Adjunct treatments aid survival and recovery in a mouse traumatic brain injury and nerve agent exposure polytrauma model

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Introduction

Polytrauma injuries pose a unique challenge for medical personnel as multiple organ systems and pharmaceutical treatments become involved. A polytrauma consisting of traumatic brain injury (TBI) and nerve agent (NA) exposure can complicate treatment with well-established medical countermeasures (MCM) designed for individual injuries, as each individual injury has peripheral and central nervous system effects that manifest across other organ systems to create a complex injury state. The availability of MCM and medical evacuation in austere environments may further complicate polytrauma treatment and lead to prolonged field care scenarios. Previously, our group established a TBI/NA polytrauma mouse model that showed an increase in mortality, NA symptom incidence and severity, and impeded recovery compared to the individual injuries even when treatments for individual injuries were used in tandem to treat the TBI/NA polytrauma. Thus, treatment of TBI/NA polytrauma may necessitate the use of additional treatment measures alongside MCM to address severe injury effects. Due to the multi-system nature of polytraumas, several pharmaceuticals across multiple drug classes hold potential as adjunct treatments. This study utilized our previously established TBI/NA polytrauma model to evaluate the effectiveness of diphenhydramine, hydromorphone, ketamine, levetiracetam, promethazine, and scopolamine as adjuncts to established single trauma MCM. These pharmaceuticals primarily fall into antihistamine, anticonvulsant, anticholinergic, analgesic, or anesthetic drug classes, and have both peripheral and central nervous system effects. Importantly, they are well-researched for use in single trauma applications and are widely available to medics and other medical personnel at both field care and hospital care levels.

Methods

Animal Model

Animals: Male C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; AKA KIKO mice, ~14 weeks old

Surgical Procedures: DSI ETA-F10 wireless EEG transmitter implantation and 4.0mm open craniotomy placement, ~2 weeks prior to polytrauma

Exposure: 1.0mm depth TBI via controlled cortical impact and effective dose Soman (GD)

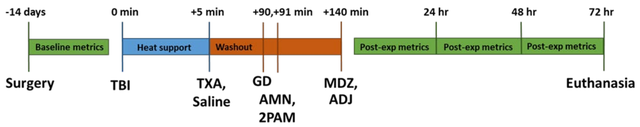
Medical Countermeasures:

- TBI MCM: tranexamic acid (TXA), 10mg/kg i.p.; saline support s.c.
- NA MCM: atropine methylnitrate (AMN), 6mg/kg i.p.; pralidoxime (2PAM), 25mg/kg i.p.; midazolam (MDZ), 5mg/kg s.c.

Experimental Adjuncts (ADJ):

- diphenhydramine hydrochloride (DPH) antihistamine, 15mg/kg i.p.
- ketamine (KET) analgesic, 40mg/kg i.p.
- hydromorphone (HMP) analgesic, 33mg/kg i.p.
- levetiracetam (LEV) antiepileptic, 800mg/kg i.p.
- promethazine (PRO) antihistamine, 6mg/kg i.p.
- scopolamine hydrobromide (SCO) anticholinergic, 1mg/kg i.p.

Post-exposure Care: twice daily 0.9% saline, s.c.



Evaluation

NA symptom categories: mice observed and designated as one of the following post-NA exposure

- CV (convulsive): Mice had full-body convulsions, with or without seizure activity
- NC (non-convulsive): Mice displayed no convulsions, seizure, or significant signs of exposure

Burrowing: Mice scored every ~24 hrs based on amount of corncob bedding removed from a plastic tube placed in cage for 30mins

Nesting: mice scored every ~12hrs based on nest shape, height, and use of materials

Statistical Analysis

Log Rank test: survival across the prolonged field care window

Fisher's Exact test: survival rates at 72hrs post-polytrauma, nesting activity

2-way ANOVA with post-hoc Dunnett's multiple comparisons test: HMP EEG activity, burrowing behavior

P Values: * p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Results

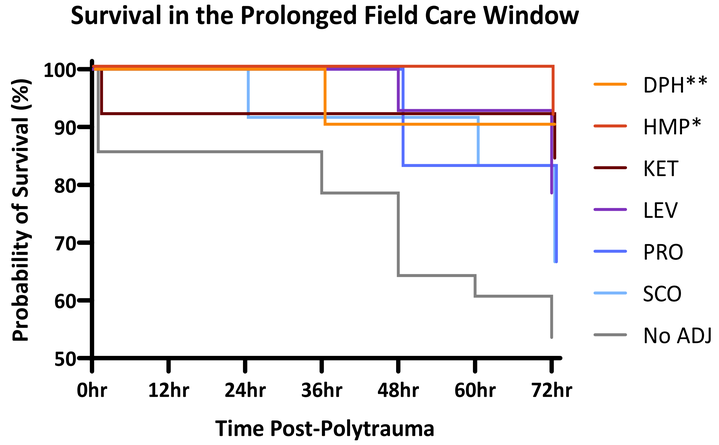


Figure 1. ***Adjuncts improved survival following polytrauma with severe symptoms.*** A single dose of DPH (**p=0.0075) or HMP (*p=0.0226) significantly improved survival following TBI/NA polytrauma with convulsive NA symptoms across the entire prolonged field care window when compared to treatment without an adjunct. KET, LEV, PRO, and SCO also improved survival across this time, though not significantly. No mortalities occurred in nonconvulsive mice regardless of adjunct treatment (data not shown).

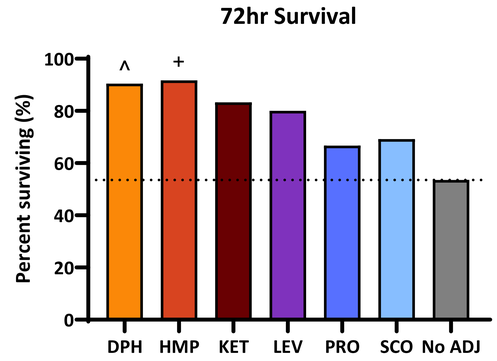


Figure 2. ***Survival rates remain higher with adjunct treatment at 72hrs post-polytrauma despite withholding of pharmaceutical intervention in the days following injury.*** At 72hrs post-polytrauma, survival rates for convulsive mice treated with DPH (^p=0.0108) and HMP (+p=0.0410) were significantly higher than that of mice without adjunct treatment. KET and LEV had notably higher survival rates than no adjunct, though this difference was not significant.

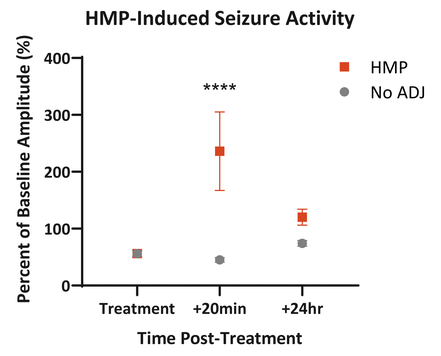


Figure 3. ***HMP induced seizure activity post-treatment.*** Despite improvements in survival, use of HMP resulted in elevated EEG activity evident of seizure in a majority of animals within minutes of HMP administration (****p<0.0001). HMP was determined a non-viable adjunct option after elevated signals were still present following reduced 25mg/kg and 15mg/kg doses.

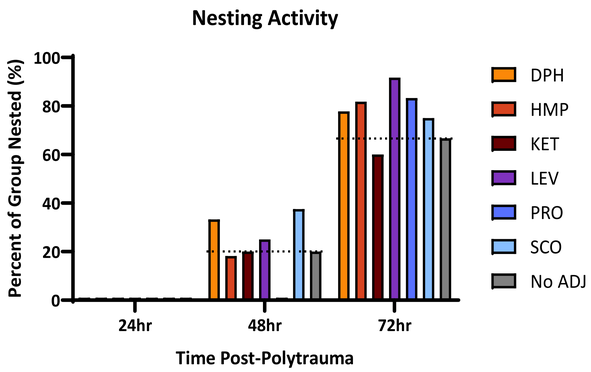


Figure 4. Adjuncts accelerated return to nesting behavior. Adjuncts did not significantly improve return to nesting behavior, though a higher proportion of DPH, PRO, and SCO-treated mice were nested by 48hrs and a higher proportion of DPH, HMP, LEV, PRO, and SCO-treated mice were nested by 72hrs in comparison to no adjunct control.

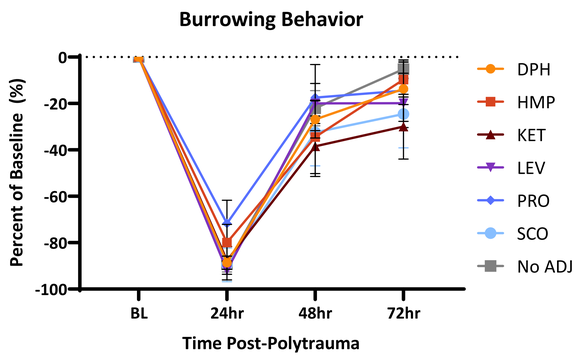


Figure 5. Adjuncts did not change return to burrowing behavior. Adjuncts did not significantly change deficits in burrowing behavior following polytrauma. All groups displayed a large burrowing deficit and gradual return toward baseline activity by 48 and 72hrs post-injury.

Discussion

Survival

- All adjuncts prolonged survival of TBI/NA polytrauma when given in combination with MCM, thus expanding the window in which additional treatment, supportive care, and transport could occur; repeated dosing of adjuncts may further increase survival through this period given that most mortalities occurred outside of the drug's therapeutic window
- TBI/NA polytrauma causes widespread systemic symptomology largely driven by the NA, so it's likely that improved survival following treatment was due to the adjuncts' ability to mitigate neuronal excitotoxicity, cardiac dysfunction, and/or respiratory distress that accompanies severe NA exposure[1]
- Efficacy at treating the injury may also be linked to drug-drug interactions with MCM, namely the increased CNS depression and sedation that can occur when any of the adjuncts are combined with MDZ[2,3]
- DPH's ability to significantly improve survival after TBI/NA polytrauma may lie in its profound anticholinergic effects, which could ameliorate the effects of harmful acetylcholine buildup that drives NA-induced symptoms and can occur post-TBI[4]
- HMP-induced seizure activity may have been caused by a metabolite of the drug that causes neuroexcitation, particularly in rodent studies[5]

Recovery of Innate Behavior

- Adjuncts did not improve 24hr behavioral deficits, indicating that their effects do not overcome the severe decline in wellbeing initially following polytrauma
- Adjuncts did not impede return to normal behavior despite probable widespread effects, increased depth of sedation, and drug interactions following initial polytrauma
- Group differences in behavioral recovery following adjunct administration may be more visible in nesting than in burrowing due to the increased complexity of nesting behavior, which requires sustained coordinated movement, goal-directedness, and spatial memory over a comparatively longer period of time[6]

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