Dissociating the effects of A_{2A} agonism

By Kai-Jye Lou, Staff Writer

The vasodilatory effects of adenosine A_{2A} receptor agonists make them useful as cardiac stress agents in imaging but have made it difficult to exploit these molecules' potent anti-inflammatory effects.¹ Researchers in Germany have dissociated the anti-inflammatory effects of adenosine A_{2A} receptor agonists from their hemodynamic effects and are determining a specific inflammation-related indication to pursue with the molecule.²

Activation of the adenosine A_{2A} receptor (ADORA_{2A}) on endothelial cells promotes vasodilation, and activation of the receptor on immune cells inhibits inflammatory processes. The vasodilatory effects become dose-limiting side effects in the inflammation setting by causing hypotension, which in turn triggers a compensatory increase in heart rate and cardiac output.

Thus, Jürgen Schrader and colleagues at the **Heinrich Heine University of Duesseldorf** and the **University of Bonn** have been looking at the endogenous adenosine signaling pathway for molecular targets that can be exploited to dissociate the vasodilatory effects of ADORA_{2A} agonists from their anti-inflammatory effects.

The search led the group to ecto-5'-nucleotidase (NT5E; NT; CD73), which dephosphorylates adenosine monophosphate (AMP) to form adenosine^{3,4} (see Figure 1, "Effects of adenosine A_{2A} receptor agonism").

Schrader, a professor of physiology and head of the Department of Molecular Cardiology at the Heinrich Heine University of Duesseldorf, said the group was drawn to CD73 because of the enzyme's expression under normal and inflammatory conditions.

"We've previously observed that whenever there is inflammation, immune cells dramatically increase their expression of both CD73 and the A_{2A} receptor," he told *SciBX*. "So our idea is why not make a phosphorylated A_{2A} receptor agonist prodrug that remains biologically inactive until it is locally activated by CD73 at sites of inflammation."

In 2009, the researchers published data on a series of AMP derivatives and showed *in vitro* that the molecules were accepted as substrates by mouse Cd73 and dephosphorylated into the corresponding ADORA_{2A} agonist.⁵

Now, the group has shown *in vivo* that one of the lead derivatives from the 2009 study, 2-cyclohexylethylthio-AMP (chet-AMP), is selectively activated at sites of inflammation and has negligible vasodilatory effects.

In mouse models of collagen-induced arthritis, delivery of chet-AMP via implanted osmotic minipumps decreased joint inflammation compared with no treatment. Importantly, plasma chet-AMP concentrations that had an anti-inflammatory effect were in the subnanomolar range and about 100-fold lower than the concentrations required for vasodilation.

Results were published in *Science Translational Medicine*.

Inflammatory history lessons

The prodrug strategy could help overcome a key barrier that has caused some ADORA_{2A} agonists to flounder in the inflammation setting.

"The hemodynamic effects of A_{2A} receptor agonists limit their dosing to the point where they are no longer effective at resolving inflammation, and this has contributed to the failure of several such compounds," said Schrader.

Examples of discontinued ADORA_{2A} agonists include GW328267X from **GlaxoSmithKline plc** and UK-432097 from **Pfizer Inc.** Both compounds were being developed to treat chronic obstructive pulmonary disorder (COPD) but were discontinued in Phase II trials due to poor efficacy.

At least two ADORA_{2A} agonists are still in clinical trials for inflammation-related indications. BVT.115959, which is being developed by **CBT Development Ltd.**, **Ergomed Group** and **Swedish Orphan Biovitrum AB**, is in Phase II testing to treat diabetic neuropathic pain. Lexiscan regadenoson is being evaluated in an investigator-led Phase I trial to treat sickle cell disease-associated pain crisis.

Holger Eltzschig, a professor of anesthesiology, medicine, cell biology and immunology at the **University of Colorado Denver School of Medicine**, said targeting ADORA_{2A} with a specific agonist could have an advantage over drugs that work via adenosine liberation, such as methotrexate. The specific compounds, he said, could be more effective at activating the receptor and may not be affected by pathways that rapidly shut down adenosine signaling.

Gilead Sciences Inc. and **Astellas Pharma Inc.** already market Lexiscan as a cardiac stress agent for use in myocardial perfusion imaging (MPI).

Despite the multitude of ADORA_{2A} agonists, Schrader cautioned that the group's prodrug strategy cannot be generalized to any ADORA_{2A} agonist. "The phosphorylated prodrug needs to be compatible as a substrate for CD73," he told *SciBX*.

For your inflammation

Schrader said the group's key step forward will be to select an appropriate inflammation-related indication to pursue with chet-AMP.

Bruce Cronstein, a professor in the Department of Medicine and Pathology and the Department of Biochemistry and Molecular Pharmacology at the **New York University Langone Medical Center**, thinks the ADORA_{2A} agonist prodrug could be applied in chronic inflammation settings because it may be selectively activated at sites of inflammation.

"If we could show in these other disease models that our A_{2A} receptor prodrug works equally well as known A_{2A} receptor agonists but without the hemodynamic side effects, it could considerably broaden its application in inflammation."

**—Jürgen Schrader,
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Figure 1. Effects of adenosine A_{2A} receptor agonism. Activation of the adenosine A_{2A} receptor (ADORA_{2A}) is known to both inhibit inflammatory processes and promote vasodilation. The cell surface enzyme ecto-5'-nucleotidase (NT5E; NT; CD73) is highly expressed by immune cells and endothelial cells and is one of the central regulators of endogenous adenosine signaling. CD73 dephosphorylates adenosine monophosphate (AMP) to form adenosine.

(I) In immune cells, adenosine signaling through ADORA_{2A} inhibits proinflammatory processes.

(II) In endothelial cells, adenosine signaling through ADORA_{2A} promotes vasodilation.

In Flögel *et al.*, researchers showed that their ADORA_{2A} agonist prodrug is selectively activated at sites of inflammation, at which CD73 is highly upregulated. Thus, the molecule is able to exert potent anti-inflammatory effects at doses that do not also cause vasodilation.

Cronstein suggested that the researchers develop their compound in indications for which marketed anti-inflammatory drugs are not particularly effective. "Where they may want to head is to conditions such as psoriatic arthritis and ankylosing spondylitis," he told *SciBX*.

Joel Linden, professor of inflammation biology at the **La Jolla Institute for Allergy & Immunology**, liked the idea that chet-AMP could be used to create high local concentrations of an active ADORA_{2A} agonist at sites of inflammation and thinks it is a clever and appealing approach to limit the exposure of other tissues to the active form of the compound.

However, he noted that because the prodrug compound is phosphorylated, it would most likely need to be delivered intravenously and in a hospital setting, which could make it difficult to develop for chronic inflammatory conditions.

Linden therefore thinks chet-AMP would be best suited for acute

inflammation, such as treating acute flare-ups in patients with rheumatoid arthritis (RA).

Linden wanted to see additional examples of ADORA_{2A} agonists that incorporate the CD73-activated prodrug strategy described by the researchers. He added that it also will be a good idea to determine whether chet-AMP could be formulated for oral delivery, or as a subcutaneous injection or implant.

Cronstein added that it will be important to show that chet-AMP is a substrate for human CD73 and to demonstrate that there would be no acute effects on blood pressure and bleeding in humans.

Finally, Eltzschig wanted to know whether the prodrug strategy could be applied to an ADORA_{2B} agonist, as such compounds also are known to have potent anti-inflammatory effects.

Schrader said the group now is looking at CD73 and ADORA_{2A} expression patterns in mouse models of other inflammation-related conditions, such as ischemia/reperfusion injury and sepsis, to help determine whether chet-AMP could be useful in such settings.

"If we could show in these other disease models that our A_{2A} receptor prodrug works equally well as known A_{2A} receptor agonists but without the hemodynamic side effects, it could considerably broaden its application in inflammation," he told *SciBX*.

The Heinrich Heine University of Duesseldorf and the University of Bonn have cofiled a patent covering phosphorylated ADORA_{2A} agonists. The work is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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