Molecular Aspects of Medicine 55 (2017) 118–125

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

# Adenosine receptors and caffeine in retinopathy of prematurity

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ARTICLE INFO

*Article history:*
Received 1 October 2016
Received in revised form
28 December 2016
Accepted 10 January 2017
Available online 11 January 2017

*Keywords:*
Adenosine
Adenosine ($A_1$, $A_{2A}$, $A_{2B}$) receptors
Retinopathy of prematurity
Oxygen-induced retinopathy
Caffeine

ABSTRACT

Retinopathy of prematurity (ROP) is a major cause of childhood blindness in the world and is caused by oxygen-induced damage to the developing retinal vasculature, resulting in hyperoxia-induced vaso-obliteration and subsequent delayed retinal vascularization and hypoxia-induced pathological neovascularization driven by vascular endothelial growth factor (VEGF) signaling pathway in retina. Current anti-VEGF therapy has shown some effective in a clinical trial, but is associated with the unintended effects on delayed eye growth and retinal vasculature development of preterm infants. Notably, cellular responses to hypoxia are characterized by robust increases in extracellular adenosine production and the markedly induced adenosine receptors, which provide a novel target for preferential control of pathological angiogenesis without affecting normal vascular development. Here, we review the experimental evidence in support of adenosine receptor-based therapeutic strategy for ROP, including the aberrant adenosine signaling in oxygen-induced retinopathy and the role of three adenosine receptor subtypes ($A_1$R, $A_{2A}$R, $A_{2B}$R) in development and treatment of ROP using oxygen-induced retinopathy models. The clinical and initial animal evidence that implicate the therapeutic effect of caffeine (a non-selective adenosine receptor antagonist) in treatment of ROP are highlighted. Lastly, we discussed the translational potential as well therapeutic advantage of adenosine receptor- and caffeine-based therapy for ROR and possibly other proliferative retinopathy.



## 1. Retinopathy of prematurity (ROP) is a leading cause of childhood blindness

Retinopathy of prematurity (ROP) is a disease of premature infants which disrupts normal retinal vascularization (Fleck and McIntosh, 2008). With increased survival of extremely premature infants due to advances in neonatology, ROP has become a major cause of childhood blindness (50,000–100,000 cases/year) in many parts of the world (Fleck and McIntosh, 2008; Gilbert, 2008). ROP is caused by oxygen-induced damage to the developing retinal vasculature (Gilbert, 2008; Chen et al., 2008; Dhaliwal et al., 2009) and is characterized by the hyperoxia-induced vaso-obliteration, subsequent delayed retinal vascularization, and hypoxia-induced pathological neovascularization (Fleck and McIntosh, 2008; Cavallaro et al., 2014) driven by hypoxia-induced factor-1α (HIF-1α) signaling pathway and increased vascular endothelial growth factor (VEGF) levels in retina (Cavallaro et al., 2014; Penn et al., 2008) (see Fig. 1A). Characteristic pathological changes include vaso-obliteration and proliferation of abnormal fibrovascular tissue at the border of the vascularised and non-vascularised retina (Fleck and McIntosh, 2008). Conventional therapies for ROP are limited to laser to ablate the avascular retina to prevent retinal detachment caused by ROP (Clark and Mandal, 2008), but the efficacy of ablative laser therapy are limited, and are associated with destruction to retina causing clinically significant loss of visual field. Anti-VEGF therapy (e.g. intra-vitreal injection of anti-VEGF-A antibody bevacizumab) was also proposed (Clark and Mandal, 2008) and has

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http://dx.doi.org/10.1016/j.mam.2017.01.001
0098-2997/

been recently shown to be effective in a randomized, controlled trial (Mintz-Hittner et al., 2011). However, the long term effect of intra-vitreal bevacizumab remains unclear with reported persistent avascular retina (Tokunaga et al., 2014) and recurrent intra-vitreal neovascularization (Hu et al., 2012). Importantly, VEGF acts as an angiogenic and a neurotrophic factor for normal retinal neural and vascular development (Tokunaga et al., 2014; Robinson et al., 2001; McCloskey et al., 2013). There are concerns on the unintended effects of anti-VEGF agents on delayed eye growth and retinal vasculature development of preterm infants who are still forming new blood vessels in many different organ systems (Nishijima et al., 2007; Saint-Geniez et al., 2008). Thus, there is a critical need to develop more effective and preferably non-invasive prophylactic and therapeutic strategies for ROP.

## 2. Normal retinal vascular development and pathological angiogenesis in ROP

An ideal therapeutic strategy for ROP is to selectively control pathological neovascularization/angiogenesis without affecting normal retinal vasculature during postnatal development. The key to this strategy is to distinguish pathological angiogenesis process from normal retinal vascular development. Normal retinal vascular development starts with the de novo formation of blood vessels from endothelial precursor cells (vasculogenesis) (Lutty and McLeod, 2003; Gariano, 2003). This is followed by development of new blood vessels by budding from existing blood vessels (angiogenesis) (Gariano, 2003). A critical event in the pathogenesis of ROP is oxygen-induced damage to the developing retinal vasculature. ROP occurs in two distinct phases: first, the developing retina is exposed to a relatively hyperoxic environment, which damages developing retinal vessels, (Aiello et al., 1994; Alon et al., 1995). Consequently, retinal vascularization is delayed, resulting in vaso-obliteration. Second, as the avascular retina becomes critically hypoxic, increased VEGF production leads to physiological revascularization of the central retina and pathological angiogenesis with formation of preretinal vascular tufts (Fleck and McIntosh, 2008; Lutty and McLeod, 2003), ultimately resulting in traction retinal detachment and blindness. Oxygen-induced retinopathy (OIR) is an animal model of ROP that recapitulates some characteristic pathophysiological features of ROP, including vaso-obliteration, physiological revascularization and pathological angiogenesis (Fleck and McIntosh, 2008; Aiello et al., 1994; Alon et al., 1995). Normal vascular development and pathological angiogenesis share some common pathways: HIF-1α and angiogenic factors such as VEGF are involved in both processes (Lutty and McLeod, 2003; Gariano, 2003). Distinct molecular and morphological processes have been documented for those processes. While developmental and physiological vascularization is a highly organized process, producing distinct superficial and deep vasculature plexuses in retina (Gariano, 2003), pathological angiogenesis generates new vessels in the preretinal area that are unorganized and leaky, with a tortuous architecture (Gariano, 2003; Powers et al., 2008). Furthermore, distinct cellular mechanisms may also underlie these two processes. For instance, astrocytes play an important role in normal development of retinal vasculatures by forming a template that provides guidance for the developing vascular network (Stone et al., 1995, 1996). However, VEGF released from astrocytes reactive to hypoxia is critical for pathological angiogenesis in the retina following OIR but not essential to developmental angiogenesis (Weidemann et al., ; Dorrell et al.,). Furthermore, a recent study indicates that deletion of bone marrow derived cells by transplantation may preferentially affect developmental angiogenesis than pathological angiogenesis (Zou et al.,). These distinct characteristics provide a biological basis for selectively targeting pathological angiogenesis without affecting normal postnatal vascular development.

## 3. Aberrantly enhanced adenosine signaling in retina of oxygen-induced retinopathy

Current therapeutic development of ROP focuses on directly targeting VEGF and HIF-1α signaling pathway (Cavallaro et al., 2014; Penn et al., 2008; Mintz-Hittner et al., 2011; Hartnett and Penn, 2012). However, cellular responses to hypoxia are characterized by robust increases in extracellular adenosine production (up to 100 folds) and signaling events through the markedly induced adenosine receptors (up to 50 folds) locally (Chen et al., 2013). Adenosine is a naturally occurring nucleoside that is distributed ubiquitously throughout the body as a metabolic intermediary and neuromodulator in the brain. Extracellular adenosine acts through multiple G-protein-coupled receptors (i.e. $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$) (Fredholm et al., 2001) to exert control over blood vessel growth in various tissues, including retina, both under normal and pathological conditions (Adair et al., 2005; Patz, 1980). All four adenosine receptor subtypes have been detected in retina (Cui et al., 2010; Brito et al., 2012).

Hypoxia triggers the surge in extracellular adenosine level as a result of transcriptional induction of CD73 and equivalent nucleotide transporter 1 as well as suppression of adenosine kinase, thereby elevating the capacity of local tissues for extracellular adenosine production (Lutty and McLeod, 2003; Elsherbiny et al., 2013a). Indeed, pioneering studies by Lutty and colleagues showed that 5′ nucleotidase and adenosine were reduced during the hyperoxia phase but markedly increased in the hypoxic retina using a neonatal canine model of OIR, (Lutty and McLeod, 2003; Takagi et al., 1996; Taomoto et al., 2000; Lutty et al., 2000). Adenosine accumulating locally during hypoxia permits the local control of retinal vessel growth (Lutty and McLeod, 2003). Pathological conditions such as OIR are also accompanied by the increases of local inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor−α (TNF-α), which lead to a delayed (~24 h), marked and sustained increases in adenosine receptor (particularly the $A_{2A}$R and the $A_{2B}$R) expression in tissues and inflammatory cells (Frick et al., 2009; Schingnitz et al., 2010; Linden, 2011). In OIR models of ROP, the expression of $A_{2A}$R was suppressed during the hyperoxic phase, but markedly increased in hypoxic retina, supporting the possible involvement of adenosine-$A_{2A}$R signaling in retinal pathological angiogenesis (Lutty and McLeod, 2003; Takagi et al., 1996; Taomoto et al., 2000; Lutty et al., 2000) (see Fig. 1A).

Locally increased adenosine levels and adenosine receptor signaling might represent a *local* “find-me” signal and serve as a unique “purinergic chemotaxis” for a *local* resolution to pathological conditions (as revealed by genetic KO studies) (Chen et al., 2013). Thus, the surge of adenosine level and the induction of adenosine receptors in the hypoxic phase of OIR (Lutty and McLeod, 2003) may constitute a negative feedback and defense mechanism countering such pro-angiogenic statues triggered by hypoxia and HIF-1α-mediated expression of VEGF in retina. Increased adenosine-adenosine receptor signaling in hypoxic retina also offers an opportunity of targeting pathological angiogenesis of ROP with minimal effects on normal retinal vascular development. Consequently, we propose that $A_{2A}$R activity in the retina has the potential to modulate normal retinal vascularization and/or pathological angiogenesis.

## 4. The role of adenosine receptors in development of ROP

Therapeutic potential of adenosine receptors-based therapy for ROP is supported by the ability of adenosine subtype receptors to