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Inosine improves functional recovery after experimental traumatic brain injury

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ABSTRACT

Despite years of research, no effective therapy is yet available for the treatment of traumatic brain injury (TBI). The most prevalent and debilitating features in survivors of TBI are cognitive deficits and motor dysfunction. A potential therapeutic method for improving the function of patients following TBI would be to restore, at least in part, plasticity to the CNS in a controlled way that would allow for the formation of compensatory circuits. Inosine, a naturally occurring purine nucleoside, has been shown to promote axon collateral growth in the corticospinal tract (CST) following stroke and focal TBI. In the present study, we investigated the effects of inosine on motor and cognitive deficits, CST sprouting, and expression of synaptic proteins in an experimental model of closed head injury (CHI). Treatment with inosine (100 mg/kg i.p. at 1, 24 and 48 h following CHI) improved outcome after TBI, significantly decreasing the neurological severity score (NSS, $p<0.04$ vs. saline), an aggregate measure of performance on several tasks. It improved non-spatial cognitive performance (object recognition, $p<0.016$ vs. saline) but had little effect on sensorimotor coordination (rotarod) and spatial cognitive functions (Y-maze). Inosine did not affect CST sprouting in the lumbar spinal cord but did restore levels of the growth-associated protein GAP-43 in the hippocampus, though not in the cerebral cortex. Our results suggest that inosine may improve functional outcome after TBI.

1. Introduction

Despite many years of research, no specific therapy is yet available for the treatment of traumatic brain injury (TBI) (Beauchamp et al., 2008; Jain, 2008). TBI is a major cause of mortality and morbidity worldwide, especially among young people. It may result in permanent functional deficits due to the acute primary injury as well as secondary injury

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mechanisms (Zhang et al., 2010). The most prevalent and debilitating features in survivors of TBI are cognitive deficits and motor dysfunction. A limited amount of synaptic reorganization is thought to occur spontaneously in the brain and it can be enhanced by training (Dobkin, 2003). A potential therapeutic method for improving the function of patients following TBI is to increase the plasticity of the CNS, thereby enhancing the ability of spared neurons to form new circuits that might compensate for ones that have been lost (Benowitz and Carmichael, 2010; Chen et al., 2002; Smith et al., 2007).

Inosine is a naturally occurring purine nucleoside that is formed by the deamination of adenosine and is released from cells in response to metabolic stress (Conta and Stelzner, 2008). Inosine can diffuse across the cell membrane and activate Mst3b, a protein kinase that is part of a signal transduction pathway that regulates the expression of multiple genes involved in axon growth (Benowitz et al., 1998; Irwin et al., 2006; Lorber et al., 2009; Petrausch et al., 2000). It has been shown to promote axon sprouting in the corticospinal tract and functional recovery in rat models of stroke and traumatic brain injury (Benowitz et al., 1999; Chen et al., 2002; Shen et al., 2005; Smith et al., 2007; Zai et al., 2009, 2011). Importantly, in all these studies inosine was delivered via continuous intracerebrally infusion. However, implantation of syringe for local infusion, may not be of clinical relevance, based on the accumulating reports on cognitive impairments associated with deep brain stimulation (DBS) in Parkinsonian (e.g. Daniele et al., 2003; Witt et al., 2008; York et al., 2008) as well as in Huntington's disease (Fasano et al., 2008) patients. Moreover, post-mortem analyses of brain biopsies from patients treated with DBS demonstrate a local brain tissue reaction to the electrode characterized by the presence of activated astrocytes and activated microglia (Nielsen et al.2007). The first aim of the present study was therefore to investigate long-term (up to 35 days) effects of peripheral administration of inosine in a closed head-injury model in mice and rat with special emphasis on behavioral and cognitive outcome.

Growth-Associated Protein-43 (GAP-43) is a useful marker for axonal growth during development as well as in axonal remodeling and regeneration in the adult. GAP-43 is a neuron specific, calmodulin-binding phosphoprotein that is enriched in lipid rafts and is thought to regulate the actin cytoskeleton in response to protein kinase C (PKC) activity. It is produced at high levels in all nerve cells during neurite outgrowth and at early stages of synaptogenesis, and represents a major constituent of the axonal growth cone. Up-regulation of GAP-43 occurs in several adult neuronal populations after axotomy and has been shown to promote not only axonal regrowth but also degenerative phenomena, depending on environmental cues (Burello et al., 2012). GAP-43 is a major substrate for PKC in the brain and has been found to participate in long-term potentiation (Denny, 2006). At steady-state, most of the GAP-43 is membrane-bound.

Synaptophysin, highly concentrated in the axonal terminals in the neuron, is involved in processes involving the formation and cycling of the synaptic vesicle from which neurotransmitters are released. Increases in synaptogenesis are seen during reactive synaptogenesis in sprouting neurons (Viberg, 2009). Recently Darwish et al. (2012) reported that severe TBI leads to spatial learning deficits and to decreases of synaptophysin in the ipsilateral CA3 region.

The second aim of the present study was to investigate the effects of inosine on cognitive and motor function, on axon sprouting, and on the levels of the synaptic proteins GAP-43 and synaptophysin.

2. Results

2.1. *Effect on cerebral edema and infarct volume*

As the maximal edema formation in our CHI is expressed 24 h after injury, mice were euthanized at that time, namely, after receiving only the first injection of inosine. Water content in the injured and contralateral hemispheres were 83.12±0.43% and 79.06±0.11% respectively in the control, saline-treated mice ($n=8$) and 82.87±0.34% and 78.8±0.19% respectively in the inosine treated mice ($n=10$). Thus, no effect of inosine was noted on edema formation at 24 h post-injury. Thirty five days after injury frozen brain tissue was stained and processed for evaluation of lesion volume. No difference was found between the saline- and inosine treated groups (18.8±4.0% vs. 17.2±3.7% respectively).

2.2. *Neurological evaluation*

In the first experiment, only the dosing regimen and route of administration of inosine were examined in mice. One hour after CHI all mice displayed NSS values in the range of 6–8 and were allocated to four groups with mean values ranging from 6.4±0.2 to 6.6±0.3, indicating moderate injury, similar in all groups. Mice were treated either 2 or 3 times with inosine or saline, as indicated in Section 4, and their NSS was recorded over 35 days. As depicted in Fig. 1, NSS values decreased with time in all groups due to some spontaneous recovery, although the recovery of the inosine-treated animals was faster. The group that was treated at 1, 24, and 48 h post-injury displayed a significantly greater recovery from day 7 on, as compared to saline-treated mice. We therefore chose this treatment protocol for our next mice experiments, in which NSS, motor coordination (rotarod) and cognitive function, were also evaluated, at different time points after injury.

The same experimental design was applied to rats, namely CHI was induced (using the appropriate weight and height of fall of the device, see Section 4) and treatment with inosine or saline was given as described for mice ($n=4$ in each group). The results obtained for the rats were similar to those for the mice (Fig. 2) namely, in the first few days there was only a tendency of faster recovery in the inosine-treated rats; however, between days 14 and 28, a significant ($p<0.03$) difference was observed, indicating an improved neurological recovery for the rats treated with inosine. The rats were then used for the study on CST (see below).