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Targeting the A2B adenosine receptor during gastrointestinal ischemia and inflammation

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Abstract

Extracellular adenosine functions as an endogenous distress signal via activation of four distinct adenosine receptors (A1, A2A, A2B and A3). Conditions of limited oxygen availability or acute inflammation lead to elevated levels of extracellular adenosine and enhanced signaling events. This relates to a combination of four mechanisms: i) increased production of adenosine via extracellular phosphohydrolysis of precursor molecules (particularly ATP and ADP); ii) increased expression and signaling via hypoxia-induced adenosine receptors, particularly the A2B adenosine receptor; iii) attenuated uptake from the extracellular towards the intracellular compartment; and iv) attenuated intracellular metabolism. Due to their large surface area, mucosal organs are particularly prone to hypoxia and ischemia associated inflammation. Therefore, it is not surprising that adenosine production and signaling plays a central role in attenuating tissue inflammation and injury during intestinal ischemia or inflammation. In fact, recent studies combining pharmacological and genetic approaches demonstrated that adenosine signaling via the A2B adenosine receptor dampens mucosal inflammation and tissue injury during intestinal ischemia or experimental colitis. This review outlines basic principles of extracellular adenosine production, signaling, uptake and metabolism. In addition, we discuss the role of this pathway in dampening hypoxia-elicited inflammation, specifically in the setting of intestinal ischemia and inflammation.

Keywords

A2B; adenosine kinase; adenosine receptor; CD39; CD73; ecto-5-nucleotidase; ecto-apyrase; equilibrative nucleoside transporter; inflammation; ischemia

1. Introduction

Transient abdominal ischemia caused by surgery, organ transplantation and spontaneous ischemia leads to profound functional and structural alterations of the gastrointestinal tract. Although restoration of blood flow to an ischemic organ is essential to prevent irreversible tissue injury, reperfusion augments injury by causing destruction of vascular integrity, tissue edema and disturbances in cellular energy balance [1]. Clinically, ischemia/reperfusion (IR) injury of the intestine is a significant problem during surgery for abdominal aortic aneurysm, small bowel transplantation, cardiopulmonary bypass, strangulated hernias and neonatal necrotizing enterocolitis [2]. Intestinal IR can proceed to a systemic response and may result in bacterial translocation, endotoxemia, acute respiratory distress syndrome or acute hepatic injury [3,4].

Like ischemic episodes, intestinal inflammation is associated with tissue hypoxia. Indeed, sites of intestinal inflammation are characterized by significant changes in metabolic activity. Shifts in energy supply and demand can result in diminished delivery and/or availability of oxygen, leading to inflammation-associated tissue hypoxia, termed 'inflammatory hypoxia' [5–8]. As an example, models of murine colitis have provided compelling evidence that particularly the mucosal surface (especially the intestinal epithelium) is prone to significant drops in pO_2 and resulting inflammatory hypoxia [6,7]. As a result, studies of hypoxia signaling and pharmacological targeting of hypoxia-dependent signaling pathways has become an area of intense investigation in diseases such as inflammatory bowel disease (IBD).

Previous studies have shown that the endogenous signaling molecule adenosine plays a critical role in attenuating inflammatory hypoxia of mucosal organs [9–14]. During conditions of limited oxygen availability, extracellular adenosine signaling is enhanced and mainly stems from enzymatic phosphohydrolysis of its extracellular precursor molecules (ATP, ADP or AMP) [15–19]. Functional studies of extracellular adenosine signaling during inflammatory hypoxia have demonstrated attenuation of vascular leakage [15,17,20,21], inflammatory cell accumulation [16], myocardial infarction [19,22,23], acute lung injury [24,25] intestinal inflammation [9,26] liver [27] or gut ischemia [28,29]. Extracellular adenosine can signal through four different adenosine receptors (AR), the A1, A2A, A2B and A3AR. Of these four adenosine receptors, the A2B adenosine receptor in particular is induced during conditions of limited oxygen availability [15,16,20,29–31], or acute inflammation [32]. While previous reviews have analyzed the current available evidence on the role played by the adenosine system in the pathophysiology of inflammatory conditions [33–35] this review mainly focuses on the role of the A2B adenosine receptor. As such, studies utilizing a combination of pharmacological approaches with A2BAR agonists and antagonist, in conjunction with studies of gene-targeted mice for individual adenosine receptors revealed a protective role of A2BAR signaling in intestinal ischemia [29] or inflammation [26].

2. Extracellular adenosine generation, signaling, uptake and metabolism

In the extracellular compartment, adenosine mainly stems from phosphohydrolysis of precursor molecules. During inflammation or acute hypoxia, multiple cell types release nucleotides (particularly ATP and ADP) into the extracellular space [36,37]. For example, cellular sources for extracellular nucleotide release can include inflammatory cells (neutrophils, lymphocytes and platelets), vascular endothelia and epithelial cells [36–39]. On the extracellular surface, a coordinated two-step enzymatic process leads to the rapid conversion of ATP/ADP to adenosine. The first step includes conversion of extracellular ATP and/or ADP to AMP. Extracellular ATP/ADP-phosphohydrolysis is mainly achieved enzymatically by ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), a recently described family of ubiquitously expressed membrane-bound enzymes [40,41]. The catalytic sites of plasma membrane expressed E-NTPDases 1 – 3 and 8 are exposed to the extracellular milieu, the others are intracellular [41]. The presumptive biological role of plasma-membrane-bound E-NTPDases (E-NTPDase1 – 3 and 8) is to fine-tune extracellular nucleotide levels. In particular E-NTPDase1 (CD39) plays a central role in converting extracellular ATP/ADP to AMP, including on the vascular endothelial surface [15,16], or the epithelial surface of mucosal organs [38,42].

Following conversion of ATP/ADP to AMP via CD39, the second step in extracellular adenosine generation is catalysed by the ecto-5'-nucleotidase (CD73). CD73 is a ubiquitously expressed GPI anchored ecto-enzyme that rapidly facilitates AMP phosphohydrolysis to adenosine. Comparative expression studies identified the highest expressional levels of CD73 in the intestine [17]. More specifically, other studies found that CD73 expression is predominantly localized on the apical surface of intestinal epithelial cells [43]. Studies in gene-targeted mice for CD73 indicated a more severe phenotype during intestinal ischemia [28] or experimental colitis (Figure 1) [9], highlighting its role in attenuating intestinal inflammation in the setting of limited oxygen availability.

Following extracellular generation of adenosine via CD39-and CD73-dependent nucleotide phosphohydrolysis, extracellular adenosine can exert its signaling effects via four different adenosine receptors (AR; A1AR, A2AAR, A2BAR, and A3AR) [44]. These receptors are G-protein-coupled and have been characterized via their potential to alter intracellular cAMP levels [45–47]. Activation of the A1AR and the A3AR result in attenuated intracellular cAMP levels, whereas activation of the A2AAR or A2BAR result in increased cAMP levels [46,47]. Consistent with this notion, signaling events through the A2AAR and A2BAR have been described in tissue protection from inflammation or hypoxia [12–14,20,22,31,32,48–52]. Moreover, recent studies identified a central role of A2B signaling in attenuating intestinal inflammation and tissue injury during murine intestinal ischemia [29] or experimental colitis [26].

Extracellular adenosine has a very short half-life on the extracellular surface. This is due to rapid uptake from the extracellular compartment towards the intracellular space. This is mainly achieved via equilibrative nucleoside transporters (ENTs). These are channels that allow adenosine to cross the cell membrane following its concentration-dependent gradient. During conditions of hypoxia, ischemia or inflammation, extracellular adenosine levels are

dramatically elevated and the flow through ENTs is directed from the extracellular compartment towards the intracellular space [53]. Following uptake into the intracellular compartment, adenosine is rapidly converted to AMP via the adenosine kinase [54], or via deamination to inosine via the adenosine deaminase [55]. Taken together, extracellular adenosine generation, signaling, uptake and metabolism represent different aspects of an enzymatically controlled, and transcriptionally regulated metabolic pathway process.

3. Consequences of hypoxia on extracellular adenosine

Previous studies had suggested that extracellular adenosine signaling is particularly important in adapting different cell types to limited oxygen availability (hypoxia) [19,22,27,52,56,57] and in attenuating hypoxia-associated inflammation [51,58]. Therefore, increases in extracellular adenosine production and signaling during hypoxia represent an endogenous pathway to attenuate hypoxia-elicited inflammation. As such, ambient hypoxia regulates extracellular adenosine levels and signaling effects at multiple levels. As a first step, CD39-dependent ATP/ADP phosphohydrolysis is significantly enhanced by hypoxia [15,59], and CD39 transcript and protein levels are dramatically enhanced by ambient levels of hypoxia [15,59,60], ischemia [19,57] or acute inflammation [25,61]. Studies with the CD39 promoter identified a binding site for the transcription factor SP1, and functional studies demonstrated SP1 in transcriptional induction of CD39 during ambient hypoxia, or myocardial ischemia [60].

Similarly, CD73-dependent generation of extracellular adenosine from AMP is enhanced by hypoxia [59] ischemia [22,27,28,56] and/or inflammation [25,61]. Again, this process is transcriptionally regulated and involves hypoxia-inducible factor (HIF)-1 dependent induction of CD73 [59]. Studies with promoter constructs for CD73 indicate that HIF-1 binding to the promoter is required for CD73 induction during hypoxia [59]. Taken together, these studies provide evidence that extracellular adenosine generation from precursor nucleotides is dramatically enhanced during limited oxygen availability [15,16] – as occurs during intestinal ischemia or inflammation (Figure 2) [26,28,29].

In addition to enhanced extracellular adenosine production, limited oxygen availability also alters adenosine signaling effects at the receptor level. A comparative study of all four adenosine receptors during ambient hypoxia revealed a selective induction of the A2BAR during hypoxia [15]. In fact, studies of the A2BAR promoter revealed a functional binding site for HIF-1 [30] and other studies demonstrated HIF-1-dependent induction and signaling of the A2BAR during cardioprotection from ischemia [52]. Other studies confirmed selective induction of the A2BAR during acute inflammation (e.g., in the setting of acute lung injury) [32], myocardial [22] or renal ischemia [31], and gene-targeted mice for the A2BAR are more prone to inflammation- or hypoxia-associated vascular leakage, inflammation and tissue injury [20,22,26,29,31,32,62]. Taken together, these studies demonstrate a selective induction of the A2BAR during hypoxia, in conjunction with a tissue protective, and anti-inflammatory role in functional studies of hypoxia-associated inflammation (Figure 2).

In addition to enhanced extracellular adenosine generation and signaling during hypoxia, limited oxygen availability also alters extracellular adenosine uptake [53]. In fact, several studies demonstrated that extracellular adenosine uptake is attenuated by low oxygen levels [63–66]. As such, transcript and protein levels of equilibrative nucleoside transporters (ENTs) are repressed during hypoxia [63–66]. Similarly to its role in regulating CD73 and the A2BAR, HIF-1 is responsible for the transcriptional regulation of ENT1 and ENT2 repression during hypoxia [65,66]. Attenuated expression of ENTs is associated with a lower capacity for extracellular adenosine transport, and represents an additional cellular mechanism to further elevate extracellular adenosine levels during hypoxia (Figure 2) [53,63–66].

Finally, hypoxia also alters intracellular adenosine metabolism. Particularly during conditions of acute hypoxia, adenosine conversion to AMP via the adenosine kinase is attenuated [54,67]. This is due to HIF-1-dependent repression of adenosine kinase transcription, protein levels and enzymatic function, resulting in elevated intracellular adenosine levels, and attenuated extracellular adenosine uptake during conditions of limited oxygen availability (Figure 2) [54]. Taken together, these studies demonstrate a coordinated cellular response to hypoxia, with enhanced extracellular signaling effects, particularly via the A2BAR. In addition, this transcriptionally regulated pathway is under the central control of HIF-1.

4. Extracellular adenosine during intestinal ischemia

As outlined above, extracellular adenosine has been implicated as an innate anti-inflammatory metabolite, particularly during conditions of limited oxygen availability such as ischemia [12–14,44]. Due to the fact that extracellular adenosine generation is primarily produced via phosphohydrolysis from its precursor molecule AMP via the enzymatic activity of the ecto-5'-nucleotidase (CD73), we recently performed studies to address the contribution of CD73-dependent adenosine production in modulation of intestinal ischemia-reperfusion (IR) injury [28]. Following transcriptional and translational profiling of intestinal tissue that revealed a prominent induction of murine CD73, we next determined the role of CD73 in protection against intestinal IR injury. Here, we used a selective inhibitor of CD73 enzymatic function (APCP), or studied mice gene-targeted for CD73. Interestingly, pharmacological inhibition or targeted gene-deletion of CD73 significantly enhanced not only local intestinal injury, but also secondary organ injury, following IR as measured by intestinal and lung myeloperoxidase, aspartate and alanine aminotransferase, IL-1, IL-6 and histological injury. To confirm the role of CD73 in intestinal adenosine production, we measured adenosine tissue levels and found that they were increased with IR injury. In contrast, *cd73*-deficient mice had lower adenosine levels at baseline and no increase with IR injury. Finally, reconstitution of *cd73*^{-/-} mice or treatment of wildtype mice with soluble 5'-nucleotidase, which also converts AMP to adenosine, was associated with significantly lower levels of injury. These data reveal a critical role of CD73-dependent conversion of extracellular AMP to adenosine in attenuating intestinal IR-mediated injury [28].

Based on these studies, we next pursued the contribution of individual adenosine receptors to intestinal protection from IR injury [29]. Here, we proposed a protective role for extracellular adenosine signaling in intestinal IR injury. Initial profiling studies of mucosal scrapings following murine IR demonstrated selective induction of the A2BAR transcript. Moreover, gene-targeted mice for the A2BAR showed more profound intestinal IR injury compared with controls. In contrast, A2AAR^{-/-} mice exhibited no differences in intestinal injury compared with littermate controls. In addition, selective inhibition of the A2BAR resulted in enhanced intestinal inflammation and injury during IR. To test the therapeutic relevance of our findings we next tested a previously reported agonist of the A2BAR (BAY 60 – 6583). In fact, previous studies with this compound revealed therapeutic effects in murine myocardial ischemia or acute lung injury that were absent in A2BAR^{-/-} mice, indicating selectivity of the compound for the A2BAR [22,32]. Here, A2BAR agonist treatment (BAY 60 – 6583) protected from intestinal injury, inflammation, and permeability dysfunction in wild-type mice, whereas the therapeutic effects of BAY 60 – 6583 were abolished following targeted A2BAR gene deletion. Taken together, these studies demonstrate the A2BAR as a novel therapeutic target for protection during gastrointestinal IR injury [29].

5. Adenosine generation and signaling during experimental colitis

Based on the known anti-inflammatory role of extracellular adenosine generation and signaling, several studies investigated the contribution of adenosine during murine colitis. For example, two studies on the global contribution of extracellular adenosine to murine colitis were recently published. The first study used inhibitors of adenosine deaminase in rats exposed to chemically induced colitis as a strategy to enhance adenosine signaling. The authors found that inhibition of adenosine deaminase was associated with significant attenuation of intestinal inflammation [68]. A second study investigated the role of the ecto-5'-nucleotidase (CD73) during murine colitis. Here, the authors used chemically induced colitis (induced with trinitrobenzene sulfuric acid (TNBS)) as a model. They found that the severity of colitis was increased, as determined by weight loss and architectural distortion, in *cd73*^{-/-} mice relative to controls. Likewise, enteral administration of the selective CD73 inhibitor alpha, beta-methylene ADP (APCP) to wild-type mice resulted in a similar increase in severity of TNBS colitis in this study [9]. Together, these studies suggest a protective and anti-inflammatory role of adenosine in murine colitis.

Several studies addressed the contribution of individual ARs (A1AR, A2AAR, A2BAR, or A3AR) in murine colitis. While there is only little known about the role of the A1 or A3AR in colitis [69,70], the A2A and A2BAR receptor have been described as being involved in the elevation of cAMP levels, attenuation of mucosal inflammation and tissue protection [14,32,51,71]. Thus, A2AAR or A2BAR agonists represent a potential group of therapeutics for the treatment of IBD. As such, one study found a critical role for A2AAR signaling in T cell-mediated regulation of colitis. Moreover, treatment with a specific A2AAR agonist attenuated the production of pro-inflammatory cytokines and attenuation of colitis [10]. In addition, recent studies from our laboratory performed a 'head-on' comparison between A2AAR^{-/-} and A2BAR^{-/-} during murine colitis [26]. For this purpose, we subjected A2AAR^{-/-} or A2BAR^{-/-} mice to dextran sulphate sodium (DSS) protocols and compared

their responses to wild-type littermate controls. Our previous studies had revealed that neither $A2AAR^{-/-}$ nor $A2BAR^{-/-}$ animals manifest outward immunological defects when housed in specific-pathogen-free conditions [20]. Initial studies using 3.5% DSS showed that $A2BAR^{-/-}$ mice became profoundly ill within 3 days of induction, associated with high mortality. For this reason, we decreased drinking water DSS concentrations to 2.5%. $A2BAR^{-/-}$ mice exposed to DSS lost weight more rapidly and failed to regain weight over the 6 days following induction of colitis. By comparison, $A2AAR^{-/-}$ animals remained healthy at 2.5% DSS with no significant differences observed in weight loss curves between $A2AAR^{-/-}$ mice and littermate controls. Notably, $A2AAR^{-/-}$ mice showed increased susceptibility to higher concentrations of DSS. Indeed, when DSS concentrations were increased from 2.5% to 4.5%, $A2AAR^{-/-}$ showed significantly increased weight loss on days 2 – 6 and increased colon contraction compared with wild-type controls. Thus, it would appear that both $A2AAR^{-/-}$ and $A2BAR^{-/-}$ mice have increased susceptibility to DSS colitis, but that this phenotype is probably more severe in $A2AAR^{-/-}$ [26]. Likewise, enteral administration of the selective A2BAR inhibitor PSB1115 resulted in a similar increase in severity of DSS colitis [26]. Together, these studies indicate a central regulatory role for the A2BAR in modulating the acute inflammatory phase of DSS colitis [26]. These studies are consistent with other studies from our laboratory that found a more severe phenotype of $A2BAR^{-/-}$ mice during intestinal ischemia–reperfusion injury [29] and implicate the A2BAR agonist BAY 60 – 6583 in the treatment of this condition [29]. Moreover, other studies from our laboratory have shown attenuation of mucosal inflammation during acute lung injury [32] or during intestinal hypoxia exposure [20,51] mediated by the A2BAR. Similarly, A2BAR signaling is protective during renal [31] or myocardial ischemia [22,52]. This is also consistent with several studies from other investigators, implicating A2BAR signaling in protection from vascular inflammation [72,73], inflammation during organ transplantation [74] or attenuation of pulmonary inflammation during hypoxia [24].

In contrast to the findings of the present studies [26,29], two recent studies suggests a pathogenic role of A2BAR signaling in murine colitis [75,76]. Consistent with our studies, the authors found that the A2BAR is the predominant adenosine receptor expressed in colonic epithelia [76]. However, when exposing mice to DSS induced colitis, they found that mice with genetic deletion of the A2BAR were protected and mucosal inflammation was attenuated. Why the results from this study [76] is different from our studies [26,29] is not clearly understood. In contrast to the study by Kolachala *et al.* [76], several previous studies support an anti-inflammatory and tissue protective role of A2BAR signaling in different organ systems. As outlined above, gene-targeted mice for the A2BAR show enhanced vascular inflammation when exposed to endotoxin [74] or during acute vascular injury [72]. Similarly, gene-targeted deletion of the A2BAR is associated with enhanced vascular leakage and inflammation during ambient hypoxia [17,20,51]. Potential explanations why the studies of Kolachala *et al.* [75,76] found a detrimental role of A2BAR signaling during murine colitis could include details in the colitis protocol, differences in murine strains with genetic deletion of the A2BAR or environmental differences, such as differences in bacterial flora of the mice. However, it seems important to point out that in some of their studies – and in contrast to the overall theme of this paper – gene-targeted mice for the A2BAR experienced a pro-inflammatory phenotype [76]. For example,

A2BAR^{-/-} mice showed increased susceptibility to systemic *Salmonella* infection. In fact, 90% of *A2BAR*^{-/-} mice died within 10 days compared with 20% of wild-type mice following orally administered *Salmonella typhimurium*. Consistent with the mortality data, *A2BAR*^{-/-} mice also showed signs of weight loss earlier than WT mice [76]. Some additional comparisons between the individual mouse strains may be necessary to rectify some of these discrepancies.

6. Hypoxia-inducible factor in intestinal inflammation and ischemia

A number of elegant studies have indirectly implicated hypoxia in mucosal inflammatory diseases such as colitis [77–80], and recent studies in murine models identified the epithelium as the central target of hypoxia during active inflammation [6,8]. As such, studies have confirmed the existence of mucosal hypoxia during inflammation utilizing 2-nitroimidazole dyes, a class of compounds known to undergo intracellular metabolism depending on the availability of oxygen within tissue. Current understanding suggests that nitroimidazoles enter viable cells where they undergo a single electron reduction, to form a potentially reactive intermediate species. In the presence of normal oxygen levels, the molecule is immediately re-oxidized, and diffuses out of the cell over time. In the absence of adequate oxygen concentrations, low oxygen concentrations incompletely re-oxidize the molecule and the highly reactive reduced form of nitroimidazole associates with various intracellular proteins, forming adducts that can be localized with antibodies [81]. Localization of hypoxia utilizing these 2-nitroimidazole dyes revealed two interesting observations. First, in the small intestine and especially the colon, ‘physiologic hypoxia’ appears to predominate. Indeed, accumulation of nitroimidazole adducts was readily evident in epithelial cells lining the luminal aspect of the intestine. This was not the case in other tissues. Such findings confirm previous studies indicating that the resting partial pressure of oxygen in the intestine is quite low, probably due to the steep gradient of oxygen across the luminal aspect of particularly the colon. Second, these imaging studies revealed that cells overlying mucosal lesions are distinctly hypoxic. Accumulation of nitroimidazole adducts, particularly in the epithelium, was as intense as those observed in some tumors, suggesting the existence of intense foci of hypoxia within these lesions. The basis for such inflammatory hypoxia may include a shift in metabolic supply and demand in inflamed tissues. However, such studies highlight the presence of hypoxia in inflamed tissues, as may occur during experimental colitis in mice or during inflammatory bowel disease (IBD) in man [6,7].

As outlined above, hypoxia-dependent signaling pathways play an important role in enhancing extracellular adenosine generation and signaling. Particularly, HIFs play an important role in these responses. HIF is an α/β heterodimer that binds hypoxia response elements (HREs) at target gene loci under hypoxic conditions (Figure 3). In the presence of oxygen, HIF is inactivated by posttranslational hydroxylation of specific amino acid residues within its α subunits. Prolyl hydroxylation promotes interaction with the von Hippel–Lindau protein (pVHL) E3 ubiquitin ligase complex and proteolytic inactivation by proteasomal degradation, while asparaginyl hydroxylation blocks coactivator recruitment. These hydroxylation steps are catalyzed by a set of non-heme Fe(II)- and 2-oxoglutarate-dependent dioxygenases (prolyl hydroxylases (PHDs)) whose absolute requirement for

molecular oxygen confers sensitivity to hypoxia [82–86]. HIF-1 α was the original HIF isoform identified by affinity purification using oligonucleotides from the EPO locus [87] while HIF-2 α was identified by homology searches or screens for interaction partners with HIF-1 β . However, HIF-1 α and HIF-2 α are closely related, and both activate HRE-dependent gene transcription [88].

As a central regulator of oxygen homeostasis, HIF-1 has been implicated in transcriptional regulation of anti-inflammatory or tissue protective signaling pathways (Figure 3) [12,13,51,52,54,65]. As outlined above, HIF-1 coordinates the metabolism and signaling properties of extracellular adenosine [12,13,44,52,54,65,89]. In addition, HIF-1 induces metabolic changes in immune cells by switching from aerobic metabolism to glycolysis, and thereby markedly affects immune responses [12]. In mice, conditional ablation of the gene encoding the α -subunit of HIF-1 (HIF-1 α) results in impaired myeloid cell aggregation, motility, invasiveness, and bacterial killing [90]. HIF-1 α is stabilized in inflamed [6,8] or infected tissues [91,92], and several studies suggest an anti-inflammatory and tissue protective role of HIF-1 α signaling during acute inflammation [6,8,12,13,52,54,65,91,93] or bacterial infections [91,94]. Consequently, HIF-1 represents an underappreciated target in a number of important diseases related to hypoxia, including inflammatory diseases.

To study this in more detail, Karhausen *et al.* generated a mouse line with intestinal-epithelium-targeted expression of mutant HIF-1 α (inability to form HIF-1). Studies of colitis in these mice revealed that decreased HIF-1 expression correlated with more severe clinical symptoms (mortality, weight loss and colon length). Two independent studies published recently tested different pharmacological strategies of HIF activators and both revealed a protective role of HIF in murine colitis [8,93]. These studies provide insight into tissue micro-environmental changes in models of IBD and identify HIF-1 as a critical factor for attenuating mucosal inflammation during experimental colitis. Future studies will have to examine the influence of HIF on the molecular regulation of adenosine generation and signaling pathways in the setting of tissue inflammation as occurs during murine colitis or during inflammatory bowel disease in human.

To address the role of (HIF-1) in adenosine-dependent protection from intestinal ischemia, we recently performed a series of experiments in gene-targeted mice for CD73. To determine if the observed protective effects of CD73-dependent adenosine production during intestinal IR are regulated by HIF-1, wildtype or *cd73*^{-/-} mice were treated intraperitoneally with dimethyloxaloylglycine (DMOG), a non-specific prolyl hydroxylase inhibitor which activates HIF-1 activity by inhibiting proteasomal degradation of the HIF-1 α subunit. We found that treatment of wild-type mice with DMOG resulted in significant protection from intestinal I/R injury as assessed by a decrease in AST, ALT or IL-6 following intestinal IR [28]. In contrast, DMOG had no protective effects in *cd73*^{-/-} mice. Taken together these data indicate that adenosine protection from intestinal I/R injury involves hypoxia-dependent signaling pathways as key regulatory elements.

7. Expert opinion

Given the similarities between intestinal ischemia and inflammatory bowel disease, it is not surprising that both disease processes are associated with elevated extracellular adenosine production, signaling and attenuation of inflammatory hypoxia. At present, these studies have focused mainly on extracellular adenosine generation and signaling pathways. Current research work has clearly demonstrated a protective role of CD73-dependent generation of extracellular adenosine in intestinal ischemia and I/R injury [28]. In addition, we believe that adenosine signaling events – particularly through the A2BAR – represent a therapeutic target for acute intestinal ischemia, or intestinal inflammation during acute experimental colitis [26,29].

Future challenges include further definition of the contribution of extracellular adenosine transport mechanisms, and intracellular metabolism to intestinal ischemia or inflammation. Notably, most of the studies on intestinal inflammation or ischemia that are discussed in the present review are focused on an acute disease setting. Particularly in the field of inflammatory bowel disease research, and for the purpose of relating these findings towards patients suffering from ulcerative colitis or Crohn's disease, it will be important to utilize more chronic models of intestinal inflammation. For example, such studies could be performed in mice with IL-10 deficiency [95], TNF-driven chronic intestinal inflammation [96] or in mice that spontaneously develop chronic terminal ileitis, reminiscent of the human disease described by Crohn *et al.* in 1932 (e.g., SAMP/YitFc mice) [97–99]. Along the same lines, the data presented in the present review were mainly derived from self-limited murine models of intestinal inflammation, or experimental colitis. Therefore, future challenges will also include the translation of these findings from mice to humans.

While it is relatively well established that adenosine signaling is protective in murine colitis or intestinal ischemia, the mechanisms by which adenosine receptor activation dampens acute inflammatory events remain somewhat unclear. Due to the fact that intestinal epithelial cells express high levels of ARs, they represent a likely cellular target. However, a mechanistic insight into how A2BAR activation renders the intestinal epithelium more resistant towards hypoxia- or ischemia- driven inflammation and tissue injury is needed. Therefore, present research work from our laboratories is trying to find answers to the question of how extracellular adenosine signaling – particularly via the A2BAR – protects mucosal epithelia from acute inflammation or hypoxia-driven tissue damage.

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Figure 1. Model for extracellular adenosine generation during intestinal ischemia

Adenosine is an extracellular signaling molecule that is generated from its precursor molecules 5'-ATP and 5'-AMP. The final step in extracellular adenosine generation – conversion of AMP to adenosine – is catalyzed by the ecto-5'-nucleotidase (CD73). Thus, extracellular adenosine is available on the cell surface to activate its receptors, for example expressed on the apical side of intestinal epithelia. Current evidence suggests that CD73-dependent adenosine generation is enhanced during conditions of hypoxia, ischemia or intestinal inflammation.

Figure 2. Consequences of hypoxia on adenosine signaling pathways

During situations of cellular distress (acute hypoxia, inflammation, ischemia reperfusion injury), hypoxia coordinates changes that lead to increases in extracellular signaling effects that occur predominantly at the luminal side of the intestinal epithelium. These changes involve four mechanisms. First, extracellular adenosine production is enhanced through the transcriptional induction of the adenosine-producing enzyme ecto-5'-nucleotidase (CD73; conversion of AMP to adenosine). In addition, adenosine effects are also enhanced at the receptor level. As such, hypoxia coordinates the selective induction of the A2B adenosine receptor (A2BAR). Moreover, hypoxia leads to transcriptional repression of equilibrative nucleoside transporters (ENTs), resulting in attenuated adenosine uptake, enhanced extracellular adenosine concentration and signaling. Finally, hypoxia also causes transcriptional repression of the adenosine kinase, the main enzyme for intracellular adenosine metabolism. Adenosine kinase catalyzes intracellular phosphorylation of adenosine to AMP. Hypoxia-dependent repression of adenosine kinase represents an additional hypoxia-elicited mechanism that enhances extracellular adenosine concentration and signaling during hypoxia.

Figure 3. Hypoxia inducible factor (HIF) in the transcriptional coordination of cellular adaptation to hypoxia

The transcription factor HIF is stabilized during different pathological conditions such as hypoxia, inflammation or cancer. Following stabilization, HIF-dependent genes are transcriptionally activated and cause alterations in angiogenesis, metabolism and erythropoiesis. In addition, HIF also drives extracellular adenosine production and signaling, which plays a critical role in protection from colitis or intestinal ischemia.