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Immunomodulatory and neuroprotective effects of inosine

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Adenosine has been considered as a potential immunomodulatory and neuroprotective agent for 30 years. Inosine, a major degradation product of adenosine, was thought originally to have no biological effects. However, recent studies demonstrate that inosine has potent immunomodulatory and neuroprotective effects. Inosine enhances mast-cell degranulation, attenuates the production of pro-inflammatory mediators by macrophages, lymphocytes and neutrophils, and is protective in animal models of sepsis, ischemia–reperfusion and autoimmunity. Inosine preserves the viability of glial cells and neuronal cells during hypoxia, and stimulates axonal regrowth after injury. The biological actions of inosine might involve effects on adenosine receptors, poly(ADP-ribose) polymerase and cellular energy levels. In this article, we review recent observations indicating that it might be possible to exploit inosine therapeutically for the treatment of tissue damage caused by inflammation and ischemia.

Purine nucleosides, such as adenosine and its primary metabolite inosine, are low-molecular-weight molecules that participate in a wide variety of intracellular biochemical processes and serve as monomeric precursors of RNA and DNA. In addition, nucleosides have important roles as extracellular signaling molecules. Although both adenosine and inosine are present constitutively at low levels in the extracellular space, metabolically stressful conditions, such as those that occur during injury, ischemia and inflammation, dramatically increase their extracellular concentrations. It is clear that adenosine, acting mostly at its receptors, is a powerful signaling molecule that participates in the regulation of many physiological and pathophysiological processes [1,2]. Extracellular adenosine and adenosine A_{2A} (purine) receptors were identified recently as both anti-inflammatory signals and sensors of excessive inflammatory tissue damage [3]. Activation of A_3 receptors has additional anti-inflammatory effects [4–10]. Identification of specific adenosine receptors as 'natural brakes' on inflammation provides a useful framework for understanding how tissues regulate inflammation and how targeting this endogenous anti-inflammatory pathway might modulate inflammation [11,12].

Unlike adenosine, few physiological roles have been ascribed to inosine, which was generally believed to be an inactive breakdown product of adenosine that lacked physiological actions. Only recently was it recognized that inosine can also bind to adenosine receptors and initiate intracellular signaling events [13–19]. In addition, inosine can affect cell function via receptor-independent pathways. Some of the modulatory actions of extracellular adenosine are mediated by inosine following a process that involves the intracellular uptake of adenosine and conversion to inosine.

In this review, we discuss recent developments that increase our understanding of the role of inosine in regulating cell functions (Box 1). We give special emphasis to the question of how inosine regulates the immune and nervous systems because the most substantial new findings involve these areas.

Inosine metabolism and release

Inosine is formed both intracellularly and extracellularly. The two major routes for the intracellular formation of inosine are shown in Figure 1. The deamination of adenosine to inosine occurs mainly at high intracellular concentrations of adenosine, which are associated with hypoxia, ischemia and other forms of cellular stress [20]. When inosine reaches high concentrations inside the cell, it is shunted into the extracellular space by bidirectional, equilibrative, nucleoside transporters [21] (Figure 1).

Box 1. The multiple mechanisms of action of inosine

- Interaction with adenosine receptors [13–19]
- Enhancement of uric acid production [36,37,62,63]
- Inhibition of poly(ADP-ribose) polymerase [64,65]
- Neuroprotection by its breakdown product ribose 1-phosphate [53]
- Upregulation of GAP-43 in neurons [56]

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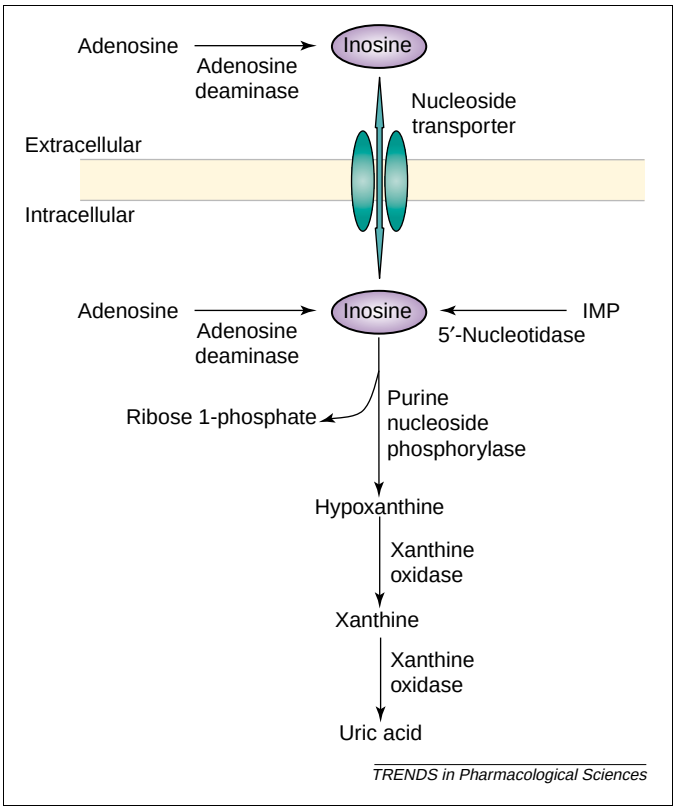

Figure 1. Some of the major pathways of inosine metabolism. Inosine is formed from adenosine in a reaction that is catalyzed by adenosine deaminase both intracellularly and in extracellular spaces. Inosine monophosphate (IMP) is dephosphorylated to inosine by 5′-nucleotidase inside the cell and shunted into the extracellular space through membrane nucleoside transporters. Inosine is degraded intracellularly to hypoxanthine and ribose 1-phosphate by purine nucleoside phosphorylase. Xanthine oxidase then catalyses the conversion of hypoxanthine, first to xanthine and then to uric acid.

Consistent with the fact that inosine exerts its most powerful regulatory actions in the immune system (see below), adenosine deaminase expression is highest in lymphoid tissues [22]. Similar to inosine, adenosine is liberated from cells during metabolic stress via nucleoside transporters [21].

The catabolic degradation of inosine to uric acid takes place inside cells (Figure 1). Extracellular inosine and adenosine access the intracellular space through both equilibrative and concentrative nucleoside transporters, and adenosine and inosine compete for these transporters [21]. Thus, extracellular inosine can augment extracellular adenosine levels by preventing adenosine uptake and, thus, generate indirect biological effects secondary to adenosine binding to its receptors.

Inosine-sensitive, cell-surface receptors

It is increasingly clear that many of the cellular actions of inosine occur through occupancy of G-protein-coupled adenosine receptors. Four subtypes of adenosine receptor, A_1, A_{2A}, A_{2B} and A_3, have been identified [1,2], one or more of which is expressed by the target cells of inosine in both the immune system and the nervous system. Inosine is reported to bind directly to A_1, A_{2A} and A_3 receptors [13,15–18,23]. In general, inosine appears to be more potent in eliciting biological effects via rodent than human A_3 receptors [13,15–18]. A recent study using A_{2A} receptor knockout mice provides evidence that some of the immunomodulatory effects of systematically injected inosine are mediated by A_{2A} receptors [19]. However, it remains to be seen whether these systemic immunomodulatory effects are the consequences of direct binding of inosine to A_{2A} receptors.

Little is known about the intracellular pathways triggered by inosine binding to adenosine receptors. Consistent with the ability of A_1 and A_3 receptor stimulation to activate $G_{i/o}$ proteins [1,2], occupancy of either of these receptors with inosine has been shown to prevent either isoproterenol-induced or forskolin-induced accumulation of cAMP [13,18]. In addition, inosine has been demonstrated recently to trigger the phosphorylation of protein kinase B (also known as Akt) via an A_3-receptor-mediated mechanism [17].

Immunomodulatory effects of inosine

Inosine augments mast-cell degranulation via the engagement of A_3 receptors

The first evidence of the immunomodulatory effects of inosine came with the observation that at concentrations of 5–10 μM inosine stimulates mast-cell degranulation by binding selectively to A_3 receptors [13]. Subsequently, it was reported that the inosine-induced enhancement of mast-cell degranulation was lost in mice that lack A_3 receptors [15]. Potentiation by inosine of mast-cell degranulation via A_3 receptors might explain the controversial observation that adenosine both enhances and decreases mast-cell degranulation [13]. According to the concept outlined by Jin *et al.* [13], adenosine must be converted to inosine to bind to A_3 receptors and facilitate mast-cell degranulation, which might provide a potential mechanism for the previously unexplained finding that adenosine-enhanced mast-cell degranulation requires adenosine deaminase [24]. Thus, the ratio of the local concentrations of adenosine and inosine might determine the outcome of the mast-cell response to adenosine because inosine appears to bind A_3 receptors preferentially in mast cells and, thus, enhance mast-cell degranulation, whereas adenosine also binds to A_{2A} receptors, which decreases mast-cell degranulation.

The adenosine:inosine concentration ratio also explains why adenosine produces a complex, inconsistent, vascular response and has both vasodilatory and vasoconstrictor effects in some vascular preparations [25]. The vasodilatory effect of adenosine is likely to be secondary to the occupancy of A_{2A} receptors on smooth muscle cells, which results in smooth muscle relaxation. By contrast, the vasoconstrictive effect of adenosine probably involves occupancy of A_3 receptors by adenosine and inosine in perivascular mast cells, with the concomitant release of histamine causing indirect constriction of smooth muscle.

Inosine suppresses macrophage, lymphocyte and neutrophil activation in vitro

In contrast to the pro-inflammatory effects on mast cells, inosine is a powerful anti-inflammatory agent in macrophages and lymphocytes *in vitro* [14]. Inosine administration to immunostimulated mouse peritoneal macrophages reduces the production of several pro-inflammatory

cytokines, including tumor necrosis factor α (TNF-α), interleukin 1 (IL-1) and macrophage inflammatory protein α (MIP-1α). The anti-inflammatory effect of inosine does not require cellular uptake, which indicates the potential role of a cell-surface receptor [14]. Furthermore, the inhibitory effect of inosine on the production of pro-inflammatory cytokines is post-transcriptional because inosine fails to reduce steady-state concentrations of mRNA of these cytokines. In addition, inosine attenuates interferon γ (IFN-γ)-induced expression of cell-surface major histocompatibility complex II (Figure 2). Finally, the anti-inflammatory effects of inosine are not confined to macrophages because inosine attenuates IFN-γ production in cells from mouse spleen that are stimulated with the specific lymphocyte stimulus anti-CD3 antibody [14].

The anti-inflammatory effects of inosine have been confirmed in a follow-up study using human cells [26]. Inosine attenuated TNF-α production by immunostimulated whole blood and blocked the generation of formyl-Met-Leu-Phe-induced superoxide by neutrophils. One important difference between mouse and human studies is that inosine is ~10 times less potent at exerting its anti-inflammatory effects in human cells. This difference in potency is explained readily by species differences in the expression and function of adenosine receptors, particularly A_3 and A_{2B} receptors [1,2].

Inosine counteracts the pro-inflammatory effects of endotoxin in vivo

Endotoxin (also know as bacterial lipopolysaccharide), the major pro-inflammatory component of the cell wall of gram-negative bacteria, is central to many of the pathophysiological events that characterize sepsis and septic shock [27]. The pro-inflammatory effect of endotoxin can be ascribed to its ability to stimulate Toll-like receptor 4 and, thus, the production of inflammatory mediators from macrophages [27]. In 2000, evidence was provided that inosine blunts the macrophage-mediated inflammatory response to endotoxin *in vivo* [14]. Intraperitoneal (systemic) treatment of mice with inosine before injection of endotoxin substantially ameliorated the pro-inflammatory cytokine 'storm' that is characteristic of endotoxin administration *in vivo*. Systemic levels of the pro-inflammatory cytokines TNF-α, IL-12, MIP-1α and IFN-γ were reduced by inosine, whereas there was an increase in the production of IL-10, a major anti-inflammatory cytokine. Consistent with this protective, anti-inflammatory cytokine profile, inosine protects mice from the lethal effect of endotoxin [14]. The endotoxin-induced cytokine storm causes death by injuring several organs, including the gut, liver, lung and cardiovascular system [27]. A study by Garcia Soriano and co-workers [28] revealed that inosine prevents gut and lung injury, in addition to liver and vascular failure that are secondary to systemic endotoxin administration in mice. Furthermore, these same investigators confirmed [29] that inosine also has local protective effects because it downregulates the pro-inflammatory response and inhibits the lung injury that results from tracheal instillation of endotoxin to mice. As with systemic administration of endotoxin, the local protective effect of inosine in the lung is secondary to suppression of macrophage-mediated pro-inflammatory cytokine production, including TNF-α, IL-1 and IL-6. In addition, in the lung, inosine reduces the formation of nitric oxide and nitrosative stress [29], both of which contribute to the acute injury after local instillation of endotoxin.

The initial pharmacological analysis using adenosine receptor antagonists indicated that the anti-inflammatory effects of inosine might be mediated by adenosine receptors [14]. A more recent study using A_{2A}-receptor and A_3-receptor knockout mice demonstrated that the protective effect of inosine on the endotoxin-induced systemic inflammatory response requires both A_{2A} and A_3 receptors [19]. Inosine-mediated suppression of the production of TNF-α following systemic administration of endotoxin was reversed in A_{2A}-receptor and A_3-receptor double-knockout mice, but not A_{2A}-receptor and A_3-receptor single-knockout mice (Figure 3). Interestingly, inosine protects mice from the systemic inflammatory response induced by concanavalin-A, which is mediated by T cells, natural killer cells, macrophages and neutrophils. However, in this experimental system, the A_3 receptor is responsible for the therapeutic effect of inosine. Thus, although inosine has protective actions in several

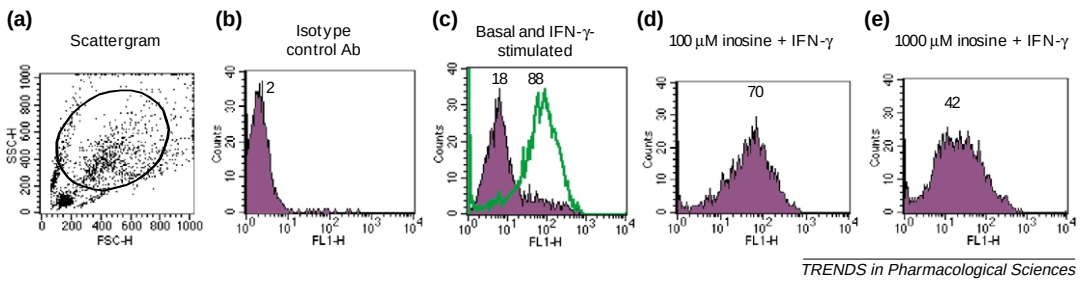

Figure 2. Inosine dose-dependently inhibits major histocompatibility complex (MHC) II expression induced by interferon γ (IFN-γ) in peritoneal macrophages *in vitro*. Thioglycollate-elicited peritoneal macrophages from BALB/c mice were plated on 12-well plates and treated with inosine in the presence or absence of IFN-γ (0.5 μg ml^{-1}) for 48 h. Cells were removed by scraping, and washed in phosphate-buffered saline. After washing, the cells were resuspended in phosphate-buffered saline containing 10% mouse serum and Fc block (rat anti-mouse CD16/CD32) to prevent nonspecific binding of FITC (fluorescein isotio-cyanate)-conjugated anti-I-A^d antibody to Fc receptors. Subsequently, cells were stained with an antibody (FITC-conjugated anti-I-A^d) raised against the BALB/c mouse MHCII alloantigen. The cells were then analyzed with a FACSCalibur flow cytometer. Values shown for each graph are mean fluorescence. **(a)** The scattergram depicts the distribution of cells based on forward scatter (size) and side scatter (granularity). The circle represents gated macrophages used for the analysis. **(b)** Cells were stained with an isotype-control antibody. **(c)** The purple peak represents cells that were not treated with IFN-γ (basal) whereas the green peak represents IFN-γ-stimulated cells. **(d)** IFN-γ-stimulated cells treated with 100 μM inosine. **(e)** IFN-γ-stimulated cells treated with 1000 μM inosine. This figure is representative of four separate experiments. Abbreviations: FL1-H, fluorescent intensity of FITC-stained cells; FSC-H, forward scatter; SSC-H, side scatter.

inflammatory states, the receptors that mediate these salutary effects depend on the cell types and stimulus involved.

Although endotoxemic models provide information about the early inflammatory response that is encountered in some forms of sepsis, results from these models neither predict nor correlate with the majority of cases of clinical sepsis and/or the development of multiple organ failure. Most patients survive the initial hyperinflammatory response that is a direct consequence of endotoxin from bacteria. The subsequent development of infection, which is secondary to an immunosuppressed state in the later stages of sepsis, is the major cause of multiple organ failure that kills many patients. One clinically relevant model of sepsis-mediated multiple-organ failure is the mouse cecal ligation and puncture model, which causes bacterial peritonitis and generalized bacteremia [30]. Liaudet and co-workers [31] report that inosine also protects in this model of sepsis. Inosine rescues mice from the lethal effects of cecal ligation and puncture and substantially ameliorates the course of multiple organ failure.

Inosine attenuates the course of chronic autoimmune (inflammatory) diseases

Macrophages have an important role in the induction of tissue injury during autoimmune diseases [32]. Because inosine has potent macrophage-deactivating effects, it is not surprising that inosine protects against the development of disease in several mouse models of autoimmune disease. These include the multiple-low-dose streptozotocin and non-obese diabetic mouse models [33], the dextran sulfate sodium model of colitis [34], and collagen-induced arthritis [35]. In line with the concept that inosine prevents disease development in part, at least, by suppressing macrophage activation, the production of pro-inflammatory products by macrophages, including TNF-α and MIP-1α, is reduced substantially following inosine administration in all these disease states. One possible exception is experimental allergic encephalomyelitis, a rodent model of multiple sclerosis, in which inosine-mediated protection does not appear to be associated with a direct macrophage-deactivating effect [36]. Rather, inosine suppresses clinical signs of this condition following metabolism to uric acid. Uric acid has potent antioxidant properties against peroxynitrite and other oxidant species [37], all of which are important pathophysiological factors that contribute to the clinical signs of experimental allergic encephalomyelitis; thus, it is likely that the therapeutic effects of inosine are mediated by the antioxidant action of uric acid.

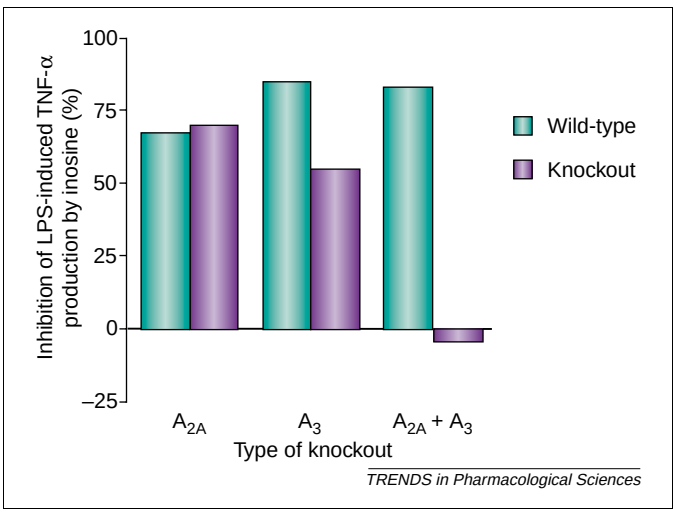

Figure 3. Inhibition of bacterial lipopolysaccharide (LPS; 10 mg kg^{-1})-induced production of systemic tumor necrosis factor α (TNF-α) by inosine: role of adenosine A_{2A} and A_3 receptors. In wild-type, control animals, inosine administration (100 mg kg^{-1}) inhibits LPS-induced TNF-α production by 70–80%. In A_{2A}-receptor-deficient animals, inhibition by inosine is not different from that observed in wild-type animals. In A_3-receptor-deficient animals, this inhibition decreases to ~55% and in double-knockout mice that lack both A_{2A} and A_3 receptors, inosine does not inhibit TNF-α production. These data demonstrate the role of A_{2A} and A_3 receptors in the anti-inflammatory effects of inosine *in vivo*.

Effects of inosine in hypoxia, reperfusion and trauma

Inosine attenuates ischemia–reperfusion injury by decreasing immune activation

Although the immune response to tissue injury has an essential role in preserving tissue homeostasis, uncontrolled inflammation and immune activation can inflict further damage on affected tissues. This is particularly true for ischemia–reperfusion injury, where activation of neutrophils, macrophages and other types of immune cells is responsible for much of the tissue injury that follows the initial insult. Inosine concentrations of up to ~6 μM have been detected in human myocardial ischemia, and many times higher concentrations are seen in experimental models of ischemia–reperfusion injury [38–41]. At present, it is unclear whether this endogenously produced inosine is sufficient to exert tissue-protective effects. Exogenous administration of larger doses of inosine are reported to prevent ischemia–reperfusion injury in several tissues, including the heart and brain [42–46], although the reason for this effect was unclear until recently. Similar to the endotoxin-induced inflammatory response, inosine attenuates the production of macrophage-derived, pro-inflammatory cytokines, including TNF-α, MIP-2 and IL-6, following ischemia–reperfusion of skeletal muscle [47] and gut [48]. Suppression of the inflammatory response is likely to contribute to the protective action of inosine because neutralizing antibodies to these cytokines provide similar protection against skeletal muscle and gut injury [49,50].

Inosine preserves glial-cell and neuronal-cell viability during hypoxia

It has long been known that adenosine has neuroprotective effects during cerebral ischemia [51]. However, under some conditions, the neuroprotective effects of adenosine cannot be explained by an action on adenosine receptors, which raises the possibility of a mechanism related to the intracellular metabolism of adenosine. Haun and colleagues reported [52] that inosine mediates the protective effect of adenosine on cell viability in cultures of rat astrocytes subjected to deprivation of glucose and oxygen. They came to this conclusion following the observation that erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride, which inhibits adenosine deaminase, reverses the

protective effect of adenosine. Furthermore, adenosine is rapidly metabolized to inosine, and inosine mimics the protective effect of adenosine. Subsequent studies demonstrating that an inhibitor of purine nucleoside phosphorylase abolishes the protective effect of inosine on the viability of glial cells [53] provide further mechanistic insight. Because hypoxanthine does not have the protective effect of inosine, it is possible that ribose 1-phosphate, the other degradation product of inosine (Figure 1), is responsible for the prevention of glial-cell death by inosine. In fact, when iodoacetate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase is added to the cells, the protective effect of inosine is lost. Because glyceraldehyde-3-phosphate dehydrogenase is part of the glycolytic pathway downstream of the entry of ribose, it is possible that the ribose 1-phosphate moiety of inosine protects against the consequences of glucose and oxygen deprivation by providing the ATP necessary to maintain plasmalemmal integrity. In a separate line of investigation, Litsky and colleagues [54] demonstrated that administration of inosine has a similar protective effect against glucose and oxygen deprivation in neurons.

Inosine stimulates axon regrowth after injury

Normally, neurons in the mature CNS cannot regenerate injured axons. However, altering the extracellular environment allows many neurons that otherwise show no potential for growth to extend injured axons over long distances. It is well known that several polypeptide factors secreted by glial cells, including nerve growth factor (NGF), ciliary neurotrophic factor and axogenesis factor, promote axon regeneration following injury. Studies from Benowitz and co-workers [55] have identified inosine as a small-molecule factor that fosters axonal regrowth in the CNS. These investigators demonstrated that inosine triggers axonal outgrowth from retinal ganglion cells of goldfish [55]. The axonal outgrowth stimulated by inosine is associated with a characteristic pattern of molecular changes, most notably upregulation of GAP-43, a protein that is associated with the submembrane cytoskeleton and is a crucial regulator of axonal regrowth [56]. The primary intracellular target responsible for the changes in gene expression after inosine treatment is a 47–50 kDa serine–threonine kinase (N-kinase) that is normally activated in minutes of treating cells with NGF. These findings *in vitro* have been extended to demonstrate that inosine stimulates axonal regrowth *in vivo*, after corticospinal tract injury [57] and stroke [58].

Concluding remarks and future prospects

Inosine is a safe, naturally occurring purine that appears to be non-toxic to humans, even when ingested at doses as high as 10 g kg^{-1} day^{-1}; in fact, inosine is widely available as a nutritional supplement in health food stores [59–61]. Inosine has been used sporadically in clinical practice for various cardiovascular disorders, including some ischemic events [42]. Furthermore, inosine has been used recently in small patient populations for the therapy of multiple sclerosis [62]. With an increasing body of preclinical evidence to show that inosine is effective in several ischemic and inflammatory diseases, it might be worthwhile to re-evaluate the therapeutic potential of inosine in ischemic, autoimmune and inflammatory diseases.

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References

1 Ralevic, V. and Burnstock, G. (1998) Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50, 413–492
2 Fredholm, B.B. *et al.* (2001) International union of pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53, 527–552
3 Ohta, A. and Sitkovsky, M. (2001) Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 414, 916–920
4 Haskó, G. *et al.* (1996) Adenosine receptor agonists differentially regulate IL-10, TNF-α, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *J. Immunol.* 157, 4634–4640
5 Szabó, C. *et al.* (1998) Suppression of macrophage inflammatory protein (MIP)-1α production and collagen-induced arthritis by adenosine receptor agonists. *Br. J. Pharmacol.* 125, 379–387
6 Haskó, G. *et al.* (2000) Adenosine inhibits IL-12 and TNF-α production via adenosine A_{2a} receptor-dependent and independent mechanisms. *FASEB J.* 14, 2065–2074
7 Salvatore, C.A. *et al.* (2000) Disruption of the A_3 adenosine receptor gene in mice and its effect on stimulated inflammatory cells. *J. Biol. Chem.* 275, 4429–4434
8 Wu, W.P. *et al.* (2002) Decreased inflammatory pain due to reduced carrageenan-induced inflammation in mice lacking adenosine A_3 receptors. *Neuroscience* 114, 523–527
9 Montesinos, M.C. *et al.* (2003) Adenosine A_{2A} or A_3 receptors are required for inhibition of inflammation by methotrexate and its analog MX-68. *Arthritis Rheum.* 48, 240–247
10 Mabley, J. *et al.* (2003) The adenosine A_3 receptor agonist, N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide, is protective in two murine models of colitis. *Eur. J. Pharmacol.* 466, 323–329
11 Haskó, G. *et al.* (2002) Adenosine: a potential mediator of immunosuppression in multiple organ failure. *Curr. Opin. Pharmacol.* 2, 440–444
12 Sitkovsky, M.V. (2003) Use of the A_{2A} adenosine receptor as a physiological immunosuppressor and to engineer inflammation *in vivo*. *Biochem. Pharmacol.* 65, 493–501
13 Jin, X. *et al.* (1997) Inosine binds to A_3 adenosine receptors and stimulates mast cell degranulation. *J. Clin. Invest.* 100, 2849–2857
14 Haskó, G. *et al.* (2000) Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. *J. Immunol.* 164, 1013–1019
15 Tilley, S.L. *et al.* (2000) Adenosine and inosine increase cutaneous vasopermeability by activating A_3 receptors on mast cells. *J. Clin. Invest.* 105, 361–367
16 Fenster, M.S. *et al.* (2000) Activation of adenosine $A_{2\alpha}$ receptors inhibits mast cell degranulation and mast cell-dependent vasoconstriction. *Microcirculation* 7, 129–135
17 Gao, Z. *et al.* (2001) A_3 adenosine receptor activation triggers phosphorylation of protein kinase B and protects rat basophilic leukemia 2H3 mast cells from apoptosis. *Mol. Pharmacol.* 59, 76–82
18 Fredholm, B.B. *et al.* (2001) Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem. Pharmacol.* 61, 443–448
19 Gomez, G. and Sitkovsky, M.V. (2003) Differential requirement for A_{2a} and A_3 adenosine receptors for the protective effect of inosine *in vivo*. *Blood* 102, 4472–4478
20 Deussen, A. (2000) Metabolic flux rates of adenosine in the heart. *Naunyn Schmiedebergs Arch. Pharmacol.* 362, 351–363
21 Pastor-Anglada, M. *et al.* (2001) Complex regulation of nucleoside transporter expression in epithelial and immune system cells. *Mol. Membr. Biol.* 18, 81–85
22 Aldrich, M.B. *et al.* (2000) The importance of adenosine deaminase for lymphocyte development and function. *Biochem. Biophys. Res. Commun.* 272, 311–315

23 Askalan, R. *et al.* (1994) Role of histidine residues in the adenosine A_{2a} receptor ligand binding site. *J. Neurochem.* 63, 1477–1484
24 Benyon, R.C. *et al.* (1984) The effect of methyltransferase inhibitors on histamine release from human dispersed lung mast cells activated with anti-human IgE and calcium ionophore A23187. *Biochem. Pharmacol.* 33, 2881–2886
25 Shepherd, R.K. *et al.* (1996) Adenosine-induced vasoconstriction *in vivo*. Role of the mast cell and A_3 adenosine receptor. *Circ. Res.* 78, 627–634
26 Marton, A. *et al.* (2001) Anti-inflammatory effects of inosine in human monocytes, neutrophils and epithelial cells *in vitro*. *Int. J. Mol. Med.* 8, 617–621
27 Ulevitch, R.J. (2001) New therapeutic targets revealed through investigations of innate immunity. *Crit. Care Med.* 29, S8–12
28 Garcia Soriano, F. *et al.* (2001) Inosine improves gut permeability and vascular reactivity in endotoxic shock. *Crit. Care Med.* 29, 703–708
29 Liaudet, L. *et al.* (2002) Inosine exerts a broad range of antiinflammatory effects in a murine model of acute lung injury. *Ann. Surg.* 235, 568–578
30 Deitch, E.A. (1998) Animal models of sepsis and shock: a review and lessons learned. *Shock* 9, 1–11
31 Liaudet, L. *et al.* (2001) Inosine reduces systemic inflammation and improves survival in septic shock induced by cecal ligation and puncture. *Am. J. Respir. Crit. Care Med.* 164, 1213–1220
32 Lam-Tse, W.K. *et al.* (2002) Animal models of endocrine/organ-specific autoimmune diseases: do they really help us to understand human autoimmunity? *Springer Semin. Immunopathol.* 24, 297–321
33 Mabley, J.G. *et al.* (2003) Inosine protects against the development of diabetes in multiple-low-dose streptozotocin and nonobese diabetic mouse models of type 1 diabetes. *Mol. Med.* 9, 96–104
34 Mabley, J.G. *et al.* (2003) Inosine reduces inflammation and improves survival in a murine model of colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 284, G138–G144
35 Mabley, J.G. *et al.* Inosine protects against development of collagen-induced rheumatoid arthritis. *J. Autoimmun.* (in press)
36 Scott, G.S. *et al.* (2002) Therapeutic intervention in experimental allergic encephalomyelitis by administration of uric acid precursors. *Proc. Natl. Acad. Sci. U. S. A.* 99, 16303–16308
37 Hooper, D.C. *et al.* (1997) Prevention of experimental allergic encephalomyelitis by targeting nitric oxide and peroxynitrite: implications for the treatment of multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 94, 2528–2533
38 de Jong, J.W. *et al.* (1977) Myocardial nucleoside and carbohydrate metabolism and hemodynamics during partial occlusion and reperfusion of pig coronary artery. *J. Mol. Cell. Cardiol.* 9, 297–312
39 Fox, A.C. *et al.* (1979) Release of nucleosides from canine and human hearts as an index of prior ischemia. *Am. J. Cardiol.* 43, 52–58
40 Hale, S.L. *et al.* (1985) Alterations in the distribution of high-energy phosphates during ischemia in a canine model of reperfusion-induced ventricular fibrillation. *Am. Heart J.* 110, 590–594
41 Eklund, T. *et al.* (1991) Interstitial lactate, inosine and hypoxanthine in rat kidney during normothermic ischaemia and recirculation. *Acta Physiol. Scand.* 143, 279–286
42 Juhasz-Nagy, A. and Aviado, D.M. (1977) Inosine as a cardiotonic agent that reverses adrenergic beta blockade. *J. Pharmacol. Exp. Ther.* 202, 683–695
43 Czarnecki, W. and Herbaczynska-Cedro, K. (1982) The influence of inosine on the size of myocardial ischaemia and myocardial metabolism in the pig. *Clin. Physiol.* 2, 189–197
44 DeWitt, D.F. *et al.* (1983) Nucleotide degradation and functional impairment during cardioplegia: amelioration by inosine. *Circulation* 67, 171–178
45 Aussedat, J. *et al.* (1985) Adenine nucleotide synthesis from inosine during normoxia and after ischaemia in the isolated perfused rat heart. *Can. J. Physiol. Pharmacol.* 63, 1159–1164
46 Hagberg, H. *et al.* (1986) Blockade of N-methyl-D-aspartate-sensitive acidic amino acid receptors inhibits ischemia-induced accumulation of purine catabolites in the rat striatum. *Neurosci. Lett.* 68, 311–316
47 Wakai, A. *et al.* (2001) Inosine attenuates tourniquet-induced skeletal muscle reperfusion injury. *J. Surg. Res.* 99, 311–315
48 Dowdall, J.F. *et al.* (2002) Inosine modulates gut barrier dysfunction and end organ damage in a model of ischemia-reperfusion injury. *J. Surg. Res.* 108, 61–68
49 Yao, Y.M. *et al.* (1995) The potential etiologic role of tumor necrosis factor in mediating multiple organ dysfunction in rats following intestinal ischemia-reperfusion injury. *Resuscitation* 29, 157–168
50 Seekamp, A. *et al.* (1993) Requirements for tumor necrosis factor-alpha and interleukin-1 in limb ischemia/reperfusion injury and associated lung injury. *Am. J. Pathol.* 143, 453–463
51 Stone, T.W. (2002) Purines and neuroprotection. *Adv. Exp. Med. Biol.* 513, 249–280
52 Haun, S.E. *et al.* (1996) Inosine mediates the protective effect of adenosine in rat astrocyte cultures subjected to combined glucose-oxygen deprivation. *J. Neurochem.* 67, 2051–2059
53 Jurkowitz, M.S. *et al.* (1998) Adenosine, inosine, and guanosine protect glial cells during glucose deprivation and mitochondrial inhibition: correlation between protection and ATP preservation. *J. Neurochem.* 71, 535–548
54 Litsky, M.L. *et al.* (1999) Inosine and guanosine preserve neuronal and glial cell viability in mouse spinal cord cultures during chemical hypoxia. *Brain Res.* 821, 426–432
55 Benowitz, L.I. *et al.* (1998) Axon outgrowth is regulated by an intracellular purine-sensitive mechanism in retinal ganglion cells. *J. Biol. Chem.* 273, 29626–29634
56 Petrausch, B. *et al.* (2000) A purine-sensitive pathway regulates multiple genes involved in axon regeneration in goldfish retinal ganglion cells. *J. Neurosci.* 20, 8031–8041
57 Benowitz, L.I. *et al.* (1999) Inosine stimulates extensive axon collateral growth in the rat corticospinal tract after injury. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13486–13490
58 Chen, P. *et al.* (2002) Inosine induces axonal rewiring and improves behavioral outcome after stroke. *Proc. Natl. Acad. Sci. U. S. A.* 99, 9031–9036
59 Reid, P.G. *et al.* (1987) Intravenous infusion of adenosine but not inosine stimulates respiration in man. *Br. J. Clin. Pharmacol.* 23, 331–338
60 Williams, M.H. *et al.* (1990) Effect of inosine supplementation on 3-mile treadmill run performance and VO_2 peak. *Med. Sci. Sports Exerc.* 22, 517–522
61 McNaughton, L. *et al.* (1999) Inosine supplementation has no effect on aerobic or anaerobic cycling performance. *Int. J. Sport Nutr.* 9, 333–344
62 Spitsin, S. *et al.* (2001) Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease. *Mult. Scler.* 7, 313–319
63 Hooper, D.C. *et al.* (1998) Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 95, 675–680
64 Virag, L. and Szabo, C. (2001) Purines inhibit poly(ADP-ribose) polymerase activation and modulate oxidant-induced cell death. *FASEB J.* 15, 99–107
65 Virag, L. and Szabo, C. (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol. Rev.* 54, 375–429