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Review Article

Adenosine receptor agonists: novel antiinflammatory therapy whose time has come

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Introduction

Adenosine is a purine nuceloside found in body fluids such as plasma. Besides being part of active molecules such as ATP and ADP, adenosine is a mediator with a set of cell-bound receptors by which it exerts biological activities, including powerful antiinflammatory effects. Most cells and organs express adenosine receptors. Currently, 3 types of adenosine receptors, called A_1, A_2 and A_3, have been identified and characterized by the specific structures of agonists and antagonists, by biological activities, and by binding coefficients.

History of adenosine biology

In 1929, Drury and Szent-Gyorgi observed the effects of AMP and adenosine on heart and circulation *in vivo*. In several species they described bradycardia and peripheral vasodilation, which are the main physiological effects of intravenous adenosine administration. In their publication, Drury and Szent-Gyorgi hypothesized that the effects of adenosine could be extended to cell function in general, and not limited to heart tissue, a truly prescient prediction (1). By working with chemical analogs, they determined that both the 6-amino group of the purine base and the ribose moiety were essential for agonist activities, thereby describing the earliest structure-activity relationship for adenosine analogs (1). In 1970, it was observed that cAMP accumulated in isolated brain slices following treatment with adenosine. The effect of adenosine could be inhibited in a concentration-dependent manner by theophylline, thereby demonstrating the existence of specific adenosine receptors (2).

The discovery in 1972 that one form of severe combined immunodeficiency (SCID) in humans was due to lack of adenosine deaminase (ADA) resulted in experiments to determine the immunomodulatory role, if any, of adenosine (3). Investigators noted that substrates for ADA, such as adenosine, accumulated in the urine and plasma of ADA-SCID patients (4). ADA-SCID patients are immunosuppressed because they lack T-cells, which both orchestrate the immune response and effect cellular immunity as well. As time goes on, ADA-SCID patients lose B-cells (antibody-producing cells) as well (that is why it is called “combined” immunodeficiency). Scientists discovered that adenosine and other ADA substrates inhibited lymphocyte proliferation *in vitro*, thereby halting clonal expansion of the immune repertoire, as well as suppressing an ongoing immune response (5). Of the ADA substrates examined, the most potent inhibitor of T-cell proliferation is deoxyadenosine (6).

In the late 1980s, interest in the effects of adenosine on immune function shifted from lymphocytes to neutrophils and other granulocytes with the observation that adenosine inhibited superoxide anion (O_2-) production stimulated by chemoattractants such as the formylated tripeptide f-Met-Leu-Phe (7). It was later shown that adenosine inhibited the respiratory burst of neutrophils mediated by the enzymes of the hexose-monophosphate shunt, therefore inhibiting the generation of all reactive oxygen intermediates: H_2O_2, OH^-, singlet O and O_2^- (8, 9). At the same time, scientists found that adenosine inhibited both leukotriene release and IgE-dependent histamine release from human basophils (10, 11).

Receptor biology and pharmacology

There are 3 known types of adenosine receptors, as elucidated by studies with analog compounds. The receptors mediate diverse biological effects, even though some of them are expressed on the same cell. Neutrophils express both A_1 and A_2 receptors; when neutrophils were exposed to low concentrations (EC_{50} = 9 pM) of 5’*N*-ethylcarboxamidoadenosine (NECA) their chemotactic and oxidative responses (O_2^- production) to stimuli were enhanced; however, at higher concentrations of NECA, these responses were abolished (EC_{50} = 17 nM). Therefore, NECA was shown to be both an A_1 and an A_2

agonist. Another analog, N^6-cyclopentyladenosine (CPA), was observed to stimulate neutrophils at low concentrations but did not inhibit activation at higher concentrations; thus, this compound is a specific A_1 agonist. Treatment of neutrophils with pertussis toxin or colchicine completely abolished the enhancement of stimulation of chemotaxis by CPA, thereby showing the A_1 receptor signaled through a G-linked protein and that microtubule assembly is required for the activation response. Treatment with these 2 agents enhanced the inhibition of O_2^- production by NECA, therefore showing that the response signaled through the A_2 receptor is independent of microtubule assembly and is mediated by a different class of G-linked proteins (12). In fact, A_2 receptor occupancy was found to uncouple the Gαs-linked protein signal from the chemoattraction receptors (13, 14). In parallel studies, investigators showed that Fcγ receptor-mediated responses of human neutrophils were modulated by NECA and CPA: NECA inhibited Fcγ receptor-mediated phagocytosis, while CPA enhanced it (15). Further studies in an *in vivo* inflammatory pleurisy model revealed that theophylline and related methylxanthines are A_1 antagonists (16); this is the probable mechanism of action of theophylline in asthma. Finally, researchers demonstrated that the therapeutic effect of methotrexate in the treatment of rheumatoid arthritis, for which methotrexate is given at 1/100th the cancer chemotherapeutic dose, is due to A_2 agonist activity (17-19).

The modulatory effects of adenosine on inflammatory responses is not limited to neutrophils. There is a third type of receptor, A_3, which is found on human basophil leukocytes. Occupancy of the A_3 receptor has been shown to inhibit IgE-mediated histamine release (10) as well as leukotriene release (11). The analogs 2-chloroadenosine and NECA were demonstrated to be more potent than adenosine in inhibiting histamine release (11), which is a similar pharmacological profile to the A_2 receptor. This receptor on basophils was shown to be distinct from the A_2 receptor by the competitive inhibition of A_3-mediated responses by methylxanthines and by the sensitivity of the response to cholera toxin (20). The A_3 receptor is apparently the only adenosine receptor subtype expressed on basophils and mast cells; its occupancy modulates the release of allergic mediators by degranulation (21).

All 3 receptor subtypes have been cloned and expressed (22-28), which should ease the path for drug discovery.

From the above discussion it is clear that compounds which engage A_2 and A_3 receptors, or block A_1 receptors, should be therapeutically useful. As mentioned above, systemic administration of adenosine resulted in bradycardia and other undesirable cardiovascular effects (1). There are several other pharmacological routes to the same end which could lessen the side effects associated with straight adenosine administration. Compounds which increase the local adenosine concentration theoretically would have as potent antiinflammatory effects as A_2 agonists. Such compounds could include adenosine kinase inhibitors and phosphoribosylaminoimidazolecarboxamide (AICAR) transformylase inhibitors. Methotrexate, which has potent therapeutic effects for rheumatoid arthritis patients with just a single dose, has been shown to work by increasing the local concentrations of adenosine at the sites of inflamed joints due to its activity as an AICAR transformylase inhibitor (19, 29). Sulfasalazine, an antibiotic used in the treatment of inflammatory bowel disease, has recently been demonstrated to increase adenosine concentrations by AICAR transformylase as well (30). GP-1-515, a recently synthesized adenosine kinase inhibitor, was shown to inhibit neutrophil-mediated inflammation *in vivo* (31). A series of potent, orally active adenosine kinase inhibitors was recently synthesized. These 5-iodotubercidin analogs were shown to be competitive inhibitors of the target enzyme and hold promise as antiinflammatory agents for the clinic (32).

Antiinflammatory activities of adenosine

Almost all inflammatory activities of neutrophils have been shown to be inhibited by A_2 agonism. Besides oxidative metabolism (8, 9, 33, 34), adherence (35, 36) was shown to be inhibited by downregulation of beta$_2$-integrin expression (36, 37). Neutrophil degranulation was shown to be inhibited by adenosine acting on both A_2 and A_3 receptors, thus demonstrating the presence of A_3 receptors on neutrophils (38). Nonneutrophil-mediated inflammatory events modulated by adenosine include inflammatory cytokine release. 3-Deazaadenosine was shown to inhibit IL-1 production by human peripheral blood monocytes (39). TNF-α production by human monocytes is also inhibited by adenosine, and this is mediated by the A_3 receptor (40), while IL-1 release is modulated by the A_2 receptor (39). The possible mechanism of TNF-α inhibition mediated by the A_3 receptor could be the enhanced production of IL-10 by A_3 agonism, as IL-10 is a potent inhibitor of inflammatory cytokine production (41, 42). Adenosine has been shown to increase IL-6 release (43); IL-6, like IL-10, inhibits inflammatory cytokine production and also antagonizes the effects of inflammatory cytokines *in vivo* (41, 44-46). The antiinflammatory effect of adenosine on cytokines has been shown by the ability of adenosine and analogs to enhance survival in various lethal shock models (47, 48). Such models exaggerate the inflammatory effects of cytokines and therefore are useful for rapid analysis of antiinflammatory agents.

Conclusions

It has been established by the work cited above that adenosine is a potent antiinflammatory agent, the biology of which has yielded valuable insight into novel antiinflammatory mechanisms. Future work focusing on the

best way to bring adenosine to sites of inflammation, or on ways to agonize A_2 and A_3 receptors while antagonizing A_1 receptors, should give rise to useful therapeutic agents. Targeted diseases should include rheumatoid arthritis, inflammatory bowel disease, psoriasis, asthma, inflammatory lung diseases, sepsis and others.

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