molecular docking. PloS one. 2018; 13(5): e0197734. https://doi.org/10.1371/journal.pone.0197734 PMID: 29795628

49. Volpe DA, McMahon Tobin GA, Mellon RD, Katki AG, Parker RJ, Colatsky T, et al. Uniform assessment and ranking of opioid mu receptor binding constants for selected opioid drugs. Regulatory toxicology and pharmacology: RTP. 2011; 59(3):385–90. https://doi.org/10.1016/j.yrtph.2010.12.007 PMID: 21215785

50. Xu H, Kim CH, Zhu YC, Weber RJ, Jacobson AE, Rice KC, et al. (+)-cis-3-methylfentanyl and its analogs bind pseudoirreversibly to the mu opioid binding site: evidence for pseudoallosteric modulation. Neuropharmacology. 1991; 30(5):455–62. https://doi.org/10.1016/0028-3908(91)90006-w PMID: 1650928

51. Traynor JR, Nahorski SR. Modulation by mu-opioid agonists of guanosine-5'-O-(3-[35S]thio)triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. Molecular pharmacology. 1995; 47(4):848–54. PMID: 7723747

52. Brasel CM, Sawyer GW, Stevens CW. A pharmacological comparison of the cloned frog and human mu opioid receptors reveals differences in opioid affinity and function. European journal of pharmacology. 2008; 599(1–3):36–43. https://doi.org/10.1016/j.ejphar.2008.09.043 PMID: 18930720

53. Maillet EL, Milon N, Heghinian MD, Fishback J, Schurer SC, Garamszegi N, et al. Noribogaine is a G-protein biased kappa-opioid receptor agonist. Neuropharmacology. 2015; 99:675–88. https://doi.org/10.1016/j.neuropharm.2015.08.032 PMID: 26302653

54. Chan K, Brodsky M, Davis T, Franklin S, Inturrisi CE, Yoburn BC. The effect of the irreversible mu-opioid receptor antagonist clocinnamox on morphine potency, receptor binding and receptor mRNA. European journal of pharmacology. 1995; 287(2):135–43. https://doi.org/10.1016/0014-2999(95)00488-2 PMID: 8749027

55. Paronis CA, Woods JH. Clocinnamox dose-dependently antagonizes morphine-analgesia and [3H] DAMGO binding in rats. European journal of pharmacology. 1997; 337(1):27–34. https://doi.org/10.1016/s0014-2999(97)01296-x PMID: 9389377

56. Yoo JH, Borsodi A, Toth G, Benyhe S, Gaspar R, Matifas A, et al. Characterization of [(3)H] oxymorphone binding sites in mouse brain: Quantitative autoradiography in opioid receptor knockout mice. Neuroscience letters. 2017; 643:16–21. https://doi.org/10.1016/j.neulet.2017.02.002 PMID: 28192197

57. Yoburn BC, Billings B, Duttaroy A. Opioid receptor regulation in mice. The Journal of pharmacology and experimental therapeutics. 1993; 265(1):314–20. PMID: 8386239

58. Afify EA. Turnover of mu-opioid receptors in neuroblastoma cells. Brain research Molecular brain research. 2002; 106(1–2):83–7. https://doi.org/10.1016/s0169-328x(02)00414-x PMID: 12393267

59. Levoye A, Zwier JM, Jaracz-Ros A, Klipfel L, Cottet M, Maurel D, et al. A Broad G Protein-Coupled Receptor Internalization Assay that Combines SNAP-Tag Labeling, Diffusion-Enhanced Resonance Energy Transfer, and a Highly Emissive Terbium Cryptate. Frontiers in endocrinology. 2015; 6:167. https://doi.org/10.3389/fendo.2015.00167 PMID: 26617570

60. Vukojevic V, Ming Y, D'Addario C, Rigler R, Johansson B, Terenius L. Ethanol/naltrexone interactions at the mu-opioid receptor. CLSM/FCS study in live cells. PloS one. 2008; 3(12):e4008. https://doi.org/10.1371/journal.pone.0004008 PMID: 19104662

61. Sianati S. μ-Opioid receptor signalling mechanisms: quantifying bias and kinetics [Doctor of Philosophy Ph.D.]. University of Sydney: University of Sydney; 2015.

62. Andresen H, Gullans A, Veselinovic M, Anders S, Schmoldt A, Iwersen-Bergmann S, et al. Fentanyl: toxic or therapeutic? Postmortem and antemortem blood concentrations after transdermal fentanyl application. Journal of analytical toxicology. 2012; 36(3):182–94. https://doi.org/10.1093/jat/bks005 PMID: 22417834

63. Avedschmidt S, Schmidt C, Isenschmid D, Kesha K, Moons D, Gupta A. Acetyl Fentanyl: Trends and Concentrations in Metro Detroit. Journal of forensic sciences. 2019; 64(1):149–53. https://doi.org/10.1111/1556-4029.13840 PMID: 29940698

64. Gill JR, Lin PT, Nelson L. Reliability of postmortem fentanyl concentrations in determining the cause of death. Journal of medical toxicology: official journal of the American College of Medical Toxicology. 2013; 9(1):34–41.

65. Lilleng PK, Mehlum LI, Bachs L, Morild I. Deaths after intravenous misuse of transdermal fentanyl. Journal of forensic sciences. 2004; 49(6):1364–6. PMID: 15568716

66. Busardo FP, Carlier J, Giorgetti R, Tagliabracci A, Pacifici R, Gottardi M, et al. Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry Assay for Quantifying Fentanyl and 22 Analogs and Metabolites in Whole Blood, Urine, and Hair. Frontiers in chemistry. 2019; 7:184. https://doi.org/10.3389/fchem.2019.00184 PMID: 31001514

67. Kuhlman JJ Jr., McCaulley R, Valouch TJ, Behonick GS. Fentanyl use, misuse, and abuse: a summary of 23 postmortem cases. Journal of analytical toxicology. 2003; 27(7):499–504. https://doi.org/10.1093/jat/27.7.499 PMID: 14607006

68. Anderson DT, Muto JJ. Duragesic transdermal patch: postmortem tissue distribution of fentanyl in 25 cases. Journal of analytical toxicology. 2000; 24(7):627–34. https://doi.org/10.1093/jat/24.7.627 PMID: 11043670

69. Henderson GL. Fentanyl-related deaths: demographics, circumstances, and toxicology of 112 cases. Journal of forensic sciences. 1991; 36(2):422–33. PMID: 2066723

70. Corli O, Roberto A. Pharmacological and clinical differences among transmucosal fentanyl formulations for the treatment of breakthrough cancer pain: a review article. Minerva anestesiologica. 2014; 80 (10):1123–34. PMID: 24346227

71. Bovill JG, Sebel PS. Pharmacokinetics of high-dose fentanyl. A study in patients undergoing cardiac surgery. British journal of anaesthesia. 1980; 52(8):795–801. https://doi.org/10.1093/bja/52.8.795 PMID: 7426257

72. Smith C, McEwan AI, Jhaveri R, Wilkinson M, Goodman D, Smith LR, et al. The interaction of fentanyl on the Cp50 of propofol for loss of consciousness and skin incision. Anesthesiology. 1994; 81(4):820–8; discussion 26A. PMID: 7943832

73. Kelly HE, Shaw GM, Brett CN, Greenwood FM, Huckabee ML. The effect of titrated fentanyl on suppressed cough reflex in healthy adult volunteers. Anaesthesia. 2016; 71(5):529–34. https://doi.org/10.1111/anae.13410 PMID: 26919658

74. Reilly CS, Wood AJ, Wood M. Variability of fentanyl pharmacokinetics in man. Computer predicted plasma concentrations for three intravenous dosage regimens. Anaesthesia. 1985; 40(9):837–43. https://doi.org/10.1111/j.1365-2044.1985.tb11043.x PMID: 4051149

75. Welchew EA. The optimum concentration for epidural fentanyl. A randomised, double-blind comparison with and without 1:200 000 adrenaline. Anaesthesia. 1983; 38(11):1037–41. https://doi.org/10.1111/j.1365-2044.1983.tb12476.x PMID: 6356972

76. Justins DM, Knott C, Luthman J, Reynolds F. Epidural versus intramuscular fentanyl. Analgesia and pharmacokinetics in labour. Anaesthesia. 1983; 38(10):937–42. https://doi.org/10.1111/j.1365-2044.1983.tb12022.x PMID: 6356969

77. Kazama T, Ikeda K, Morita K. The pharmacodynamic interaction between propofol and fentanyl with respect to the suppression of somatic or hemodynamic responses to skin incision, peritoneum incision, and abdominal wall retraction. Anesthesiology. 1998; 89(4):894–906. https://doi.org/10.1097/00000542-199810000-00014 PMID: 9778007

78. Drummond GB. Comparison of decreases in ventilation caused by enflurane and fentanyl during anaesthesia. British journal of anaesthesia. 1983; 55(9):825–35. https://doi.org/10.1093/bja/55.9.825 PMID: 6615670

79. Seith RW, Theophilos T, Babl FE. Intranasal fentanyl and high-concentration inhaled nitrous oxide for procedural sedation: a prospective observational pilot study of adverse events and depth of sedation. Academic emergency medicine: official journal of the Society for Academic Emergency Medicine. 2012; 19(1):31–6.

80. Mostert JW, Evers JL, Hobika GH, Moore RH, Ambrus JL. Cardiorespiratory effects of anaesthesia with morphine or fentanyl in chronic renal failure and cerebral toxicity after morphine. British journal of anaesthesia. 1971; 43(11):1053–60. https://doi.org/10.1093/bja/43.11.1053 PMID: 5131458

81. Del Gaudio A, Ciritella P, Perrotta F, Puopolo M, Lauta E, Mastronardi P, et al. Remifentanil vs fentanyl with a target controlled propofol infusion in patients undergoing craniotomy for supratentorial lesions. Minerva anestesiologica. 2006; 72(5):309–19. PMID: 16675939

82. Kodaka M, Okamoto Y, Handa F, Kawasaki J, Miyao H. Relation between fentanyl dose and predicted EC50 of propofol for laryngeal mask insertion. British journal of anaesthesia. 2004; 92(2):238–41. https://doi.org/10.1093/bja/aeh033 PMID: 14722176

83. Lunn JK, Stanley TH, Eisele J, Webster L, Woodward A. High dose fentanyl anesthesia for coronary artery surgery: plasma fentanyl concentrations and influence of nitrous oxide on cardiovascular responses. Anesthesia and analgesia. 1979; 58(5):390–5. PMID: 314761

84. Streisand JB, Bailey PL, LeMaire L, Ashburn MA, Tarver SD, Varvel J, et al. Fentanyl-induced rigidity and unconsciousness in human volunteers. Incidence, duration, and plasma concentrations. Anesthesiology. 1993; 78(4):629–34. https://doi.org/10.1097/00000542-199304000-00003 PMID: 8466061

85. Matsuda C, Sato J. Postoperative Apnea Induced by Fentanyl and Other Multiple Respiratory-Modulating Factors. Open Journal of Anesthesiology. 2014; 04(08):177–82.

86. Deschamps JY, Gaulier JM, Podevin G, Cherel Y, Ferry N, Roux FA. Fatal overdose after ingestion of a transdermal fentanyl patch in two non-human primates. Veterinary anaesthesia and analgesia. 2012; 39(6):653–6. https://doi.org/10.1111/j.1467-2995.2012.00749.x PMID: 22789128

87. Andersen HB, Christensen B, Findlay JW, Jansen JA. Pharmacokinetics of intravenous, intrathecal and epidural morphine and fentanyl in the goat. Acta anaesthesiologica Scandinavica. 1986; 30(5):393–9. https://doi.org/10.1111/j.1399-6576.1986.tb02437.x PMID: 3766095

88. Metz C, Gobel L, Gruber M, Hoerauf KH, Taeger K. Pharmacokinetics of human cerebral opioid extraction: a comparative study on sufentanil, fentanyl, and alfentanil in a patient after severe head injury. Anesthesiology. 2000; 92(6):1559–67. https://doi.org/10.1097/00000542-200006000-00012 PMID: 10839904

89. Peng PW, Sandler AN. A review of the use of fentanyl analgesia in the management of acute pain in adults. Anesthesiology. 1999; 90(2):576–99. https://doi.org/10.1097/00000542-199902000-00034 PMID: 9952166

90. Saccone PA, Lindsey AM, Koeppe RA, Zelenock KA, Shao X, Sherman P, et al. Intranasal Opioid Administration in Rhesus Monkeys: PET Imaging and Antinociception. The Journal of pharmacology and experimental therapeutics. 2016; 359(2):366–73. https://doi.org/10.1124/jpet.116.235192 PMID: 27625351

91. Ngai SH, Berkowitz BA, Yang JC, Hempstead J, Spector S. Pharmacokinetics of naloxone in rats and in man: basis for its potency and short duration of action. Anesthesiology. 1976; 44(5):398–401. https://doi.org/10.1097/00000542-197605000-00008 PMID: 1267205

92. Schaefer CP, Tome ME, Davis TP. The opioid epidemic: a central role for the blood brain barrier in opioid analgesia and abuse. Fluids and barriers of the CNS. 2017; 14(1):32. https://doi.org/10.1186/s12987-017-0080-3 PMID: 29183383

93. Schleimer R, Benjamini E, Eisele J, Henderson G. Pharmacokinetics of fentanyl as determined by radioimmunoassay. Clinical pharmacology and therapeutics. 1978; 23(2):188–94. https://doi.org/10.1002/cpt1978232188 PMID: 620479

94. Blanco ME, Encinas E, Gonzalez O, Rico E, Vozmediano V, Suarez E, et al. Quantitative determination of fentanyl in newborn pig plasma and cerebrospinal fluid samples by HPLC-MS/MS. Drug testing and analysis. 2015; 7(9):804–11. https://doi.org/10.1002/dta.1778 PMID: 25755165

95. Bjorkman S, Stanski DR, Verotta D, Harashima H. Comparative tissue concentration profiles of fentanyl and alfentanil in humans predicted from tissue/blood partition data obtained in rats. Anesthesiology. 1990; 72(5):865–73. https://doi.org/10.1097/00000542-199005000-00017 PMID: 2339802