# A Model for Evaluating Synergistic Opioid Induced Respiratory Depression

R. Brandon Borders, Jennifer Shaffer, Yuan Lu , Kenneth R. Kearney, Brian Roche

charles river

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## 1 ABSTRACT

Drug overdose is one of the leading causes of injury-related death in the United States with almost 68% of reported cases in 2017 involving a prescription or illicit opioid[1]. Opioid overdose or combining opioids with other sedative medication causes respiratory depression leading to mortality. With the recent emergence of the opioid abuse crisis, it has become increasingly important to understand the potential interactive effects on respiratory parameters of new chemical entities (NCEs) that may be taken with opioids. For such assessments, positive and negative assay controls are useful to demonstrate proper evaluation. In this regard, respiratory function of male Sprague Dawley rats was assessed by plethysmography following administration of an intravenous (IV) morphine challenge (3 mg/kg) and/or oral baclofen (20 mg/kg). The conditions tested included oral vehicle (negative control), oral vehicle with IV saline (negative control), oral vehicle with IV morphine (positive control), oral baclofen alone (positive control), and oral baclofen concomitant with IV morphine (double positive control). In this model we demonstrate that administering morphine together with baclofen resulted in synergistic decreases in respiratory frequency (RF) and substantial decreases in minute volume (MV) of 65.7 and 61.4% from baseline, compared to administering morphine (RF:26.0%; MV:40.2%) or baclofen (RF: 39.5%; MV: 47.6%) independently. Hence, baclofen with a morphine challenge model can be effectively used as a comparator to assess potential interactive effects of NCEs with opioids.

## 3 RESULTS

Co-administration of morphine and baclofen resulted in synergistic decreases in respiratory frequency (RF; figure 1) and additive decreases in minute volume (MV, figure 3) of 65.7% and 61.4% respectively from baseline, compared to morphine alone which resulted in decreases in RF of 26.0% and MV of 40.2%, and baclofen alone that resulted in decreases in RF of 39.5% and MV of 47.6%. Paradoxical increases in tidal volume were observed in the baclofen administered groups resulting in a 3-fold increase when combined with morphine (figure 2). A general decrease in respiratory parameters was observed over the duration of the experiment for the (-/-) control animals. All groups trended towards the vehicle/saline group (-/- control) data by the end of the 5 hour acquisition period.
One of the 16 rats in the baclofen and morphine (+/+ control) group was removed from the acquisition and euthanized due to the severity of respiratory depression indicated by the respiratory signal values and clinical observations of reduced tissue perfusion and reduced body temperature.

**Maximum % Change from Baseline at 1-2 Hours Post Challenge**

| n | Treatment | | Control | Dose Level (mg/kg) | RF | TV | MV |
|---|---|---|---|---|---|---|---|
| | Oral | Intravenous | | | | | |
| 16 ♂ | vehicle | saline | -/- | 0/0 | ↓19.6% | ↓11.9% | ↓19.1% |
| 16 ♂ | vehicle | morphine | -/+ | 0/3 | ↓26.0% | ↓40.0% | ↓40.2% |
| 16 ♂ | baclofen | NA | +/NA | 20/NA | ↓39.5% | ↑6.4% | ↓47.6% |
| 16 ♂ | baclofen | morphine | +/+ | 20/3 | ↓65.7% | ↑19.0% | ↓61.4% |

## 2 METHODS

**Experimental Design**

| n | Treatment | | Control | Dose Level (mg/kg) | Dose Concentration (mg/mL) | Dose Volume (mL/kg) |
|---|---|---|---|---|---|---|
| | Oral | Intravenous | | | | |
| 16 ♂ | vehicle | Saline | -/- | 0/0 | 0/0 | 10/1 |
| 16 ♂ | vehicle | morphine | -/+ | 0/3 | 0/3 | 10/1 |
| 16 ♂ | baclofen | NA | +/NA | 20/NA | 2/NA | 10/NA |
| 16 ♂ | baclofen | morphine | +/+ | 20/3 | 2/3 | 10/1 |

All animal procedures were approved by the Charles River Ashland Institutional Animal Care and Use Committee. Eight to ten week old male Crl:CD(SD) Sprague Dawley rats were progressively acclimated to nose only plethysmograph restrainers for 6 hours. Rats were randomized by body weight and assigned to dose groups represented in the table above. Baseline respiratory data were collected continuously for 1 hour using head-out plethysmographs interfaced to the Ponemah (Data Sciences International) acquisition system. Rats were returned to their socially housed home cages during the baseline data review and signal verification. Rats subsequently received a single dose via oral gavage with either an inert vehicle polymer or 20 mg/kg baclofen (both at 10mL/kg). In 3 of the 4 groups, percutaneous catheters were placed in the lateral tail vein connected to a 36” infusion line filled with 0.9% sterile saline. Rats were returned to their assigned plethysmographs with the infusion lines externalized for access. Respiratory frequency, tidal volume and calculated minute volume were collected continuously for 1 hour and the catheterized rats then received an intravenous administration of either saline or 3 mg/kg morphine (both at 1 mL/kg) via the catheter access. Data were then collected continuously for an additional 4 hours. Animals were removed from the plethysmographs following collection and humanely euthanized. 16 rats per dose group were treated over 8 different replicate acquisition sessions. The data were filtered for outliers or noise artefact using Ponemah analysis software and figures were generated with GraphPad Prism

## 4 DISCUSSION/ CONCLUSION

The mechanism of action on respiratory depression from baclofen (antispasmodic; $GABA_B$ agonist)[2] and morphine (analgesic; μ-opioid agonist)[3] are inherently different and this should be considered when evaluating specific NCEs for an interactive effect. Profiling of additional compounds with different receptor targets may be useful in developing specific assays to test drug-drug interaction.
In this model we demonstrate the drug interaction of morphine and baclofen results in additive and synergistic respiratory depression compared to morphine or baclofen alone. The paradoxical increase in tidal volume demonstrates the dose effect was large enough to drive the compensatory change from the induced hypoventilation. The magnitude of depression on respiratory parameters and subsequent survival of 15/16 animals in the baclofen/morphine group indicates the combined dose was potent enough to be considered borderline for severe respiratory depression but was still recoverable within the 5 hour assay. This observed magnitude of respiratory depression can be used as a benchmark that should not be exceeded when evaluating the safety of the interaction of an NCE when co-administered with morphine. General decrease in respiratory parameters for control animals due to habituation of the restraint duration should be considered in the overall evaluation. Recent literature has demonstrated that head-out plethysmography can exacerbate pharmacologically driven respiratory depression due to the restraint device. This potentially confounding variable should also be considered when comparing head-out to nose-only and whole-body plethysmography data[4].

1: https://www.cdc.gov/drugoverdose/data/statedeaths.html
2: https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022462s000lbl.pdf
3: Kelly E (August 2013). "Efficacy and ligand bias at the μ-opioid receptor". British Journal of Pharmacology. 169 (7): 1430–46. doi:10.1111/bph.12222. PMC 3724102. PMID 23646826
4: Lynch III J *et al* 2019. Increased stress associated with head-out plethysmography testing can exacerbate respiratory effects and lead to mortality in rats. Journal of Pharmacological and Toxicological Methods 99 (2019) 106580

FIGURE 1
SUMMARY OF MEAN DATA - RESPIRATORY FREQUENCY
Data Presented as Means ±SEM

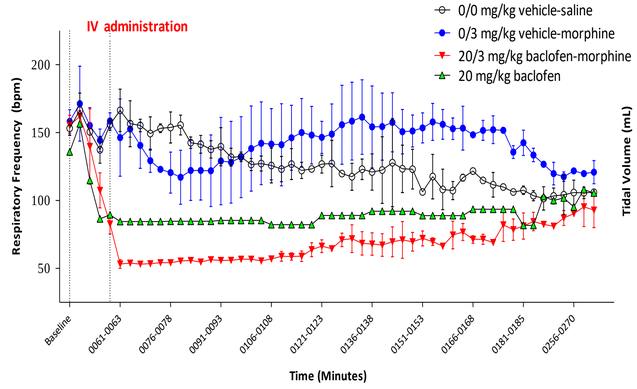


FIGURE 2
SUMMARY OF MEAN DATA - TIDAL VOLUME
Data Presented as Means ±SEM

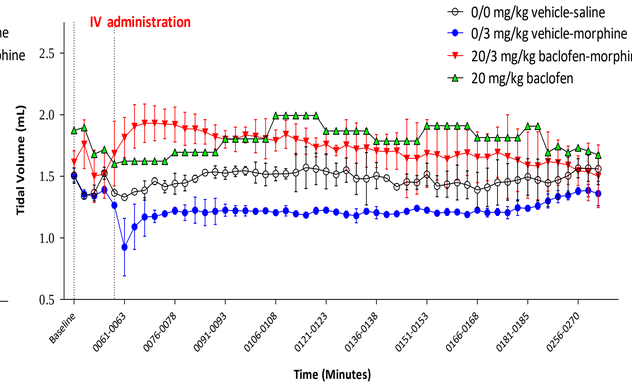


FIGURE 3
SUMMARY OF MEAN DATA - MINUTE VOLUME
Data Presented as Means ±SEM