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Research Report

Inhaled nitric oxide improves short term memory and reduces the inflammatory reaction in a mouse model of mild traumatic brain injury

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ABSTRACT

Although the mechanisms underlying mild traumatic brain injury (mTBI) are becoming well understood, treatment options are still limited. In the present study, mTBI was induced by a weight drop model to produce a closed head injury to mice and the effect of inhaled nitric oxide (INO) was evaluated by a short term memory task (object recognition task) and immunohistochemical staining of glial fibrillary acidic protein (GFAP) and CD45 for the detection of reactive astrocytes and microglia. Results showed that mTBI model did not produce brain edema, skull fracture or sensorimotor coordination dysfunctions. Mice did however exhibit a significant deficit in short term memory (STM) and strong inflammatory reaction in the ipsilateral cortex and hippocampus compared to sham-injured controls 24 h after mTBI. Additional groups of untreated mice tested 3 and 7 days later, demonstrated that recognition memory had recovered to normal levels by Day 3. Mice treated with 10 ppm INO for 4 or 8 h, beginning immediately after TBI demonstrated significantly improved STM at 24 h when compared with room air controls ($p<0.05$). Whereas mice treated with 10 ppm INO for 24 h showed no improvement in STM. Mice treated with INO 10 ppm for 8 h exhibited significantly reduced microglia and astrocyte activation compared with room air controls. These data demonstrate that mTBI produces a disruption of STM which is evident 24 h after injury and persists for 2—3 days. Treatment with low concentration or short durations of INO prevents this memory loss and also attenuates the inflammatory response. These findings may have relevance for the treatment of patients diagnosed with concussion.

1. Introduction

'Traumatic brain injury' (TBI) is a frequent consequence of vehicle, sport and war related injuries (Zohar et al., 2011). 'Closed head injury' (CHI) is a type of TBI in which the skull and dura matter remains intact. It is reported that about two-thirds of TBI patients sustain only a mild injures (Shay and Shany, 2011). The WHO group estimated that the rate of mild

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traumatic brain injury (mTBI) in the general population is over 6 per 1000 people annually (Cassidy et al., 2004). mTBI therefore represents a significant public health burden.

Patients with mTBI are difficult to diagnose because they lack clear morphological brain defects. Although mTBI is the least severe of the traumatic brain injuries, it can result in short and long term symptoms including cognitive, behavioral and emotional difficulties. Animal experiments have shown (Stahel et al., 2000; Beni-Adani et al., 2001; Leinhase et al., 2006) that after a weight drop, mice typically showed neurological impairment during the first 24 h after trauma, followed by a partial spontaneous recovery within a week. But there are studies that have shown profound and irreversible memory impairments accompanied by depressed behavior evident as long as 90 days post injury (Gao and Chen, 2011; Zohar et al., 2011).

With increasing information on the pathological changes induced after TBI, it is becoming clear that brain trauma is a complicated neurodegenerative disease, involving many cellular and molecular pathways, including inflammation (Raghupathi, 2004; Stahel et al., 2000).

Accumulating data suggest that the neuroinflammatory response induced by TBI is characterized by microglia and astrocytic activation, and acute up-regulation of proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF) α, and IL-6 (Lucas et al., 2006; Morganti-Kossmann et al., 2001; Schmidt et al., 2005). This neuroinflammatory cascade is implicated in the development of cerebral edema, breakdown of the blood—brain barrier, and secondary neuronal injury. (Li et al., 2009) This suggests that using anti-inflammatory treatment to attenuate the deleterious consequences of cerebral inflammation and reduce delayed cell death may be a promising therapy for mTBI (Stahel et al., 2000).

Nitric oxide (NO) is the major regulator of cerebral blood flow. In addition, it inhibits platelet adherence and aggregation, reduce adherence of leukocytes to the endothelium, and suppresses vessel injury (Sehba and Bederson, 2011).

NO is a gaseous chemical messenger. When brain is in a variety of physiological processes, it has some important functions such as control of cerebral blood flow, interneuronal communications, synaptic plasticity, memory formation, receptor functions, intracellular signal transmission, and release of neurotransmitters (Moncada et al., 1991). When brain is in pathological processes, NO also has key and different roles (Gross and Wolin, 1995). Potential clinical relevance of inhaled nitric oxide(INO) as a therapy for TBI is indicated by the results of several studies that have shown INO can significantly improve outcome in patients with TBI and acute respiratory distress syndrome (ARDS).(Peillon et al., 1999; Vavilala et al., 2001; Thomas et al., 2009).

The aim of this study was to test the hypothesis that INO would improve outcome in a mouse model of mTBI. Cognition was evaluated by the use of the object recognition short term memory test. Motor and sensorimotor coordination and balance behavior were evaluated by the traverse-beam and rotor rod performance. The neurological effects of concussion was examined by measuring inflammation and edema.

2. Results

2.1. Blood gas

Physiological parameters showed that INO treatment (10 ppm for 4 h) did not affect the arterial blood gas. Arterial pH, PaO_2 and $PaCO_2$ values were summarized in Table 1.

2.2. Neurological function measurement

Our closed head mTBI mouse model did not result in brain edema, motor, balance, sensorimotor coordination impairment and acute or delayed mortality. On one day after concussion, neurological function (traverse-beam and accelerating rotor rod test) had no significant difference compared with the sham-injured mice (injured mice: $n=14$, 7.0 ± 0, 5.0 ± 2.5; sham-injured mice: $n=10$, 7.0 ± 0.25, 4.75 ± 2.75; $p=0.8213$, $p=0.3294$ respectively).

2.3. Cognitive deficit following mTBI

Mice suffered from closed head mTBI showed obvious short term memory deficit when assessed by Object Recognition Task (ORT) on Day 1 but Day 3 and Day 7. The average discrimination ratio on Day 1 ($n=14$), Day 3 ($n=11$) and Day 7 ($n=12$) were 48.94 ± 20.90, 54.66 ± 9.88 and 58.34 ± 15.86 respectively. Day 7 increased by 9.4% compared with that of Day 1 (Fig. 1).

Table 1 – Physiological variables between sham- and injured-mice administrated with or without INO.

	Baseline	Sham-mice with INO	Injured mice without INO	Injured mice with INO
n	4	4	4	4
BW(g)	37.88 ± 2.53	36.63 ± 0.63	36.75 ± 0.29	37 ± 1.08
pH	7.37 ± 0.09	7.37 ± 0.03	7.38 ± 0.04	7.42 ± 0.04
$PaCO_2$ (kPa)	5.08 ± 0.9	4.79 ± 0.61	5.25 ± 1.05	4.89 ± 0.61
PaO_2 (kPa)	15.06 ± 3.03	17.06 ± 3.07	13.77 ± 2.49	14.53 ± 2.53

Values are presented as the means ± standard deviation. INO: inhaled nitric oxide; BW: body weight; pH: potential of hydrogen; $PaCO_2$: arterial partial pressure of carbon dioxide; PaO_2: arterial partial pressure of oxygen; factorial one way analysis of variance (ANOVA) displayed that there were no difference between physiological variables in all groups ($F(3,_{12})=0.74$, $p=0.547$; $F(3,_{12})=0.25$, $p=0.859$; $F(3,_{12})=1.01$, $p=0.420$ respectively).