Targeting Purinergic Signaling for Perioperative Organ Protection

ADVANCEMENTS in the field of anesthesia have led to significant improvements in our monitoring capabilities, safer anesthetic drugs, and improved pain control. Despite this remarkable progress, acute organ injury remains one of the leading causes for morbidity and mortality in surgical patients. Patients who require a major surgical intervention—such as cardiothoracic, vascular, general surgery, or solid organ transplantation—may have had a "perfect" operation but are threatened during their perioperative course by the development of acute organ injury. For example, acute kidney injury, myocardial infarction, intestinal ischemia, and reperfusion injury or stroke are some of the threats that are among the leading causes of perioperative morbidity and mortality.[1] A recent review article published in the *New England Journal of Medicine* discussed "Purinergic Signaling during Inflammation,"[2] provides an update on how signaling events through extracellular receptors for the purines adenosine triphosphate (ATP) and adenosine can be targeted to alter inflammatory endpoints. As discussed in this review article, recent studies implicate purinergic signaling *via* ATP or adenosine receptors as important therapeutic targets to prevent or treat acute organ injury in perioperative patients.

In many instances, perioperative organ injury is caused by ischemia and concomitant hypoxia-induced inflammation.[3] Hypoxia and inflammation share an interdependent relationship, where inflammatory diseases such as intestinal inflammation or acute lung injury can cause inflamed areas to become severely hypoxic. This typically occurs because of increased metabolic demand of resident and inflammatory cells while metabolic supply is simultaneously decreased. However, diseases that are attributed primarily to hypoxia and ischemia will undergo secondary inflammatory changes that—in the context of ischemia and reperfusion injury—contribute significantly to organ injury (fig. 1).[1]

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During hypoxic or inflammatory disease states, many cells release ATP from their intracellular toward the extracellular compartment. In the extracellular space, ATP can activate extracellular ATP receptors, which are classified as P2 receptors. They function as G-protein coupled receptors (P2Y receptors) or as ligand-gated ion channels (P2X receptors). Activation of P2 receptors has been shown to cause inflammatory activation and organ injury during ischemia or inflammation, and therapeutic strategies to dampen ATP receptor activation have been implicated in the treatment of inflammatory diseases.[4–6]

In the extracellular compartment, ATP is rapidly converted to adenosine. In contrast to the proinflammatory functions of ATP receptors, adenosine receptors have been shown to attenuate hypoxic inflammation,[7] provide metabolic tissue adaptation to increase ischemia tolerance,[8] and to promote injury resolution.[9–11] Therefore, treatment approaches to enhance ATP conversion to adenosine represents an experimental therapeutic strategy to dampen acute organ injury. Conversion of ATP to adenosine is controlled by a two-step enzymatic system. The first step is the conversion of ATP to adenosine monophosphate by the ectonucleoside triphosphate diphosphohydrolase 1 (CD39). The subsequent second step in the extracellular generation of adenosine is catalyzed by the ecto-5′-nucleotidase CD73. Conditions of inflammatory hypoxia result in the induction of both enzymes (fig. 2). CD39 is transcriptionally induced by the Sp1 transcription factor,[12] whereas CD73 is induced by hypoxia-inducible factor (HIF)—the key transcription factor for hypoxia adaptation.[13,14] For example, HIF is known to regulate the transcriptional activity of the erythropoietin promoter. Conditions of hypoxia, ischemia, or anemia will lead to tissue hypoxia and subsequent stabilization of HIF. Binding of HIF to the erythropoietin promoter results in transcriptional induction of erythropoietin, leading to increases in erythropoiesis. Indeed, HIF activators have been implicated in treating inflammatory hypoxia *via* increasing tissue expression of CD73, thereby promoting the extracellular generation of adenosine.[5]

Once generated in the extracellular compartment, adenosine can activate four distinct adenosine receptors. These

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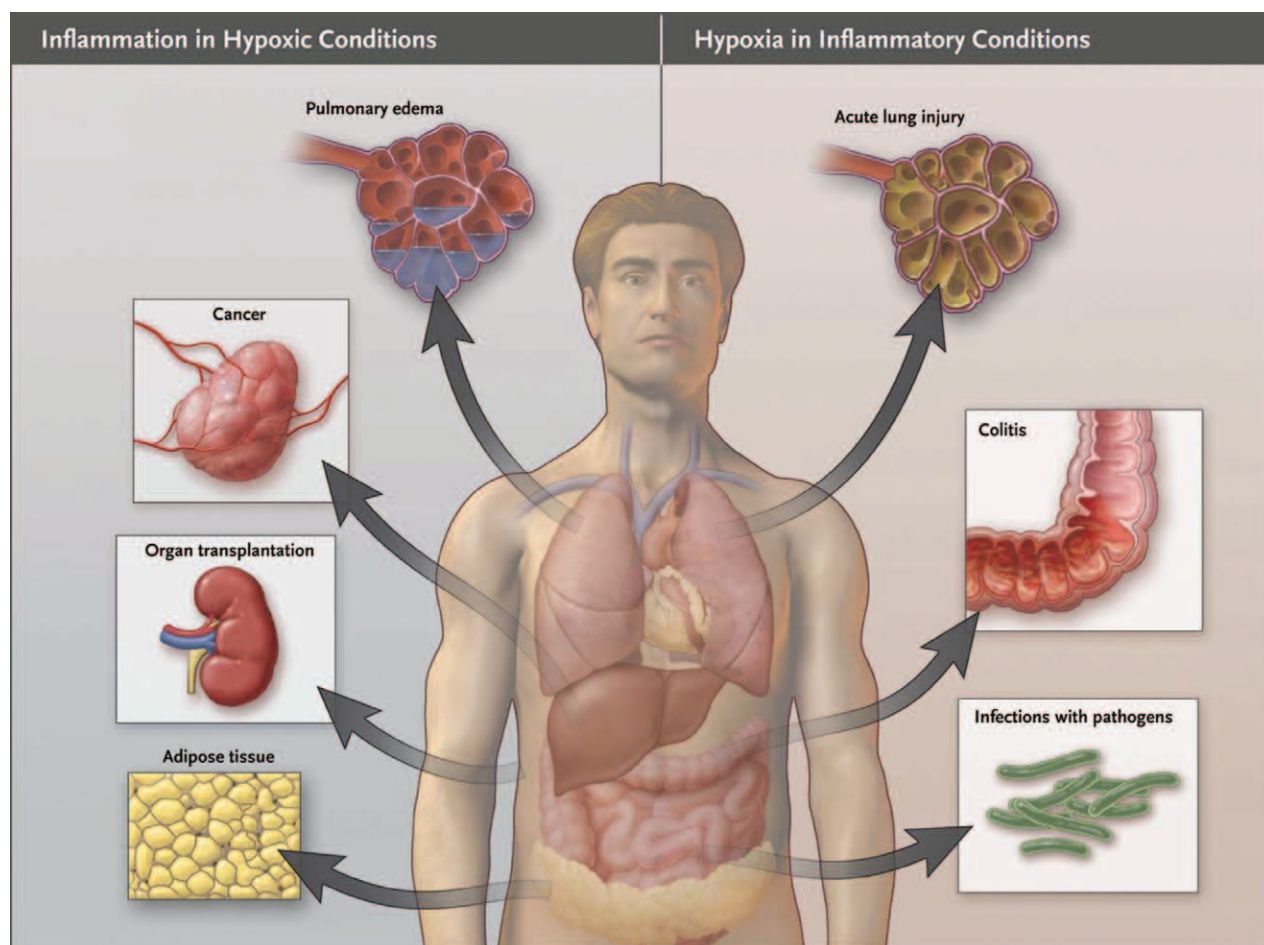
Fig. 1. Links between hypoxia and inflammation. Shown is an overview of clinical conditions characterized primarily by tissue hypoxia that causes inflammatory changes (*left*) and inflammatory diseases leading to tissue hypoxia (*right*). From Eltzschig HK, Carmeliet P[3] Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

are G-protein coupled receptors that differ in their sensitivity for adenosine, and the type of second message response they elicit. The Adora1 and Adora3 are known to inhibit adenylate cyclase, thereby leading to decreased intracellular cyclic adenosine monophosphate levels. In contrast, the Adora2a and Adora2b are known to activate adenylate cyclase and thereby increase cyclic adenosine monophosphate levels.[15] In the context of organ protection, activation of Adora2a receptors expressed on inflammatory cells has been shown to provide tissue protection. Moreover, the Adora2b receptor is transcriptionally induced by HIF during conditions of ischemia or hypoxia,[16] and has been shown to protect the tissues from the detrimental effects of hypoxia-driven inflammation (fig. 2).

In summary, current experimental evidence indicates that purinergic signaling events can be targeted to treat or prevent acute organ injury such as occurs during the perioperative period. These studies suggest the following pharmacologic approaches:

- Inhibition of signaling events through ATP receptors (*e.g.* P2Y or P2X receptors) by ATP receptor antagonists.

- Pharmacologic approaches that promote the extracellular conversion of ATP to adenosine, such as apyrase treatment (conversion of ATP to adenosine monophosphate) or nucleotidase treatment (conversion of adenosine monophosphate to adenosine). Several other pharmacologic compounds that are used in the treatment of inflammatory diseases (*e.g.* methotrexate or sulfasalazine) are known to exhibit their antiinflammatory properties by stimulating CD73-dependent adenosine production.

- Pharmacologic approaches to activate adenosine receptors, particularly the Adora2a or the Adora2b. For example, a highly selective Adora2b agonist has recently been characterized to function in murine or human tissues.[8,17] This compound (BAY 60–6483) has been shown to have organ-protective functions in a wide range of disease models from the field of ischemia and reperfusion injury, for example intestinal ischemia[13,18] or renal ischemia.[10,11]

- Pharmacologic approaches to indirectly increase extracellular adenosine signaling effects. This can be achieved
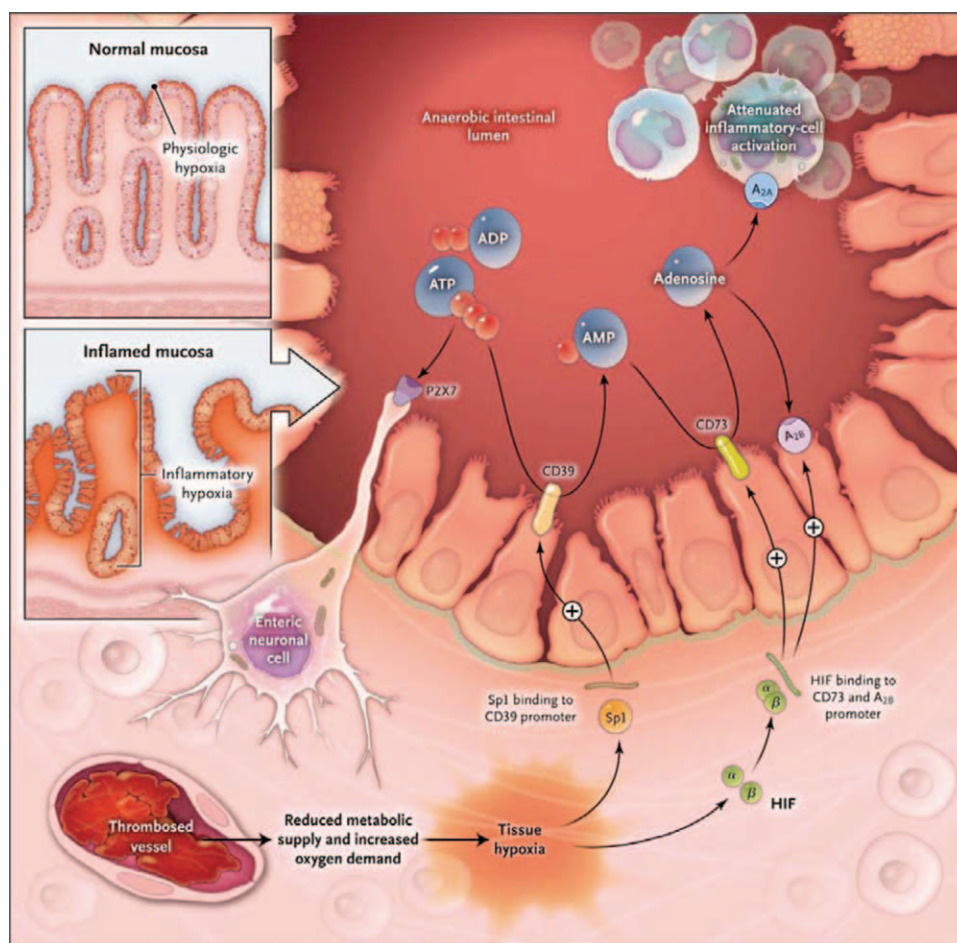
Fig. 2. Hypoxia control of extracellular adenosine generation and signaling. Histologic staining of intestinal sections for hypoxia shows that hypoxia is present within the apical surface of the intestinal mucosa (*orange* area in upper insert). This presence is most likely due to the fact that the intestinal lumen is anaerobic, which results in a steep oxygen gradient across the epithelial monolayer. In patients with intestinal inflammation such as that which occurs in the course of inflammatory bowel disease, a decrease in metabolic supply (*e.g.,* due to thrombosed vessels) and profound increases in oxygen demand result in an imbalance in oxygen availability. This imbalance causes severe hypoxia of the inflamed mucosa, as indicated by histologic staining for tissue hypoxia (as shown in the lower insert, the *orange* staining that extends from the apical aspects of the mucosa into the crypts and submucosal tissues indicates severe tissue hypoxia). Release of adenosine triphosphate (ATP) or adenosine diphosphate (ADP) from inflammatory cells, platelets, or epithelial cells results in the activation of P2 receptors such as the P2X7 receptor, expressed on enteric neurons, thereby promoting tissue inflammation and injury. Hypoxia causes the activation of transcriptional programs that result in an Sp1-dependent induction of CD39[17] and a hypoxiainducible factor (HIF)–dependent induction of CD73[25] and the ADORA2B (A2B) adenosine receptor. These transcriptional changes lead to an increased rate of turnover of the extracellular nucleotides ATP and ADP to adenosine monophosphate (AMP) (through CD39) and subsequently to adenosine (through CD73). Experimental studies indicate that adenosinereceptor activation—particularly through Adora2a (A2A) and A2B—dampens hypoxia-induced inflammation and promotes tissue integrity. From Eltzschig HK, Sitkovsky MV, Robson SC[2] Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

for example by adenosine transport inhibitors[19] or by inhibitors of the metabolic breakdown of adenosine.[20]

- Pharmacologic strategies to enhance transcriptional pathways that result in increased ATP to adenosine turnover and increased expression of adenosine receptors. This can be achieved for example by pharmacologic activators of the transcription factor HIF.

Most of these pharmacologic interventions have been identified and described in animal models. However, several pharmacologic agents that modulate purinergic signaling are being used in patients. For example, the antiinflammatory medications methotrexate or sulfasalazine are known to increase extracellular adenosine levels *via* activation of CD73. Similarly, the adenosine uptake inhibitor dipyridamole is used in patients during stress echocardiography. Finally, clinical studies have used HIF activators in patients without safety concerns.[21] As such, we are hoping that several of these pharmacologic interventions can be used for the prevention of organ injury in surgical patients in the near future. However, efforts to go forward with translational studies, such as

clinical randomized trials in perioperative patients will be required to make progress on this front.

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References

1. Eltzschig HK, Eckle T: Ischemia and reperfusion–from mechanism to translation. Nat Med 2011; 17:1391–401

2. Eltzschig HK, Sitkovsky MV, Robson SC: Purinergic signaling during inflammation. N Engl J Med 2012; 367:2322–33

3. Eltzschig HK, Carmeliet P: Hypoxia and inflammation. N Engl J Med 2011; 364:656–65

4. Gulbransen BD, Bashashati M, Hirota SA, Gui X, Roberts JA, MacDonald JA, Muruve DA, McKay DM, Beck PL, Mawe GM, Thompson RJ, Sharkey KA: Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. Nat Med 2012; 18:600–4

5. Colgan SP, Eltzschig HK: Adenosine and hypoxia-inducible factor signaling in intestinal injury and recovery. Annu Rev Physiol 2012; 74:153–75

6. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F, Hoogsteden HC, Luttmann W, Ferrari D, Di Virgilio F, Virchow JC Jr, Lambrecht BN: Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. Nat Med 2007; 13:913–9

7. Rosenberger P, Schwab JM, Mirakaj V, Masekowsky E, Mager A, Morote-Garcia JC, Unertl K, Eltzschig HK: Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. Nat Immunol 2009; 10:195–202

8. Eckle T, Hartmann K, Bonney S, Reithel S, Mittelbronn M, Walker LA, Lowes BD, Han J, Borchers CH, Buttrick PM, Kominsky DJ, Colgan SP, Eltzschig HK: Adora2b-elicited Per2 stabilization promotes a HIF-dependent metabolic switch crucial for myocardial adaptation to ischemia. Nat Med 2012; 18:774–82

9. Eckle T, Grenz A, Laucher S, Eltzschig HK: A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. J Clin Invest 2008; 118:3301–15

10. Grenz A, Bauerle JD, Dalton JH, Ridyard D, Badulak A, Tak E, McNamee EN, Clambey E, Moldovan R, Reyes G, Klawitter J, Ambler K, Magee K, Christians U, Brodsky KS, Ravid K, Choi DS, Wen J, Lukashev D, Blackburn MR, Osswald H, Coe IR, Nürnberg B, Haase VH, Xia Y, Sitkovsky M, Eltzschig HK: Equilibrative nucleoside transporter 1 (ENT1) regulates post-ischemic blood flow during acute kidney injury in mice. J Clin Invest 2012; 122:693–710

11. Grenz A, Osswald H, Eckle T, Yang D, Zhang H, Tran ZV, Klingel K, Ravid K, Eltzschig HK: The reno-vascular A2B adenosine receptor protects the kidney from ischemia. PLoS Med 2008; 5:e137

12. Eltzschig HK, Köhler D, Eckle T, Kong T, Robson SC, Colgan SP: Central role of Sp1-regulated CD39 in hypoxia/ischemia protection. Blood 2009; 113:224–32

13. Hart ML, Grenz A, Gorzolla IC, Schittenhelm J, Dalton JH, Eltzschig HK: Hypoxia-inducible factor-1a-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. J Immunol 2011; 186:4367–74

14. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, Hansen KR, Thompson LF, Colgan SP: Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. J Clin Invest 2002; 110:993–1002

15. Eltzschig HK: Adenosine: An old drug newly discovered. Anesthesiology 2009; 111:904–15

16. Eckle T, Köhler D, Lehmann R, El Kasmi K, Eltzschig HK: Hypoxia-inducible factor-1 is central to cardioprotection: A new paradigm for ischemic preconditioning. Circulation 2008; 118:166–75

17. Eckle T, Krahn T, Grenz A, Köhler D, Mittelbronn M, Ledent C, Jacobson MA, Osswald H, Thompson LF, Unertl K, Eltzschig HK: Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. Circulation 2007; 115:1581–90

18. Hart ML, Jacobi B, Schittenhelm J, Henn M, Eltzschig HK: Cutting Edge: A2B Adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury. J Immunol 2009; 182:3965–8

19. Morote-Garcia JC, Rosenberger P, Nivillac NM, Coe IR, Eltzschig HK: Hypoxia-inducible factor-dependent repression of equilibrative nucleoside transporter 2 attenuates mucosal inflammation during intestinal hypoxia. Gastroenterology 2009; 136:607–18

20. Morote-Garcia JC, Rosenberger P, Kuhlicke J, Eltzschig HK: HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak. Blood 2008; 111:5571–80

21. Bernhardt WM, Wiesener MS, Scigalla P, Chou J, Schmieder RE, Günzler V, Eckardt KU: Inhibition of prolyl hydroxylases increases erythropoietin production in ESRD. J Am Soc Nephrol 2010; 21:2151–6