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Adenosine receptors and caffeine in retinopathy of prematurity

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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_1R, $A_{2A}R$, $A_{2B}R$) 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

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Jiang-Fan Chen obtained MD from Wenzhou Medical College, China and PhD from Medical College of Pennsylvania, USA. Dr. Chen is currently a professor at Wenzhou Medical University (China) and Boston University School of Medicine (USA). Dr. Chen's work has advanced the prospective of $A_{2A}R$ antagonists as a novel treatment strategy for neurological disorders such as Parkinson's disease. Dr. Chen's recent investigation into the role of $A_{2A}R$ in retinopathy led to the discovery of the abnormal $A_{2A}R$ signaling in retina as a therapeutic target for retinopathy of prematurity. Dr. Chen has published >150 papers on adenosine receptor neurobiology.

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well therapeutic advantage of adenosine receptor- and caffeine-based therapy for ROR and possibly other proliferative retinopathy.

Keywords

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

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 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

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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.

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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 modulate inflammation, neuroprotection and angiogenesis in retina. In particular, numerous studies support the role of adenosine receptors in modulating the angiogenic effects in various cell types and tissues (Adair, 2005), including cardiomyocyte (Deussen, 2000), skeletal muscle fiber (Lynge et al., 2001), skin (Feoktistov et al., 2009; Valls et al., 2009) and retina (Adair, 2005; Grant et al., 2001). The translational potential of adenosine receptor-based therapy for controlling proliferative retinopathy is substantiated by clinical evidence that clinical treatment of apnea of

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prematurity with caffeine (a non-selective adenosine receptor antagonist) reduced ROP related problems (Schmidt et al., 2007) (see below), and by genetic identification of the variants of the human $A_{2A}R$ gene that are associated with reduced risk of developing diabetic retinopathy (Charles et al., 2011).

4.1. $A_{2A}R$ and ROP

In the developing retina, immunoreactivity for adenosine and the A_{2A} receptor ($A_{2A}R$) are detected on endothelial cell precursors, angioblasts, and endothelial cells in formed blood vessels in the retina of newborn animals (Lutty and McLeod, 2003). In a neonatal canine model of OIR, the extracellular adenosine level is markedly increased in hypoxic retinal tissues supporting the possible involvement of $A_{2A}Rs$ in the retinal vasoproliferation in OIR (Lutty and McLeod, 2003; Takagi et al., 1996). Thus, the $A_{2A}R$ activity in retina may contribute to modulation of normal retinal vascularization as well as pathological angiogenesis (Lutty and McLeod, 2003). Using $A_{2A}R$ knockout (KO) mice, we have demonstrated that genetic inactivation of the $A_{2A}R$ attenuates development of OIR pathology (Liu et al., 2010), as evidenced by: (a) reduced vaso-obliteration area in the center of the retina; (b) reduced pathological angiogenesis in retina; and (c) inhibition of hypoxia-induced VEGF gene expression. Notably, attenuation of pathological angiogenesis by the $A_{2A}R$ inactivation is selective for OIR since it does not affect the normal retinal vascularization during postnatal development (see Fig. 1B). This angiogenic role of the $A_{2A}R$ is also consistent with the ability of $A_{2A}R$ activation to increase angiogenesis in various cell types and tissues, including liver (Day et al., 2005), kidney (Okusa, 2002), skin (Montesinos et al., 1997) and retina (Taomoto et al., 2000), and with the ability of $A_{2A}R$ activation to modulate the expression of VEGF, a key regulator of tissue angiogenesis (Takagi et al., 1996; Grant et al., 1999). Collectively, these findings provide the direct evidence that the $A_{2A}R$ is critical for the development of OIR, and suggest a novel therapeutic approach of $A_{2A}R$ inactivation for ROP by targeting the pathological angiogenesis without affecting normal vascularization in the retina.

4.2. $A_{2B}R$ and ROP

Similar to $A_{2A}Rs$, the $A_{2B}Rs$ are adenylyl-cyclase activating receptors, known to be expressed in the eye, and more specifically in the vasculature, microglia and macrophages (Saura et al., 2005; Brambilla et al., 2003; Boison et al., 1071; van Calker and Biber, 2005). Their expression is induced by oxidative stress (St Hilaire et al., 2008), and they control the expression of VEGF in various cells, including macrophages (Granata et al.,). In the cultured human retinal endothelial cells, pharmacological profiles are consistent with $A_{2B}R$-mediated (but not with A_1R- or $A_{2A}R$-mediated) effects on growth factor expression and cell proliferation (Grant et al., 1999, 2001). Furthermore, studies with first generation of $A_{2B}R$ antagonists (Grant et al., 1999, 2001; Mino et al., 2001) and with ribozyme approach to inactivating $A_{2B}Rs$ demonstrate that adenosine acting at the A_{2B} receptors promotes pathological angiogenesis in retina through modulating VEGF level (Afzal et al., 2003). These results obtained with the $A_{2B}R$ antagonists are, however, intrinsically limited in their specificity. The exact role of the $A_{2B}R$ in normal retinal vascular development and the pathogenesis of OIR remains to be determined and no confirmatory OIR studies with genetic ablation of $A_{2B}Rs$ are available.

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4.3. A_1R and ROP

The A_1R mRNA and ligand binding are detected in developing retina (Brito et al., 2012) and is reduced in retina of OIR model (Zhang et al., 2015). Our characterization of the A_1R KO and WT mice in normal and OIR model demonstrate that genetic deletion of the A_1R does not affect normal retinal vascularization during postnatal development since ontogeny of the superficial, deep and intermedial layers of retinal vasculatures is largely indistinguishable between WT and A_1R KO mice (Zhang et al., 2015). However, in OIR model, A_1R activity distinctly controls hyperoxia-induced vaso-obliteration at postnatal day (P) 12 and hypoxia-induced revascularization at P17. Specifically, genetic deletion of the A_1R reduces hyperoxia-induced retinal vaso-obliteration at P12. This effect of A_1Rs on OIR is associated with A_1R control of cellular apoptosis in the inner nuclear layer of retina at P12. At the vaso-proliferative phase (P17), A_1R KO attenuates hypoxia-induced intra-retinal revascularization without affecting intra-vitreal neovascularization at P17. This effect of A_1R KO on OIR is associated with the reduced number of endothelium tip cells, without modification of cellular proliferation and astrocytic activation (Zhang et al., 2015). Thus, A_1R activity is not required for normal postnatal development of retinal vasculatures, but selectively controls hyperoxia-induced vaso-obliteration and hypoxia-driven revascularization by distinct cellular mechanisms.

5. Effective therapeutic window of AR actions on ROP

Retinal vasculature undergoes critical developmental changes postnatally: from P7 onward the superficial capillaries start sprouting vertically in retina to form first the deep then the intermediated vascular plexus in the retina of C57BL/6 mice (Smith et al., 1994; Stahl et al.,). Pathologic neovascularization formation might be particular sensitive to pharmacological manipulation at this stage. Furthermore, ROP is defined as a two-stage disease: the first stage is characterized by the vaso-obliteration while the second stage is characterized by hypoxia of the avascularized retina and resultant increase in neovascularization (Lutty and McLeod, 2003). Thus, it is critical to define the specific postnatal developmental stages and the specific disease course (hyperoxic vs hypoxic phases) that is sensitive to adenosine receptor modulation.

Adenosine-based actions are also presumably to be most evident at the hypoxic phase with the surge of adenosine level. Indeed, our preliminary study indicates the protection against pathological angiogenesis at the hypoxic phase (P17) by A_{2A}R KO and by A_{2A}R antagonists (KW6002) and by caffeine (non-selective adenosine receptor antagonists). However, detailed analysis indicated that A_1R KO reduced avascular areas at during the hyperoxic phase (P12) (Zhang et al., 2015). Similarly, A_{2A}R KO and caffeine was effective in protecting OIR not only at P17, but also at P12 (unpublished data). Our findings highlight the important function of adenosine signaling in modulating retinal vascular function even under hyperoxic environments. This may indicate that retinal vasculature development at P7–12 might be particular sensitive to interference since angiogenic sprouting from retinal superficial capillaries into the vitreous take places at P7. However, this finding is somewhat surprising since despite clearly vaso-obliteration at the retina center at the hyperoxic phase, there is no “hypoxia” in retina as shown by *in vivo* detection with nitroimidazole EF5 (Scott

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and Fruttiger, 2010) and the adenosine concentration and the expression of ecto-5′ nucleotidase (CD73) are low during the hyperoxic phase. The exact reason for this is not clear. If the hypothesis that the hyperoxia phase is the critical to AR-mediated protection against OIR is validated by future investigations, this finding suggests that the hyperoxic damage to developing retinal vasculatures is the primary and critical effect during ROP pathogenesis despite the fact that pathological angiogenesis is most evident at the hypoxic phase of ROP (Zhang et al., 2015).

6. Cellular (type) mechanism of adenosine receptor actions on ROP

ROP pathology is characterized by abnormal/pathological angiogenesis and the endothelial cell is a final common pathway of abnormal endothelial proliferation (Xu et al., 2012). In retina, endothelial tip cells are mainly located at the leading edge of vascular plexus and the fusion sites of the remodeling area. Numerous studies support the role of adenosine signaling in endothelial cell proliferation and migration *in vitro* and vascular growth *in vivo* (Adair, 2005). In retinal endothelial cells, $A_{2A}R$ activation increases production of VEGF and GLUT-1 (Takagi et al., 1996, 1998), indicating a pro-angiogenic effect of $A_{2A}R$. Consistent with this, genetic inactivation of the $A_{2A}R$ reduces endothelial cell proliferation in OIR model (Liu et al., 2010). Thus, increased endothelial sprouting and proliferation likely play a major role in control of pathological angiogenesis in ROP (Xu et al., 2012; Horowitz and Simons, 2008; Carmeliet and Tessier-Lavigne, 2005). However, the exact role of the endothelial $A_{2A}Rs$ remains to be determined by study using endothelial $A_{2A}R$ KO mice. Future studies of caffeine and KW6002 control of gene expression in the tip cell fraction of sprouting vessels in OIR would shed light on the transcriptional mechanism underlying AR control of tip cells in promoting retinal vascularization.

Control of retinal vascularization during development and OIR likely involves close interactions among endothelial cells, neurons and glial cells (microglial and astrocytes) (see Fig. 2). In particular, the interaction between endothelial tip cells and astrocytes plays a critical role in developmental blood vessel formation and physiological revascularization (Wood and Martin, 2002). Astrocytes play a significant role in angiogenesis in response to hypoxia through their high expression of VEGF (Dorrell and Friedlander, 2006). Studies in OIR models showed that the density of astrocytes in the retina decreases during hyperoxia and then increases following hypoxia (Chan-Ling et al., 1992; Downie et al., 2008), and that restoring retinal astrocytes reduces vascular pathology associated with OIR (Weidemann et al., ; Dorrell et al.,). Neuronal mechanisms in retina may also contribute to retinal vascularization of ROP, particularly during the hyperoxic phase. In the vaso-obliteration phase, hyperoxia induces apoptosis of ganglia cells and developing endothelial cells and inhibits endothelial cell proliferation and migration, resulting in vaso-obliteration (Aiello et al., 1994; Alon et al., 1995). Activation of retinal A_1Rs has been shown to inhibit Ca^{++} channels in retinal ganglion cells of mini-slices (Sun et al., 2002; Santos et al., 2000), protect NMDA-induced cell death in cultured retinal neurons (Oku et al., 2004), and mediate the interleukin-6 effect on the survival of cultured retinal ganglion cells (Perigolo-Vicente et al., 2013). Consistent with the A_1R-mediated neuroprotective effect, early studies indicated that cytotoxicity and cell death were generally more pronounced in neurons and astrocytes derived from A_1R KO mice (Bjorklund et al., 2008; Dunwiddie and Masino, 2001;

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Johansson et al., 2001). In addition, in parallel with the avascular area, $A_{2A}R$ KO attenuates TUNEL-positive cells in the inner nuclear layer of retina (unpublished data), suggesting that A_1R and $A_{2A}R$ KO probably protect against hyperoxia-induced damage to developing retinal vessels by modulating neuronal apoptosis.

7. Caffeine and ROP

The translational potential of adenosine receptor-based therapy for controlling proliferative retinopathy is substantiated by its clinical potentials of caffeine treatment in reducing ROP related problems in premature infants. In a recent large prospective clinical phase III trial with caffeine treatment for apnea in premature infants, caffeine treatment apparently reduces the severity of ROP as compared to that of the control in a two-year follow-up observation (Schmidt et al., 2007). The therapeutic potential of caffeine for ROP is further supported by the ability of caffeine to control angiogenic factors HIF-1α and VEGF (Merighi et al., 2007; Ryzhov et al., 2007), angiogenesis (Ryzhov et al., 2007; Hsu et al., 2015) and apoptosis of endothelium cells (Li et al., 2013) and other vascular actions (Echeverri et al., 2010). This raises an exciting possibility that caffeine, the ubiquitous trimethylxanthine that is widely used in premature infants with apnea of prematurity (Abdel-Hady et al., 2015) may protect against pathological neovascularization in ROP. The protection against ROP by caffeine is in general agreement with the finding that caffeine treatment reduced the vulnerability of the immature brain to hypoxic ischemia (Bona et al., 1995), reduced the effects of NMDA on e.g. seizure susceptibility (Georgiev et al., 1993) in neonates. Moreover, our recent study demonstrate that caffeine treatment at the concentration of 0.1 g/L −1.0 g/L from P0–P17 reduced nonvascular areas by 31.28–53.78%, respectively, and also reduced neovascular nuclei counting (Zhou et al., 2015). Furthermore, we also found that repeated treatment of the $A_{2A}R$ antagonist KW6002 at P7–P14 reduced avascular areas as well as neovascularization at P17 as revealed by isolectin B4-immunostaining, consistent with notion that adenosine receptors are the main pharmacological targets of caffeine's actions (Zhou et al., 2015). Lastly, chronic treatment with caffeine or KW6002 did not affect normal retinal neovascularization during postnatal development (Zhou et al., 2015). These findings provide the biological basis for the clinical finding that the use of caffeine in treatment of apnea in premature infants is associated with reduced ROP. Collectively, these findings support the novel caffeine- and adenosine receptor-based pharmacologic treatment for ROP. Further studies to identify the molecular targets, the cellular mechanism and effective therapeutic window underlying the protective effects of caffeine are needed to optimize caffeine treatment regime to target specific molecular pathways to achieve maximize prophylactic benefits for ROP while maintaining their impressive safety profiles.

8. Therapeutic advantages and clinical implications of caffeine treatment in ROP

The demonstration of the role of the adenosine receptor in development of ROP and protection by caffeine suggests two potential therapeutic strategies with high translational potential: a) modification of caffeine treatment paradigm for apnea of prematurity to the specific prophylaxis and treatment for ROP; and b) development of $A_{2A}R$ antagonists -based

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treatment for ROP. $A_{2A}R$ antagonists such as KW6002 and preledenet are safe according to the safety profiles in clinical phase III trials and have been approved for clinical use in Japan. Importantly, our analyses demonstrate that genetic inactivation of $A_{2A}Rs$ (Liu et al., 2010) or A_1Rs (Zhang et al., 2015) or treatment with $A_{2A}R$ antagonists (caffeine and KW602) (Zhou et al., 2015) selectively controls pathological OIR without affecting normal retinal development. The mechanism underlying this selectivity may be related to local increase of adenosine-adenosine receptor signaling in response to stress, hypoxia and inflammation (Chen et al., 2013). Thus, the preferential effect of caffeine and KW6002 on OIR confers a critical advantage over anti-VEGF antibody strategy which is limited by delayed eye growth and abnormal vasculature and neural development of preterm retina since VEGF activity is necessary not only for pathological angiogenesis, but also for normal retinal vascularization during development. Since the publication of the Caffeine for Apnea of Prematurity randomized clinical trial in 2006, the use of caffeine for prophylactic purposes has been suggested (i.e, administration is commenced very soon after birth or before a diagnosis of apnea of prematurity is made). Thus, caffeine may also represent a novel prophylactiv strategy for ROP.

Additionally, dissection of the adenosine receptor and VEGF signaling pathways leading to distinct physiological development and pathological angiogenesis are needed to fulfill the potential of caffeine and $A_{2A}R$ antagonists to achieve maximal therapeutic effects with minimal unwanted side effects. In particular, it should be noted that despite of lack of the general developmental effect on postnatal brain (specifically retinal vasculature) functions, there is lingering concerns on the possible specific effect of caffeine on embryonic development (Ma et al., 2014; Back et al., 2006) and possible postnatal development and maturation of cortical GABAergic neurons at the microstructural level by perinatal exposure to caffeine (Ardais et al., 2014; Silva et al., 2013).

9. Adenosine receptors and other proliferative retinopathies

Lastly, it would be important to explore whether the protection of OIR by $A_{2A}R$ inactivation can be extended to other pathological proliferative retinopathy including diabetic retinopathy in adults and MD in aging population. In diabetic retina, A_1R and $A_{2A}R$ levels were elevated (Vindeirinho et al., 2013). Interestingly, the variants of the human $A_{2A}R$ gene are associated with reduced risk of developing diabetic retinopathy in a prospective study (Charles et al., 2011), suggesting the involvement of the $A_{2A}R$ in diabetic retinopathy. In diabetic retinopathy model, genetic inactivation of the $A_{2A}R$ increased apoptotic cells, TNF-α release, and intercellular adhesion molecule-1 expression compared with wild-type mice (Liou et al., 2011; Ibrahim et al., 2011; Elsherbiny et al., 2013b). Thus, it is expected that caffeine and KW6002 may exacerbate retinal damage in diabetic retinopathy, in contrast with the caffeine-mediated protection against OIR-induced retina (Liu et al., 2010; Zhou et al., 2015). This suggesting the distinct molecular/cellular mechanisms (including neuronal, inflammatory and vascular mechanisms) may underlie distinct effects of $A_{2A}R$-mediated modulation of retinal vascularization. Future studies to clarify the possible distinct role of $A_{2A}R$ in development of DR and MD may stimulate required clinical studies to translate this novel adenosine receptor-based pharmacologic therapies for the treatment of ROP and other proliferative retinopathies.

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10. Concluding remarks

ROP is a major cause of childhood blindness in the world. Current pharmacological therapy focus on anti-VEGF strategy, but this strategy is associated with the unintended effects on delayed eye growth and retinal vasculature development of preterm infants. Preclinical studies using OIR demonstrate that elevated $A_{2A}R$ and $A_{2B}R$ signaling promotes pathological angiogenesis while A_1R signaling apparently confers protection OIR. We recently identified a pathway that affects pathologic, but not developmental, angiogenesis of the eye, and involves the $A_{2A}R$: genetic inactivation of the $A_{2A}R$ attenuated OIR without affecting normal postnatal retinal vascularization (Liu et al., 2010). This raises the exciting possibility that $A_{2A}R$ activity in the retina may be selectively targeted for treatment of ROP. This notion is further substantiated by clinical evidence that caffeine treatment of apnea of prematurity is associated with reduced ROP (Schmidt et al., 2007). Further understanding of the $A_{2A}R$, $A_{2B}R$ and A_1R signaling interacting with other molecular and cellular pathways leading to distinct physiological development and pathological angiogenesis may lead to new strategy to achieve maximal therapeutic effects of adenosine receptor-based treatment with minimal unwanted side effects. Identification of the effective therapeutic window and cellular (endothelium and neuronal and glial) mechanisms of adenosine receptor strategies for the prevention and treatment of pathological retinal neovascularization will provide the required preclinical evidence to translate adenosine receptor-based treatment for ROP. The caffeine- and $A_{2A}R$-based therapeutic strategies have high translational potential since caffeine is widely used in neonate care and KW6002 shows noted safety profile in phase III clinical trials.

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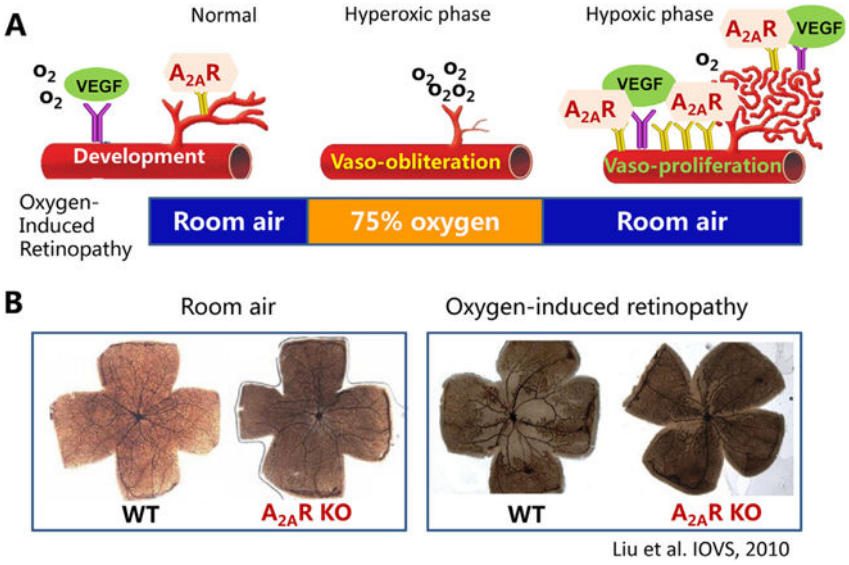

Fig. 1.

A_{2A}Rs preferentially modulate pathological angiogenesis without affecting normal retinal vascular development. (A) Adenosine receptors play a role in normal retinal vascular development (left panel) and pathological angiogenesis in oxygen-induced retinopathy model of ROP (middle and right panels). (B) In oxygen-induced retinopathy in mice, genetic inactivation of the A_{2A}R selectively attenuates oxygen-induced retinopathy without affecting retinal vascular development under room air.

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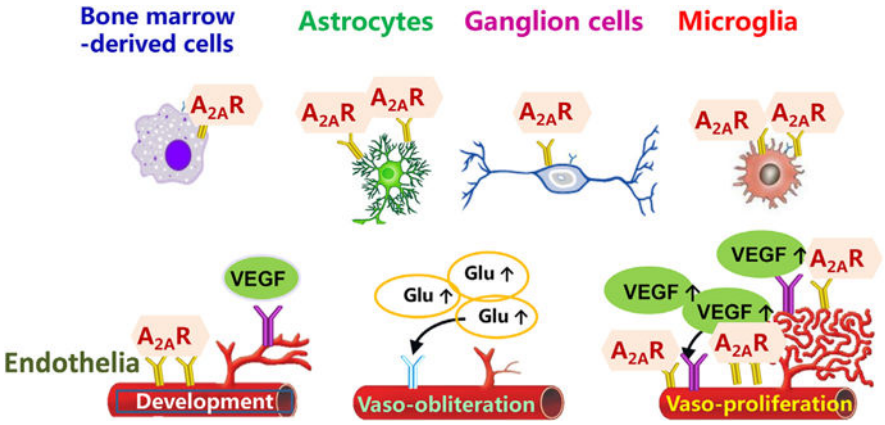

Fig. 2.

A_{2A}Rs in distinct cell types play differential roles in development of retinopathy of prematurity. A_{2A}Rs are expressed in endothelial cells as well as astrocytes, microglial cells, neuronal cells and bone marrow-derived cells. We postulate that the A_{2A}R in certain cells (such as endothelial cells and bone marrow-derived cells) may play an important role in normal development of retinal vascularization while A_{2A}Rs in microglial cells may be upregulated in OIR model and contribute to pathological angiogenesis.