Better together? Treating traumatic brain injury with minocycline plus N-acetylcysteine

Abstract
Traumatic brain injury has a complex pathophysiology that produces both rapid and delayed brain damage. Rapid damage initiates immediately after injury. Treatment of traumatic brain injury is typically delayed many hours, thus only delayed damage can be targeted with drugs. Delayed traumatic brain injury includes neuroinflammation, oxidative damage, apoptosis, and gluteamate toxicity. Both the speed and complexity of traumatic brain injury pathophysiology present large obstacles to drug development. Repurposing of Food and Drug Administration-approved drugs may be a highly efficient approach to get therapeutics to the clinic. This review examines the preclinical outcomes of minocycline and N-acetylcysteine as individual drugs and compares them to the minocycline plus N-acetylcysteine combination. Both minocycline and N-acetylcysteine are Food and Drug Administration-approved drugs with pleiotropic therapeutic effects. As individual drugs, minocycline and N-acetylcysteine are well tolerated, with known pharmacokinetics, and enter the brain through an intact blood-brain barrier. At concentrations greater than needed for anti-microbial action, minocycline is a potent anti-inflammatory minocycline, also acts as an antioxidant and inhibits multiple enzymes that promote brain injury including metalloproteases, caspasases, and polyADP-ribose-polymerase-1. N-acetylcysteine alone is also an antioxidant. It increases brain glutathione, prevents lipid oxidation, and protects mitochondria. N-acetylcysteine also acts as an anti-inflammatory as well as increases extracellular glutamate by activating the X, cystine-glutamate anti-transporter. These multiple actions of minocycline and N-acetylcysteine have made them attractive candidates to treat traumatic brain injury. When first dosed within the one hour after injury, either minocycline or N-acetylcysteine improves a diverse set of therapeutic outcome measures in multiple traumatic brain injury animal models. A small number of clinical trials for traumatic brain injury have established the safety of minocycline or N-acetylcysteine and suggested that either drug has some efficacy. Preclinical studies have shown that minocycline plus N-acetylcysteine have positive synergy resulting in therapeutic effects and a more prolonged therapeutic time window not seen with the individual drugs. This review compares the actions of minocycline and N-acetylcysteine, individually and in combination. Evidence supports that the combination has greater utility to treat traumatic brain injury than the individual drugs.

Key Words: clinical trial; outcome measures; preclinical testing; rodent models of traumatic brain injury; therapeutic time window

Introduction
Approximately 2.5 million cases of traumatic brain injury (TBI) occur yearly in the United States resulting in over 50,000 fatalities (Faul et al., 2010). Despite this large morbidity and mortality burden, there are no treatments for TBI. TBI has a complex pathophysiology that induces both rapid and delayed damage (Dixon, 2017). In addition, TBI injury severity ranges from mild to severe. Mild TBI is characterized by the absence of hematomas, hemorrhages, or contusions, while severe TBI is characterized by an extended loss of consciousness (Dixon, 2017). Most TBI models produce hematomas, hemorrhage, and/or contusions with a short loss of consciousness which is characteristic of moderate TBI (Xiong and Chopp, 2013). The remaining studies do not have hematomas, hemorrhage, and/or contusions are considered to be modeling mild TBI.

Mechanical damage to neurons, glia, and vessels occurs immediately after a TBI. This primary injury triggers a rapid secondary injury to both gray and white matter that evolves for weeks to months (Dixon, 2017; Somayaji et al., 2018). Within minutes neuronal ion homeostasis is lost leading to elevated intracellular calcium, depolarization, and excitotoxic glutamate release. Mitochondrial damage results in energy failure and increased reactive oxygen species. Vascular damage leads to hypoxia, hypoglycemia, and blood-brain barrier breakdown. Damage to axons and oligodendrocytes produces axotomy, demyelination, and impaired axonal transport. Cell lysis releases damage-associated molecular patterns and cytokines that activate astrocytes and microglia within minutes or hours after injury. Increased inflammation promotes additional blood-brain barrier breakdown and entry of peripheral inflammatory cells. Cytogenic and vasogenic edema leads to increased intracerebral pressure, and further necrotic and apoptotic cell loss, as well as loss of axons, myelin, and oligodendrocytes. Thus, both gray and white matter injuries evolve for weeks post-injury. TBI rapidly initiates multiple pathophysiological mechanisms that are subsequently altered and amplified over time. The pathophysiology of TBI in the first few days is a major determinant of time to first dose since drug targets may rapidly arise, dissipate or change (Mohamadpour et al., 2019). In contrast, pathophysiology weeks to months after TBI may be more stable or not change at all.

The time lag between injury and treatment indicates that only delayed damage can be targeted by drugs to treat TBI (Somayaji et al., 2018; Mohamadpour et al., 2019). The rapid and complex progression of TBI presents large obstacles to drug development (Somayaji et al., 2018). Thus, the repurposing of Food and Drug Administration (FDA)-approved drugs may be a highly efficient approach to bring therapeutics to the clinic. Two FDA-approved drugs, minocycline (MINO) and N-acetylcysteine (NAC) have undergone extensive preclinical testing to examine their efficacy to treat TBI (Additioinal Tables 1 and 2). Many, but not all preclinical studies have shown some efficacy of MINO or NAC in improving outcomes after experimental TBI. Limited clinical trials utilizing MINO or NAC to treat TBI have been inconclusive (Additional Table 3). MINO or NAC have also failed multiple clinical trials for a variety of neurological and psychological diseases (Garff-Mesa, 2013; Deepmala et al., 2015). As a result, neither MINO nor NAC have FDA approval to treat any central nervous system disease.

Drugs to treat TBI must have a favorable therapeutic time window (Mohamadpour et al., 2019). Two recent clinical trials (PROTECT III) and (SYNAPSE) were able to treat patients at 3.6 or 7 hours after injury; sperminone and taurine (NS108190) to PJB.

Funding: This work was funded by an award “Minocycline plus N-acetylcysteine improves brain structure and function after experimental brain injury with clinically useful time window” (NS108190) to PJB.

How to cite this article: Lawless S, Bergold PJ (2022) Better together? Treating traumatic brain injury with minocycline plus N-acetylcysteine. Neural Regen Res 17(12):2589-2592.

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respectively, which provides some notion of a clinically useful time window (Mamadopour et al., 2019). Most preclinical studies evaluated in this review dose drugs either before, or within one hour after experimental injury, which does not test whether the drug can be used later. These studies provide important information about potential drug mechanisms targeting early events after TBI and provide an initial proof-of-principle that these drugs may be effective. Early dosing of a large number of drugs is effective against experimental TBI, however some have shown efficacy with clinically relevant delayed dosing (Mamadopour et al., 2019).

This review also evaluates the utility of the MINO plus NAC combination. Combinations can increase drug efficacy and reduce potential unwanted effects (Somayaji et al., 2018). Drug combinations are now used clinically to treat cancer, infectious disease, and hypertension (Lima et al., 2017; Tsoufuis and Tripodis, 2012; Tsoufuis et al., 2018; Tsoufu et al., 2019). The efficacy of drug combinations is determined by comparison to the efficacy of the individual drugs. Many different outcomes have been used to assess the efficacy of drug combinations, so drug combinations may have additive or synergistic action on multiple outcomes. Few outcomes show indirect action, and even fewer outcomes should show drug antagonism (Somayaji et al., 2018). Assessing the efficacy of pleiotropic drug combinations has the potential to confound that some outcomes may show drug synergy, while others show additivity or indifference. With these caveats in mind, this review first evaluates the preclinical studies of MINO and NAC and then examines the studies that directly compare the individual drugs with the MINO plus NAC combination. The few clinical trials using MINO or NAC to treat TBI will also be summarized.

Search Methodology
The search string “Minocycline traumatic brain injury” received 71 articles from PubMed on 09/10/21. Forty-five articles were excluded; 12 earlier reviews; 12 clinical studies; 21 studies that only focused on TBI or did not test whether they can be used clinically. These studies provide additional Table 3 from PubMed on 09/10/21. Forty-five articles were excluded; 12 earlier reviews; 12 clinical studies; 21 studies that only focused on TBI or did not test whether they can be used clinically. These studies provide

Minocycline
MINO is a lipophilic semisynthetic tetracycline derivative that is developed to enter the brain to treat bacterial meningitis (Garrido-Mesa et al., 2013). At concentrations higher than needed for anti-microbial action, MINO is an anti-inflammatory and anti-apoptotic that also chelates iron (Garrido-Mesa et al., 2013; Zhang et al., 2020). MINO has shown efficacy in experimental animal models of ischemia, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, Alzheimer’s disease, multiple sclerosis, and spinal cord injury (Garrido-Mesa et al., 2013). Many studies have established the efficacy of MINO; for those studies that received the drug dose NAC or NACA as a single drug. The remaining 16 articles are reviewed in Additional Table 2. One study of clinical TBI is included in Additional Table 3 and this study was excluded; 8 earlier reviews; 31 studies that did not focus on TBI or did not test whether they can be used clinically. These studies provide

N-Acetylcysteine
NAC has FDA approval for the treatment of acetaminophen overdose and chronic obstructive pulmonary disease; it is also available as an over-the-counter dietary supplement (Tardiolo et al., 2013; Sangobowale et al., 2018a, b) used MINO doses that likely do not test whether they can be used clinically. These studies provide

Clinical Studies
Clinical trials have the potential confound that some outcomes may show drug synergy, while others show additivity or indifference. With these caveats in mind, this review first evaluates the preclinical studies of MINO and NAC and then examines the studies that directly compare the individual drugs with the MINO plus NAC combination. The few clinical trials using MINO or NAC to treat TBI will also be summarized.

Additional Table 3
The search string “Acetylcysteine traumatic brain injury” received 6 articles from PubMed on 09/10/21. Thirty-nine articles were included in this review are summarized in Additional Table 1 and two clinical studies are summarized in Additional Table 3. The search string “Acetylcysteine traumatic brain injury” received 6 articles from PubMed on 09/10/21. Thirty-nine articles were included in this review are summarized in Additional Table 1 and two clinical studies are summarized in Additional Table 3.
The synergy of MINO plus NAC was seen in two TBI models involving different using the same panel of antigenic markers, one-hour dosing of MINO plus NAC improved acquisition and retention of Morris water maze when dosed at 30 minutes or 1 hour post-injury (Eakin et al., 2014). Importantly, an improved hippocampal function was retained at 30 days after injury, suggesting such synergy is preserved in brain function.

Injured rats first dosed with NAC at 6 hours post-injury acquired active place avoidance and Barnes maze (Sangobowale et al., 2018b). Twelve-hour dosing allowed rats to acquire active place avoidance and Barnes maze but could not restore long-term retention of active place avoidance (Sangobowale et al., 2018b). Rats receiving the first dose of NAC at 24 hours only acquired Barnes Maze. Injured mice first dosed with NAC at 12 hours, but not 24 hours, acquired active place avoidance. Neither 12- nor 24-hour dosing restored Barnes maze acquisition to injured mice. NAC dosing had no effect on hippocampal and hippocampal MAP2 expression (Haber et al., 2018; Sangobowale et al., 2018a, 2018b). Taken together, these data demonstrate that NAC acts as an antioxidant and protector of NAC for gray, but not white, matter. These data suggest that the retained effect of NAC may be increased in a single randomized controlled clinical trial from 2013, NAC improved neurological and psychological outcomes at 7 days post-injury when first administered within 1–3 days after TBI (Hoffer et al., 2013). Retention of drug efficacy with later dosing provides a rationale to further examine its efficacy in clinical trials for TBI. In the clinic, NAC is dosed at much higher concentrations than the doses used in these TBI preclinical (Shen et al., 2013). Studies also examined the neuroprotective and anti-inflammatory effects of NAC, but it is unknown if the therapeutic effects of the combination are long-lasting. Finally, the therapeutic effects of MINO plus NAC have not been independently replicated in other laboratories. It is hoped that this review will spur others to further examine the efficacy of the combination.

**Conclusions**

At present, no drugs are available to treat TBI; it remains unknown how much preclinical efficacy a drug needs to be translated to the clinic. Both MINO and NAC as individual drugs in preclinical testing showed efficacy in improving therapeutic outcomes (Additional Tables 1 and 2). Both have shown that efficacy with some evidence of efficacy in small clinical trials for TBI (Hoffer et al., 2013; Scott et al., 2018; Koulaeinejad et al., 2019; Meythaler et al., 2019). The MINO plus NAC combination clearly has greater efficacy than the individual drugs, depending upon the outcome measured. MINO and NAC displayed additive or synergistic activity over a wide range of therapeutic outcomes. Most importantly, the combination clearly has more efficacy than the individual drugs when dosed at 12 to 24 hours post-injury (Sangobowale et al., 2018a, b; Whitney et al., 2021). The evidence for increased efficacy of the MINO plus NAC combination is a potent argument for its testing in clinical trials.

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Review

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C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y
### Additional Table 1 Preclinical studies using minocycline

| Study              | Rigor                      | Species, Sex, Weight, Age | Model, Controls                  | Dose, time to first dose, | Outcomes                                                                 |
|--------------------|----------------------------|---------------------------|----------------------------------|---------------------------|--------------------------------------------------------------------------|
| Sanchez-Meija, 2001 | Randomization, yes; Blinded, NS | Mouse, Age NS, Sex NS     | Moderate CCI, sham-CCI           | 45 mg/kg, 12 h pre-injury 90 mg/kg 30 min 12 h and 24 h post-injury 90 mg/kg, 30 min 12 h and 24 h post-injury | 1 d Rotarod ↑, 4 d Rotarod ↑, 4 h IL-1β ↑, 4 d LV ↑, 1 d Caspase-1,3 ↑ |
| Sheng, 2006        | Randomization, yes; Blinded, yes | Rat, Male, 200-250 g      | Moderate CCI, sham-CHI-saline    | Pretreatment 45mg/kg twice daily 2 d and 1 d, once 30 minutes; posttreatment 1H, twice daily 1 d, 2 d | 1 d LV ↑, 4 d LV ↑, 1-14 d NSS↑, 4-5 d Inclined plane ↑, 7 d LV ↑, 7 d CA1 neurons ↑ |
| Bye, 2007          | Randomization, NS; Blinded, NS | Mouse, Male, 12–14 weeks  | Moderate CHL Sham-CHI           | 45 mg/kg 30 min PL, 45 mg/kg every 12 h until sacrifice | 1–4 d NSS ➔, 1 d Beam test ➔, 1 d LV ➔, 4 d LV ➔, 4 d Apoptosis ➔, 4 d MP/MG ➔, 4 d Neutrophils ➔, 4 h Cytokines (IL-1b, IL-6, G-CSF, MCP-1, CXCL4) ➔ |
| Homsi 2009         | Randomization, NS; Blinded, NS | Mouse, Male, 28–30 g      | Moderate WD, sham-WD-saline, WD-saline | 45 mg/kg 5 min | 6 h IL-1b ➔, 6 h MMP-9 ➔ |
|                    |                            | Mouse, Male, 28–30 g      | Moderate WD, sham-WD-saline, WD-saline | 90 mg/kg 5 min | 6 h IL-1b ➔, MMP-9 ➔ |
|                    |                            | Mouse, Male, 28–30 g      | Moderate WD, sham-WD-saline, WD-saline | 45 or 90 mg/kg 5 min, 45 mg/kg 3 h | 6 h GSH ➔ |
|                    |                            | Mouse, Male, 28–30 g      |                                                   | 90 mg/kg 5 min, 45 mg/kg 3, 9 h | 1 d Edema ➔, 1 d MMP-9 ➔, 1 d String test ➔ |
| Abdel-Baki, 2009   | Randomization, NS; Blinded, NS | Rat, Male, 250–300 g      | Moderate CCI, Sham-CCI           | 45 mg/kg 1 h, 1 d, 2 d | 7 d APA entrances ➔, 12 d APA Time to first entrance ➔, 14 d Myelin ➔ |
| Homsi, 2010        | Randomization, yes; Blinded, yes | Mouse, Male, 28–30 g      | Moderate WD, WD-saline           | 90 mg/kg 5 min, 45 mg/kg 3 and 9 h | 1 d MG ➔, 1 d LV ➔ |
|                    |                            |                            |                                                   | 2–84 d Open field ➔, 2–84 d Body weight ➔ |
| Sioppi, 2011       | Randomization, yes; Blinding, NS | Mouse, Male, 28–30 g      | Moderate WD, Naïve, WD-vehicle   | 90 mg/kg 5 min, 45 mg/kg 3 h, 9 h | 1 d sAAPa ➔, 84 d Corpus callosum volume ➔, 84 d Thalamus volume ➔, 84 d Lateral ventricle volume ➔, 84 d GFAP ➔, 84 d MP/MG ➔ |
| Study                        | Randomization, Blinding | Animal, Sex, Weight | Treatment Descriptions                                                                 | Outcome Measures                                                                 |
|------------------------------|-------------------------|---------------------|----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Sioppi, 2012                 | Randomization, Blinding | Mouse, Male, 28–30 g| Moderate WD, Naive, WD-vehicle                                                          | 90 mg/kg 5 min, 45 mg/kg 3 h, 9 h 7–84 d Odorant avoidance ↑ 84 d Olfactory bulb surface area ↑ 21–91 d NORT ↑ 21–91 d Elevated plus maze ↑ 21–91 d Elevated zero maze ↑ |
| Ng, 2012                     | Yes                     | Mouse, Sex, NS, 28–34 g| Moderate WD, Sham, WD-vehicle                                                           | 45 mg/kg 30 min, 45 mg/kg every 12 h for 7 d 3–42 d NSS ↑ 7 d MP/MG ↑ 7 d Neurogenesis ↑ |
| Kosvedi, 2012                | Randomization, Blinding | Rat, Male, 245–265 g| Mild Blast, Sham-Blast, saline, Sham-Blast-MINO, Blast-saline                          | 50 mg/kg 1 h, 1–4 d. 9 d Elevated plus maze ↑ 46 d Elevated plus maze ↑ 10 d BM ↑ 47 d BM ↑ 51 d C-reactive peptide ↑ 51 d CCL2 ↑ 51 d Claudin 5 ↑ 51 d neuronal specific enolase ↑ 51 d Neurofilament heavy chain ↑ 51 d Tau ↑ 51 d S100B ↑ 51 d GFAP ↑ 51 d Corticosteroid ↑ 51 d Vascular endothelial growth factor receptor 2 ↑ |
| Haber, 2013                  | Randomization, Blinding | Rat, 250–300 g       | Moderate CCI, sham-CHI-saline, CCI-saline,                                              | 45 mg/kg 1 h, 1 d, 2 d 7 d Conflict active place avoidance ↑ 7 d Spaced active place avoidance ↑ 2 d MG activation ↑ |
| Lam, 2013                    | Yes                     | Rat                  | Moderate CCI, CCI-vehicle                                                              | 25 mg/kg 1-7 d 7–168 d MP/MG ↑ 7 d GFAP ↑ 12 d GFAP ↑ 168 d GFAP ↑ 168 d LV ↑ 56 d Vermicelli handling ↑ 56 d Thigmotaxis ↑ 56 d MWM ↑ |
| Vonder Haar, 2014            | Randomization, Blinding | Rat, Male, 350 g     | Moderate CCI, Sham-CCI, CCI-vehicle                                                     | 50 mg/kg 1 h, every 12 h for 3 d 7–16 d Grid walk ↑ 7 d Rotarod ↑ 15 d MWM ↑ 25 d LV ↑ |
| Lopez-Rodriguez, 2015        | Randomization, Blinding | Mouse, Male, 28–30 g | Moderate WD, Naive                                                                     | 90 mg/kg 5 min, 45 mg/kg 3 h, 9 h 1 d Edema ↑ 1 d NSS ↑ 1 d MP/MG ↑ 1 d b-APP ↑ |
| Shochat, 2015                | Randomization, Blinding | Mouse, Male, 40 g    | Mild WD                                                                               | 45 mg/kg 20 min 1 h Oxyhemoglobin ↑ 1 h Total hemoglobin ↑ |
| Study | Design | Species | Gender | Age | Injury | Treatment | Timepoints | Results |
|-------|--------|---------|--------|-----|--------|-----------|------------|---------|
| Hanlon, 2017 | Randomization, yes; Blinding, yes | Rat, Male and Female, 11 d | Moderate CCI, Sham-CCI, CCI plus vehicle | 45 mg/kg 5 min, every 12 h for 3 d | 1 h Arterial oxygen saturation ↑, 1 h Edema ↑, 3 d MP/MG ↑, 3 d Cortical Fluorojade positive cells ↑, 7 d Cortical Fluorojade positive cells ↓ |
| Chhor, 2017 | Randomization, yes; Blinding, yes | Mouse, Male and Female, 4–5 g | Mild WD, Sham-WD saline, WD-saline | 45 mg/kg 5 min, 1 d, 2 d | 1 d Inflammatory cytokines ↑, 1 d Ventricular volume ↑, 5 d Ventricular volume ↑, 1 d Apoptosis cortex, hippocampus, striatum ↑, 1 d MP/MG ↑, 5 d LV ↑, 5 d Myelin ↓ |
| Haber, 2018 | Randomization, yes; Blinding, no | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 45 mg/kg 1 h, 1 d, 2 d | 14 d Myelin 14 d ↑, 14 d Oligodendrocytes 14–4 d ↑, 14 d Oligodendrocyte apoptosis ↑, 4 d MP/MG activation ↑, 7 d MP/MG activation ↑ |
| Sangobowale, 2018a | Randomization, yes; Blinded, yes | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 22.5 mg/kg 6 h, 1 d and 2 d | 7 d BM ↑, 7 d APA, Entrances ↑, 7 d APA Time to first entrance ↑, 14 d Hippocampal MAP2, 14 d Myelin ↑ |
| Sangobowale, 2018a | Randomization, yes; Blinded, yes | Mouse, Male, 28–30 g | Moderate CHI, Sham-CHI, CHI-saline | 22.5 mg/kg 12 h, 1 d, 2 d | 7 d BM ↑, 7 d APA, Entrances ↑, 7 d APA Time to first entrance ↑, 14 d Hippocampal MAP2, 14 d Myelin ↑ |
| Sangobowale, 2018a | Randomization, yes; Blinded, yes | Mouse, Male, 28–30 g | Moderate CHI, Sham-CHI, CHI-saline | 22.5 mg/kg 1 d, 2 d, 3 d | 7 d 12–24 h, BM ↑, 7 d 24 h APA, Entrances ↑, 7 d 24 h APA, Time to first entrance ↑, 14 d Hippocampal MAP2 14 d, 14 d Myelin ↑ |
| Study                     | Randomization, Yes/No; Blinded, Yes/No | Species, Sex, Weight | Condition | Treatment | Outcome Measures |
|--------------------------|----------------------------------------|----------------------|-----------|-----------|------------------|
| Sangobowale, 2018b       | Randomization, yes; Blinded, yes       | Mouse, Male, 28–30 g | Moderate CHI, Sham-CHI, CHI-saline | 22.5 mg/kg 12 h, 1 d, 2 d | 14 d Oligodendrocytes ↑ |
| Simon, 2018              | Randomization, yes; Blinded, NS        | Rat, 35–40 g         | Moderate CCI, Sham-CCI, CCI-saline | 90 mg/kg 10 min and 20 h | 1 d High mobility group B1 ↑ 7 d MP/MG ↑ 7 d FluorJade positive cells ↑ 14 d Thalamic neurons ↑ 14 d LV ↑ 5 d Balance Beam, Inclined Plane ↑ 14 d MWM ↑ |
| Taylor, 2018             | Randomization, yes; Blinded, NS        | Rat, Male and Female, 60–70 d | Moderate CCI, Sham-CCI saline, Sham-CCI MINO, CCI-saline | 50 mg/kg 1 h 1 d, 2 d, 3 d | IL-1β 35 d male ↑, female ↑ IL-6 35 d male ↑, female ↑ TNFα 35 d male ↑, female ↑ |
| Zhang, 2020              | Randomization, NS; Blinded, NS         | Rat, Male, 150–180 g | Moderate CCI Sham-CCI saline, CCI-saline | 20 mg/kg 12 h and daily 2–7 d | 5–14 d Body weight ↑ 7–14 d Foot fault ↑ 7–14 d Cylinder test ↑ 7–14 d Wire hang ↑ 21 d MWM ↑ |
| Pernici, 2020            | Randomization, yes; Blinded, Yes       | Mouse, Male and Female, 20–30 g | Mild FPI, Sham-FPI, FPI-saline | 45 mg/kg 45 min 2 d, 3 d | 2–7 d NSS ↑ 2–7 d Rotarod ↑ 60 d NORT ↑ 60 d Open field ↑ 60 d Tail suspension ↑ |
| Study | Randomization | Species | Injury Model | Dosing | Outcome Measures |
|-------|---------------|---------|--------------|--------|------------------|
| He, 2021 | Randomization, yes; Blinded, NS | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 45 mg/kg 3 d, 4 d or 5 d | 30 d Axon loss ↑<br>2–7 d NSS ➔<br>2–7 d Rotarod ➔<br>60 d NORT ➔<br>60 d Open field ➔<br>60 d Tail suspension ➔<br>30 d Axon loss ↑ |
| Wang, 2021 | Mouse, Male, 20–35 g | Moderate CCI, Sham-CCI | 40 mg/kg 5 min prior to injury | | 3 d Cortical apoptosis ↑<br>3 d Bax ↑<br>3 d Cleaved caspase 3 ↑<br>3 d Bcl-2 ↑ |

Rigor for each study is provided followed by species, injury model plus severity and control groups and details of dosing of MINO. Time after injury is followed by whether MINO improved (↑) or had no difference (➔) in the experimental outcome. APA: Active place avoidance; BM: Barnes maze; CCI: controlled cortical injury; CHI: closed head injury; FPI: fluid percussion injury; GFAP: glial fibrillary acid protein; GSH: glutathione; LV: lesion volume; MMP-9: matrix metaloprotease 9; MP/MG: macrophage/microglia; MWM: Morris water maze; NORT: novel object recognition; NS: not specified; NSS: neurological severity score; NSS: neurological severity score; WD: weight drop.
### Table 2 Preclinical studies of N-acetylcysteine or N-acetylcysteineamine

| Study          | Rigor                      | Species, Sex, Weight or Age | Model, Controls                        | Dose, Time to first dose | Time of Outcome Assay, Outcome                                                                 |
|----------------|----------------------------|-----------------------------|----------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------|
| Xiong, 1999    | Randomization, NS; Blinded, NS | Rat, Male, 200-350 g        | Moderate CCI; Sham-CCI, CCI-saline     | 163 mg/kg 30 min         | 1 h–14 d Brain GSH, 3 h Mitochondrial GSH, 14 d Mitochondrial GSH, 12 h Mitochondrial State, 3 d Mitochondrial Ca<sup>2+</sup> uptake, 14 d Mitochondrial Ca<sup>2+</sup> uptake |
| Thomale, 2006  | Randomization, Yes; Blinded, NS | Rat, Male, 300–350 g        | Moderate CCI, Sham-CCI                 | 163 mg/kg 15 min, 2 h, 4 h | 1 d Intracranial Pressure, 1 d Edema, 1 d LV                                                  |
| Yi, 2005       | Randomization, NS; Blinded, NS | Rat, Male, 350–400 g        | Moderate FPI, Sham-CCI, CCI-saline     | 163 mg/kg 5 min          | 6 h–24 h Heme Oxidase 1, 1 d LV                                                                |
| Yi, 2006       | Randomization, NS; Blinded, NS | Rat, Male, 350–400 g        | Moderate FPI, Sham-FPI, FPI-saline     | 163 mg/kg 5 min          | 6 h, 24 h Cortical complex 1, 6 h Cortical complex 2, 1 d Cortical neurons, 1 d Hippocampal neurons |
| Hicdonmez, 2006| Randomization, Yes; Blinded, NS | Rat, Male, 280–320 g        | Moderate WD, Not specified control group | 150 mg/kg 15 min        | 2–12 h Malondialdehyde, 12 h SOD, 2–12 h Glutathione Peroxidase, 2–12 h Catalase, 2–12 h Frontal neurons, 2–12 h Caspase 3 positive cells |
| Chen, 2007     | Randomization, NS; Blinded, NS | Rat, Male, 250–300 g        | Moderate WD, Sham-WD                  | 150 mg/kg 15 min, 1, 2, and 3 d | 3 d NF-kB, 3 d IL-1β, TNFa, IL-6, 3 d ICAM-1, 3 d Edema, 3 d Blood brain barrier permeability |
| Abdel Baki, 2009| Randomization, NS; Blinded, NS | Rat, Male, 250–300 g        | Moderate CCI, Sham-CCI                | 150 mg/kg 1 h, 1 d, 2 d  | 7 d APA Entrances, 7 d APA Time to first entrance, 14 d Myelin                                  |
| Haber, 2013    | Randomization, NS; Blinded, NS | Rat, 250–300 g              | Moderate CCI, sham-CHI-saline, CCI-saline | 150 mg/kg 1 h, 1 d, 2 d  | 7 d, Conflict APA, 7 d Spaced APA, 2 d MG                                                     |
| Senol, 2014    | Randomization, Yes; Blinded, NS | Rat, Male, 4 months         | Moderate WD, Sham-WD                  | 150 mg/kg 15 min, 1 h, 1, 2, and 3 h | 3 d Malondialdehyde, 3 d Glutathione, 3 d Retinol, 3 d b-carotene, 3 d Ascorbate, 3 d a-Tocopherol, 3 d IL-1β, IL-4 |
| Pandya, 2014   | Randomization, Yes; Blinded, Yes | Male, Rat, 300–350 g        | Moderate CCI, CCI-Vehicle              | 150 mg/kg 30 min, followed by 18.5 mg/kg/h for 7 d | 1 d LV, 10 d MWM                                                                               |
| Study: Eakin, 2014 | Randomization, Yes; Blinded, Yes | Male, Rat, 350–400 g | Moderate FPI, Sham-FPI | 150 mg/kg NACA 30 min, followed by NACA 18.5 mg/kg/h for 7 d | 1 d LV ↑
7 d Lipid peroxidation ↑
7 d Protein nitrosylation ➔
10 d MWM ➔ |
---|---|---|---|---|---|
| Study: Kawoos, 2017 | Randomization, Yes; Blinded, NS | Rat, Male, 300–350 g | Multiple Blast Intensities, Blast-saline | 500 mg/kg NACA 2 h | 10–13 d MWM ➔
14 d MWM probe trial ➔ |
| Study: Chen, 2017 | Randomization, Yes; Blinded, Yes | Male, Rat, 4 months | Moderate CCI, Sham-CCI | 100 mg/kg 5 min | 1 d GSH↑
1 d Malondialdehyde ↑
1 d NO ↑
1 d NSS ↑
1 d LV ↑
1 d Edema ↑
1 d Connexin 40 expression ➔ |
| Study: Haber, 2018 | Randomization, Yes; Blinding, No | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 150 mg/kg 1 h, 1 d, 2 d | 14 d Myelin ➔
14 d Oligodendrocytes ➔
14 d Oligodendrocyte apoptosis ➔
2–7 d MG ➔ |
| Study: Sangobowale, 2018a | Randomization, Yes; Blinded, Yes | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 75 mg/kg 6 h followed by 1 d, 2 d | 7 d BM ➔
7 d APA Entrances ➔
7 d APA Time to first entrance ➔
14 d Hippocampal MAP2 ➔
14 d Myelin ➔ |
| Mouse, Male, 28–30 g | Moderate CHI, Sham-CHI, CHI-saline | 75 mg/kg 12 h followed by 1 d, 2 d | 7 d BM ➔
7 d APA Entrances ➔
7 d APA Time to first entrance ➔
14 d Hippocampal MAP2 ➔
14 d Myelin ➔ | 7 d APA, entrances ➔
7 d Time to first entrance ➔
14 d Hippocampal MAP2 ➔
14 d Myelin ➔ |
| Study        | Randomization, Blinded | Mouse, Male, 28-30g | Moderate CHI, Sham-CHI, CHI-saline | 2–14 d Oligodendrocytes | 14 d oligodendrocyte apoptosis |
|--------------|------------------------|---------------------|-----------------------------------|-------------------------|--------------------------------|
| Sangobowale, 2018b | Yes; Yes               | Mouse, Male, 28-30g | 75 mg/kg 12 h, 1 d, 2 d          |                         |                                |
| Zhou         | Yes; Yes               | Mouse, Male, 28–32 g | Moderate WD, Sham-WD, WD-saline  | 1–3 d NSS ↑              |                                |
|              |                        |                     |                                   | 1 d Heme Oxidase 1      |                                |
|              |                        |                     |                                   | 1 d NAD(P)H quinine oxidoreductase-1 ↑ |                                |
|              |                        |                     |                                   | 1 d Apoptosis ↑         |                                |
|              |                        |                     |                                   | 1 d Nuclear respiratory factor ↑ |                                |
|              |                        |                     |                                   | 1 d Malondialdehyde ↑   |                                |
|              |                        |                     |                                   | 1 d Superoxide dismutase ↑ |                                |
|              |                        |                     |                                   | 1 d Lutathione peroxidase ↑ |                                |
|              |                        |                     |                                   | 1 d Fluro Jade positive cells ↑ |                                |

Rigor for each study is provided following by injury model, species plus severity, control groups, and the MINO dosing. Time after injury is followed by whether NAC or NACA improved (↑) or had no difference (➔) in the experimental outcome. APA: Active place avoidance; BM: Barnes maze; CCI: controlled cortical impact; CHIL: closed head injury; FPI: fluid percussion injury; GSH: glutathione; LV: lesion volume; MAP2: microtubule associated protein 2; MWM: Morris water maze; NORT: novel object recognition; NS: not specified; NSS: neurological severity score; WD: weight drop.
Table 3 Clinical trials of minocycline or N-acetylcysteine

| Author          | Compound       | TBI severity                                                                 | Design                                      | Enrolled | Dose, duration                                  | Time of first dose                  | Findings                                                                                           |
|-----------------|----------------|-----------------------------------------------------------------------------|---------------------------------------------|----------|-------------------------------------------------|--------------------------------------|---------------------------------------------------------------------------------------------------|
| Hofer, 2013     | N-acetylcysteine | Mild TBI (Balance dysfunction, confusion, headache, hearing loss, impaired memory, sleep disturbances) | Randomized, double blind, placebo controlled clinical trial | 81       | 8 g loading dose, 1.3 g PO three time daily for 3 d, 1 g three times daily for 4 d | Within 3 d post-blast exposure       | Significantly greater number of patients with no residual symptoms                               |
| Meythalar, 2019 | Minocycline     | Moderate-severe TBI (Glasgow Coma Score 3–9)                                 | Dose escalation                             | 15       | 800 mg IV loading followed by 200 mg or 400 mg twice daily for 7 d | Within 6 h post-injury              | Safe in TBI population; higher doses trended towards improved disability rating score. No statistical significance |
| Scott, 2018     | Minocycline     | Moderate-severe TBI (Mayo classification)                                    | Randomized clinical trial                   | 15       | 100 mg PO twice daily for 12 weeks              | At least 6 months post-injury       | Lowered chronic microglial activation Increased serum neurofilament light protein               |
| Koulaeinejad,   | Minocycline     | Moderate-severe TBI (Glasgow Coma Score < 12)                                | Randomized, double blind, placebo. Includes both | 34       | 100 mg twice daily for 7 d                      | Within 24 h post-injury             | Lowered Serum S100B, trending (P < 0.1) Lowered Serum Neuronal Specific Enolase (P = 0.01) No effect, Glasgow Outcome Scale-Extended |

Clinical trial design is provided followed by the number of subjects enrolled, dose and duration and the most salient findings. TBI: Traumatic brain injury.
| Study                | Rigor                      | Species                  | Model               | Time to first dose, dose | Outcomes                                                                 |
|---------------------|----------------------------|--------------------------|---------------------|-------------------------|--------------------------------------------------------------------------|
| Abdel Baki, 2009    | Randomization, NS; Blinded, NS | Rat, Male, 250–300 g    | Moderate CCI, Sham-CCI | 45 mg/kg MINO, 150 mg/kg NAC 1 h, 1 d, 2 d | 8–23 d spaced APA, Entrances ↑, 8–23 d spaced APA, Time to first entrance ↑, 14 d Myelin ↑ |
|                     |                            |                          |                     |                         |                                                                           |
|                     |                            | Rat, Male, 250–300 g    | Moderate CCI, Sham-CCI | 45 mg/kg MINO, 150 mg/kg NAC 3 h preinjury | 1 h IL-1b ↑                                                                 |
| Haber, 2013         | Randomization, NS; Blinding, NS | Rat, 250–300 g     | Moderate CCI, sham-CHI-saline, CCI-saline | 45 mg/kg MINO, 150 mg/kg NAC 1 h, 1 d, 2 d | 7 d Conflict APA, Entrances ↑, 8-23 d Spaced APA, Time to first entrance ↑, 2 d MP/MG ↑, 2 d Glial fibrillary acid protein ↑ |
| Haber, 2018         | Randomization, yes; Blinding, no | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 45 mg/kg MINO, 150 mg/kg NAC 1 h, 1 d, 2 d | 14 d Myelin ↑, 1–4 d Oligodendrocytes ↑, 14 d Oligodendrocyte apoptosis ↑, 4 d MP/MG activation ↑, 7 d MP/MG activation ↑, CD86 expression ↑ |
| Sangobowale, 2018a  | Randomization, yes; Blinded, yes | Rat, Male, 250–300 g | Moderate CCI, Sham-CCI, CCI-saline | 22.5 mg/kg MINO, 75 mg/kg NAC 6 h followed by 1 d and 2 d | 7 d BM ↑, 7 d APA entrances 6H dosing ↑, 7 d APA time to first entrance ↑, 14 d Hippocampal MAP2 ↑ |
|                     |                            |                         |                     |                         |                                                                           |
|                     |                            | Rat, Male, 250–300 g    | Moderate CCI, Sham-CCI, CCI-saline | 22.5 mg/kg MINO, 75 mg/kg NAC 12 h followed by 2 d and 3 d | 7 d BM ↑, 7 d APA Entrances ↑, 7 d APA Time to first entrance ↑, 14 d Hippocampal MAP2 ↑, 14 d Myelin ↑ |
| Sangobowale, 2018b  | Randomization, yes; Blinded, yes | Mouse, Male, 28–30 g   | Moderate CHI, Sham-CHI, CHI-saline | 22.5 mg/kg MINO, 75 mg/kg NAC 12 h, by 2 d and 3 d | 7 d BM ↑, 7 d APA Entrances ↑, 7 d APA Time to first entrance ↑, 14 d Hippocampal MAP2 ↑, 14 d Myelin ↑ |
|                     |                            |                          |                     |                         |                                                                           |
|                     |                            | Mouse, Male, 28–30 g    | Moderate CHL, Sham-CHL, CHI-saline | 22.5 mg/kg MINO, 75 mg/kg NAC 12 h, by 2 d and 3 d | 2–14 d Oligodendrocytes ↑, 14 d oligodendrocyte apoptosis ↑ |
| Whitney, 2021       | Randomization, yes; Blinded, yes | Mouse, Male, 28–30 g | Moderate CHI, Sham-CHI, CHI-saline | 22.5 mg/kg MINO, 75 mg/kg NAC 3 d, 4 d, 5 d | 7 d BM↑, 7 d BM probe trial ↑, 14 d Contrallesional CA3 size, number, complexity ↑, 14 d Ipsilateral CA1 size, number, complexity ↑, 14 d CA1 synapse density ↑ |

Rigor for each study is provided following by the, species, injury model plus severity and control groups, MINO plus NAC dosing. Time after injury is followed by whether MINO plus NAC improved (↑) or had no difference (→) in the experimental outcome. APA: Active place avoidance; BM: Barnes maze; CCI: controlled cortical injury; CHI: closed head injury; MG: macrophage/microglia; MINO: minocycline; MWM: Morris water maze; NAC: N-acetylcyesteine; NS: not specified.