Inosine: a novel treatment for sciatic nerve injury

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Trauma to the peripheral nervous system often results in loss of motor and sensory functions of the affected area of the body, leading to a series of functional impairments (Allodi et al., 2012). Injuries to peripheral nerves initiate a series of complex events, known as Wallerian degeneration, which allows the injured axons to regenerate and reinnervate their targets (Allodi et al., 2012). Damage to a nerve induces multiple alterations, which include: axonal degeneration, breakdown of myelin sheath, Schwann cells (SC) proliferation and conversion to a repair phenotype which express cytokines and chemokines that allow the infiltration and activation of macrophages (Mietto et al., 2015; Jessen and Mirsky, 2016). Then, both macrophages and SC clean up axon and myelin debris, and promote in the distal degenerated portion of the axon, a favorable microenvironment that is required for regeneration. After clearance, SC forms the bands of Büngner, which will guide the regenerating axons to the target and will allow the reestablishment of new synapses with the muscle, followed by the process of axon remyelination (Allodi et al., 2012; Jessen and Mirsky, 2016). Concomitant with these events, the neuronal cell body turns into a pro-regenerative state, allowing the injured axons to grow. Some positive signals such as cyclic adenosine monophosphate (cAMP) increase and the entrance of extracellular Ca^{2+} to the axoplasm can trigger and maintain the pro-regenerative state. cAMP has a key role in the growth state of the neuronal soma, regulating axon attraction or repulsion by guidance cues from the environment. One example is the nerve growth factor and brain-derived neurotrophic factor. Low levels of cAMP turn the attractive effects of these neurotrophic factors, important for axonal growth, in repulsion. Moreover, these signals activate a cascade of mitogen-activated protein kinases, which induces the expression of transcriptions factors such as activating transcription factor 3 and signal transducers and activators of transcription, increasing the expression of neurotrophic factors, growth proteins such as growth associated protein-43, cytoskeleton proteins and extracellular matrix components (Allodi et al., 2012).

Knowing the remarkable ability of peripheral neurons to regenerate, many researchers focused on developing treatments that could enhance axon regeneration. Among the known experimental pro-regenerative strategies, molecular therapies have shown promising results. One of them is the use of inosine, a purine nucleoside derived from adenosine deamination (Ribeiro et al., 2016). Inosine acts through a series of mechanisms such as binding to adenosine or P1 receptors or binding directly to A2A receptors. It acts as an agonist of these receptors and activates adenylyl cyclase, increasing the expression of cAMP, which triggers signaling cascades that are important for axon regeneration and enhances the expression of trophic factors (Ribeiro et al., 2016; Welihinda et al., 2018). It also presents immunomodulatory effects, because A2A and A3 adenosine receptors are present on both myeloid and lymphoid cells and exert anti-inflammatory effects (Di Virgilio and Vuerich, 2015). Inosine can also promote neuroprotection by the degradation product of ribose-5-phosphate that increases ATP levels (Jurkowitz et al., 1998), the survival of cell bodies after injury (Kuricova et al., 2014) and regulation of growth associated protein-43 protein on neurons (Irwin et al., 2006; **Figure 1**). Inosine has been used as a treatment in central nervous system injuries and has shown great regenerative capacity. In a model of dorsal column transection, inosine stimulated axons from corticospinal tract to sprout to the contralateral side. Besides, they established synapses with long propriospinal interneurons, partially reestablishing cortical control at the lumbar level, improving motor function recovery (Kim et al., 2013). Oral administration of inosine after a compressive spinal cord injury, also showed a major sparing of white matter and survival of motoneurons in spinal cord ventral horn and recovery of motor function (Kuricova et al., 2014). In another study, using a model of primary motor cortical injury in the rhesus monkey, most of the monkeys that received daily doses of inosine orally, demonstrated recovery of function in movements of the hand and digits, contrasting with the control group, which developed a compensatory grasp pattern that included using multiple digits to grab food in to the palm of their hand. Thereby, inosine appears to enhance neural plasticity, which may be the basis for the recovery of function in this study (Moore et al., 2016).

The use of inosine in the central nervous system has shown axonal regeneration, neuroprotection, white matter sparing and functional recovery after injury. However, until recently, the effect of inosine in the peripheral nervous system was unknown. In a recent work of our group (Cardoso et al., 2019), we aimed to evaluate the regenerative potential of inosine treatment after sciatic nerve crush injury in mice. To perform this study, the sciatic nerve of 36 female mice (8–12 weeks of age) were crushed for 1 minute using a Dumont #5 forceps; then, 1 hour after the injury and daily, for 7 days after injury, mice were treated with either saline or inosine, by intraperitoneal injection. Then, the animals were submitted to functional tests for 2 weeks after injury. To evaluate the motor recovery after injury we used the sciatic functional index and rotarod test. Animals treated with inosine showed an early recovery of function in sciatic functional index (–61.5 ± 4.7) when compared to the saline-treated group (–85.9 ± 4.5). They also showed an early recovery in the rotarod test. Regarding the sensory recovery, we used the pinprick test to assess the pain sensitivity and, similarly to the motor function, the animals treated with inosine showed an early recovery. Then, we performed electroneuromyography to assess the regeneration after injury. Animals treated with inosine showed a significantly higher difference in the amplitude of CMAP as compared to the animals that received saline, at the first and second weeks after injury, demonstrating that the regeneration was anticipated. To explain these findings in the functional tests, we performed semithin sections of sciatic nerves, 2 weeks after injury. We observed that inosine group presented a larger number of myelinated fibers and a decrease in myelin ovoids numbers concerning the saline group, suggesting that the clearance of the microenvironment occurred earlier and was more efficient in the inosine group and, by this way, the regenerative process seemed to be anticipated in inosine treated nerves.

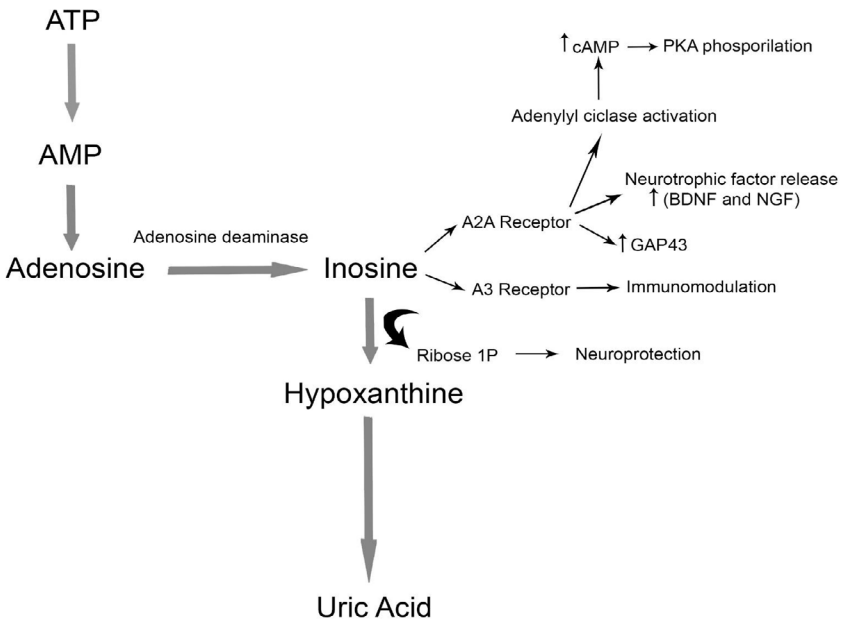

Figure 1 | **Schematic representation of inosine degradation and its action mechanisms.**
The image shows the ATP degradation pathway and its downstream substrate degradation. Notice that inosine, a derived substrate of adenosine deaminase action, can agonistically bind to the P1 receptors (here shown the A2A and A3), and stimulates the downstream cascade. Here, it is possible to see that in the conversion of inosine to hypoxanthine, a liberation of a ribose1P occurs which is responsible for neuroprotection. AMP: Adenosine monophosphate; ATP: adenosine triphosphate; BDNF: brain-derived neurotrophic factor; cAMP: cyclic adenosine monophosphate; GAP43: growth associated protein 43; NGF: nerve growth factor; PKA: protein kinase A; Ribose 1P: ribose 1 phosphate.

Regarding the clearance of axon and myelin debris, we performed immunohistochemistry to observe the presence of macrophages on the nerve. We used the F4/80 antibody and observed that inosine treated nerves showed a reduced number of macrophages two weeks after injury, corroborating the hypothesis that the microenvironment clearance in inosine groups occurred in earlier stages. Finally, we analyzed the neuroprotection of motoneurons in the ventral horn of the spinal cord and in the sensory neurons of the dorsal root ganglia, using cryoprotected sections of spinal cord and dorsal root ganglion, stained with cresyl violet. We observed that inosine was capable to protect the neurons from death after injury.

Altogether, our results demonstrated that the treatment with inosine was able to promote neuroprotection and early morphological and functional improvements, after sciatic nerve crush. Furthermore, added to our results, the efficacy of inosine has been demonstrated in multiple models of traumatic central nervous system injuries, and therefore, the use of this molecule would appear to be a feasible choice to treat injured sciatic nerve. Our group is now beginning to study the use of inosine in a model of nerve transection and repair, using a tube of polycaprolactone, to assess the efficacy of the treatment on a severe model of peripheral nerve injury. We also intend to elucidate the mechanisms behind the improvement in nerve regeneration after inosine treatment.

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