Acta Ophthalmologica

ACTA OPHTHALMOLOGICA 2014

Review Article

Could donor multipotent mesenchymal stromal cells prevent or delay the onset of diabetic retinopathy?

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ABSTRACT.
Diabetes mellitus is a complex metabolic disease that has become a global epidemic with more than 285 million cases worldwide. Major medical advances over the past decades have substantially improved its management, extending patients' survival. The latter is accompanied by an increased risk of developing chronic macro- and microvascular complications. Amongst them, diabetic retinopathy (DR) is the most common and frightening. Furthermore, during the past two decades, it has become the leading cause of visual loss. Irrespective of the type of diabetes, DR follows a well-known clinical and temporal course characterized by pericytes and neuronal cell loss, formation of acellular-occluded capillaries, occasional microaneurysms, increased leucostasis and thickening of the vascular basement membrane. These alterations progressively affect the integrity of retinal microvessels, leading to the breakdown of the blood–retinal barrier, widespread haemorrhage and neovascularization. Finally, tractional retinal detachment occurs leading to blindness. Nowadays, there is growing evidence that local inflammation and oxidative stress play pivotal roles in the pathogenesis of DR. Both processes have been associated with pericytes and neuronal degeneration observed early during DR progression. They may also be linked to sustained retinal vasculature damage that results in abnormal neovascularization. Currently, DR therapeutic options depend on highly invasive surgical procedures performed only at advanced stages of the disease, and which have proved to be ineffective to restore visual acuity. Therefore, the availability of less invasive and more effective strategies aimed to prevent or delay the onset of DR is highly desirable. Multipotent mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), are promising healing agents as they contribute to tissue regeneration by pleiotropic mechanisms, with no evidence of significant adverse events. Here, we revise the pathophysiology of DR to identify therapeutic targets for donor MSCs. Also, we discuss whether an MSC-based therapy could prevent or delay the onset of DR.

Key words: diabetes mellitus – diabetic retinopathy – mesenchymal stem cells – multipotent mesenchymal stromal cells – prevention – regenerative medicine

Acta Ophthalmol. 2014: 92: e86–e95
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doi: 10.1111/aos.12113

Introduction

Diabetes mellitus (DM) is a complex metabolic disease that has become an epidemic worldwide. The World Health Organization reports that nowadays the total number of people with DM is 285 million and expects a rise to 439 million in 2030 as a result of ageing, obesity and sedentary lifestyle (Shaw et al. 2010). According to its aetiology, DM is classified into type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM). Approximately 10% of patients with DM present T1DM. This type of the disease is due to the autoimmune destruction of beta-pancreatic cells, in genetically predisposed children and young people, resulting in a deficiency in the production of insulin (Mathis et al. 2001). On the other hand, T2DM accounts for 90% of patients with DM. It affects mainly adult, sedentary and overweight people, although the prevalence in young people is beginning to increase together with child obesity (Mayer-Davis 2008). T2DM clinically debuts when patients who are unable to use insulin efficiently (a condition referred to as insulin-resistance) stop overproducing the hormone, due to the death of the insulin-producing cells. At this time, patients with T2DM develop hyperglycaemia and turn insulin- dependent (Kahn 2003).

Major medical advances over the past decades have substantially improved the management of DM, thereby prolonging patients' survival

(Christiano & Shessler 2010). However, this extended survival also carries an increased risk of chronic macro- and microvascular diseases including stroke, neuropathy, nephropathy and retinopathy (Stolar 2010). Nowadays, these complications have become the leading causes of morbidity and mortality amongst patients with DM (Marcovecchio et al. 2010; Stolar 2010).

Prevalence and Natural Progression of Diabetic Retinopathy

Diabetic retinopathy (DR) is the most common and frightening complication of DM (Luckie et al. 2007), and the leading cause for irreversible vision loss in developed countries (Resnikoff et al. 2004). In the Western world, the prevalence of DR in patients with DM is around 28% (Mohamed et al. 2007), and strongly increases with the progression of DM. Hence, almost all patients with T1DM and 60% of those with T2DM will have any degree of DR after 20 years of DM evolution (Kempen et al. 2004; Roy et al. 2004; Klein et al. 2009).

Diabetic retinopathy is clinically diagnosed when retinal microvascular alterations are observed in a patient with DM. The main retinal lesions that develop in patients with T1DM are not different from those developed by patients with T2DM, although the severity, incidence and relative contribution of pathological mechanisms to the observed lesions may differ (Klein et al. 1985). In both types of DM, the earliest morphological change observed in the initial stage of DR is a reduction in the number of pericytes in retinal capillaries (Lorenzi & Gerhardinger 2001) (Fig. 1). Then occurs the formation of acellular-occluded capillaries, occasional microaneurysms, increased leucostasis and thickening of the vascular basement membrane (Alder et al. 1997; Kollias & Ulbig 2010). These alterations progressively affect the integrity of retinal microvessels, leading to the breakdown of the blood–retinal barrier. The homeostasis of the retina is regulated by this barrier, which restricts the movements of molecules and minimizes leucocyte migration. Therefore, its breakdown results in an increase in

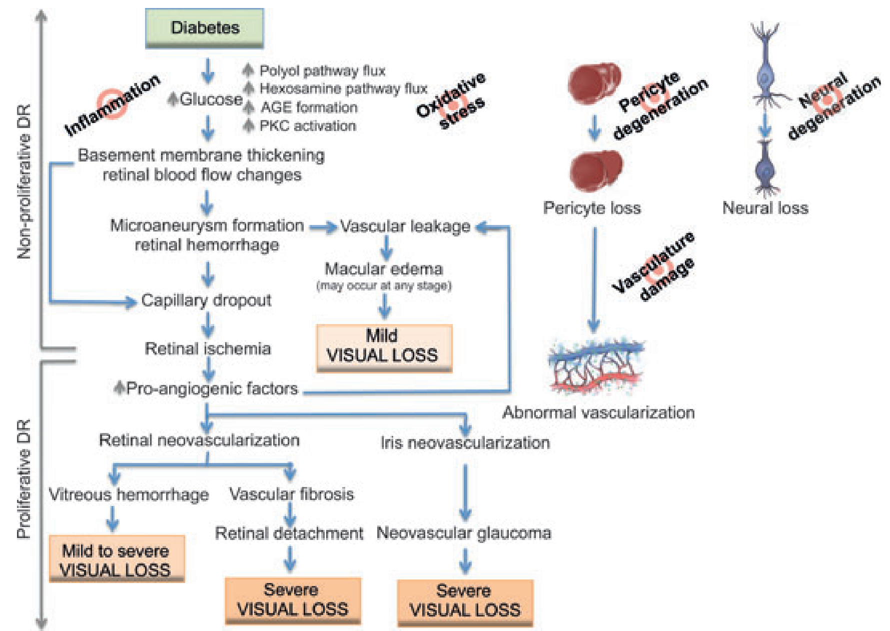

Fig. 1. Potential therapeutic targets for donor mesenchymal stem cells (MSCs) to prevent or delay the progression of diabetic retinopathy. Pathophysiological features triggered at the onset of the disease (inflammation, oxidative stress, pericyte degeneration, neural degeneration and/or vasculature damage) might be modified by donor MSCs (target sign). AGE, advanced glycation end products; PKC, protein kinase C.

vascular permeability, fluid leakage into the surrounding tissue and swelling. When this occurs in the eye macula, macular oedema develops and vision often becomes affected (Klein et al. 1995). Further vessel deterioration results in poor blood flow and ischaemia, leading to proliferative DR. At this stage, new vessels grow on the surface of the retina, in an attempt to restore blood flow. These vessels are fragile and leaky, and tend to rupture. Hence, abnormal neovascularization results in widespread haemorrhage. Together, fibrovascular scars are formed around the area of neovascularization producing tractional retinal detachment, which can result in blindness (Cheung et al. 2010). These clinically demonstrable changes in the vasculature of the retina have led to the general assumption that DR is solely a microvascular disease. Nevertheless, it has been well documented that hyperglycaemic state adversely affects the entire neurosensory retina, accelerates neuronal apoptosis and activates glial supporting cells (Lieth et al. 2000; Antonetti et al. 2006) (Fig. 1). These alterations occur before the onset of visible vascular lesions. Furthermore, patients with DM may exhibit reduced electrical responses in the electroretinography, and reduced colour and contrast sensitivity within two years of DM onset (Fortune et al. 1999; Lopes de Faria et al. 2001). Thus, today DR is also considered to be a sensory neuropathy (Gardner et al. 2011).

Available Treatments for Diabetic Retinopathy

Poor control of blood glucose level and elevated blood pressure are the major factors responsible for the onset and progression of DR (Mohamed et al. 2007; White et al. 2008). Therefore, tight glycaemic and hypertension controls remain the key strategies for preventing or arresting the development of DR, irrespective of the DM type. Unfortunately, this is difficult to achieve because diabetic patients do not adhere to their treatments. Despite considerable efforts in education, very little or no changes have been achieved in the reduction of DR incidence (Boscia 2010).

In the advanced stages of DR (macular oedema or proliferative DR), laser photocoagulation remains the mainstay of therapy (Mohamed et al. 2007). When undertaken in a timely

and appropriate manner, this technique shows a high efficacy in the prevention of visual loss. Nevertheless, the burns performed over the entire retina are associated with significant ocular side effects including worsening of visual acuity, thickening of central retina, reduction of visual field and acute glaucoma (Aiello 2003). On the other hand, vitreoctomy, a surgery in which the turbid vitreous is removed, is the last resort in cases of complications of proliferative DR, such as severe vitreous haemorrhage and secondary retinal detachment (Stefansson 2009). This is a very complicated surgery with high risk of iris neovascularization and cataract formation (Stefansson 2009). Thus, laser photocoagulation and vitreoctomy are not only highly invasive techniques, but also ineffective in restoring visual acuity. Though promising, the use of inhibitors of vascular endothelial growth factor (VEGF) has proven to be limited due to their short-lived effects, and adverse events, particularly tractional retinal detachment and systemic hypertension (Salam et al. 2011). Therefore, the availability of less invasive and more effective strategies aimed to prevent or delay the onset of DR is highly desirable.

Inflammation as a Target to Manage Diabetic Retinopathy

There is growing evidence that inflammation contributes significantly to the triggering of DR (Joussen et al. 2004) (Fig. 1). In response to hyperglycaemia, inflammatory mediators are upregulated in the retina, resulting in a pro-inflammatory response that causes abnormal leucocyte-endothelial interactions. In patients with DM and in animal models, the expression of TNF-α, IL-1β and ICAM-1 is increased in the retina (McLeod et al. 1995; Joussen et al. 2002; Zhang et al. 2006). TNF-α is a potent pro-permeability factor that induces ICAM-1, which function is the retention of leucocytes within the vascular endothelium. A higher density of leucocytes in the lumen of retinal vasculature induces capillary occlusion, non-perfusion, endothelial cell damage and vascular leakage. Also, it induces apoptosis of endothelial cells and pericytes (Joussen et al. 2002, 2003; Zhang et al. 2006). On the other hand, it has been shown that the intravitreal injection of IL-1β to normal animals increases retinal capillary cell apoptosis in a similar way to that observed in DR (Kowluru & Odenbach 2004). Accordingly, the inhibition of TNF-α, IL-1β or ICAM-1 expression or activity in animal models of DM significantly reduces retinal leucostasis and vascular leakage, indicating a causative role of pro-inflammatory factors in blood–retinal barrier breakdown at the early stages of DR (Joussen et al. 2002, 2004; Li et al. 2009a). In addition, increased levels of pro-inflammatory cytokines have been involved in the damage of the neurosensory retina. In patients with DM and in animal models of DR, increased retinal levels of TNF-α, IL-1β, IL-6 and IL-18 activate resident macrophages resulting in leucocyte infiltration, glial dysfunction and neuronal cell death (Krady et al. 2005; Kern & Barber 2008; Yang et al. 2009). Accordingly, the intravitreal administration of anti-inflammatory agents has been used clinically for decades to suppress intraocular inflammation and to reduce blood vessel leakage (Jonas 2007). However, due to their temporary effect, reinjections are usually needed and adverse events such as infection, increased intraocular pressure, glaucoma and cataract formation are observed. This has been the major obstacle to the widespread implementation of an anti-inflammatory treatment in the management of DR (Jonas et al. 2003; Jonas 2007; Mohamed et al. 2007).

Oxidative Stress as a Target to Manage Diabetic Retinopathy

Oxidative stress plays an important role at both early and late stages of DR (Du et al. 2003; Abu El-Asrar et al. 2004). Oxidative and nitrative modifications of retinal macromolecules occur promptly in the course of DM and they are not reversed by the restitution of glycaemic control (Kowluru 2003). The mechanisms underlying the increased oxidative stress in retinal tissue are complex and interconnected. High glucose flux increases the production of superoxide anion by mitochondrial electron-transport chain (Rolo & Palmeira 2006). The overproduced superoxides enhance the major pathways of hyperglycaemic vascular cell damage, including an increase of the polyol pathway, the stimulation of advanced glycation end product formation and the activation of protein kinase C, leading to more superoxide anion formation (Brownlee 2001). Excessive production of superoxide anions results in the formation of secondary reactive oxygen species (ROS) including peroxynitrite and hydroxyl radicals, which modify DNA, proteins and lipids. During the initial stages of DR, oxidative stress leads to the apoptosis of pericytes and neuronal cells (Evans et al. 2002; Ejaz et al. 2008) (Fig. 1). Consistently with these observations, the treatment of DM animals with antioxidants or with inhibitors of some of the metabolic pathways that generate ROS, reduces both oxidative and nitrosative stress and attenuated pericyte loss, basement membrane thickening and blood–retinal barrier breakdown (El-Remessy et al. 2003; Kowluru et al. 2003; Obrosova et al. 2003; Li et al. 2010). Unfortunately, these drugs have low oral bioavailability or are not able to cross the blood–retinal barrier. Therefore large doses must be administered to maintain therapeutic concentrations inside the retina, limiting their clinical use.

Inflammation and oxidative stress are closely inter-related in DR. IL-1β triggers signalling cascades resulting in ROS production, and ROS stimulate the release of pro-inflammatory cytokines (Fan et al. 2004). Both pro-inflammatory molecules and reactive species lead to the formation of advanced glycation end products and to an increase in the production of proangiogenic factors, accelerating the course of DR (Schleicher et al. 1997; Hubbard & Rothlein 2000). Thus, any therapeutic tool that could reduce simultaneously the inflammation and the oxidative stress in the retina might be promising to modify the natural evolution of DR.

Cell Replacement as a Target to Manage Diabetic Retinopathy

The earliest morphological changes in the retina of diabetic individuals are a reduction in the number of pericytes

at the vascular level, and the loss of retinal neurons and Muller glial cells at the nonvascular level (Feit-Leichman et al. 2005) (Fig. 1). As the retina provides the input for all visual sensory information to the brain, this loss of cells results in visual impairment and eventually, in blindness. At present, the mechanisms behind the dysfunction and death of retinal cells in diabetic patients have not been unequivocally proven. Inflammation, oxidative stress, formation of advanced glycation end products, and upregulation of protein kinase C may be involved (Ejaz et al. 2008). What has been proven is that the mere alteration of pericyte.

contractility results in angiogenic switching in the retina of diabetic individuals (Kutcher et al. 2007; Lee et al. 2010). On another hand, it has been reported that retinal pericytes inhibit the activation of T lymphocytes (Tu et al. 2011). Therefore, the loss of pericyte may result in an increased pro-inflammatory response in diabetic patients.

While in non-mammalian vertebrates (amphibians and fish), a robust regenerative response is activated after a retinal injury, in humans there appears to be little or no recovery of loss retinal cells (Karl & Reh 2010). Nevertheless, it has been reported that damaged retina secretes SDF1 and other factors that might promote the recruitment of bone marrow-derived stem cells (Li et al. 2006). Therefore, extra-retinal stem cells might contribute to retinal cells replacement at both vascular and non-vascular levels.

MSCs as a Tool for Degenerative Disease Management

Regenerative medicine pursues the development of therapeutic strategies aimed to manage severe injuries or chronic diseases presented by patients in whom endogenous regenerative mechanisms are unsuccessful in the restoration of impaired functions. Over the past years, stem cells have been envisioned as the best tool for this.

In general terms, a stem cell is an undifferentiated cell able to self-renew and to give rise to one or more type of mature cell (Mimeault & Batra 2006). Adult stem cells are found in any adult tissues and serve to the replacement of dead cells to maintain cellular homeostasis. Bone marrow harbours at least two distinct adult stem cells: the haematopoietic stem cells (HSCs) that give rise to blood and endothelial cells (Wagers & Weissman 2004), and the multipotent mesenchymal stromal cells also referred to as mesenchymal stem cells (MSCs) that can give rise to mesodermal cells such as adipocytes, chondrocytes, osteocytes and myocytes (Minguell et al. 2001; Dominici et al. 2006). Interestingly, MSCs also have the potential to cross the germ line barrier and generate cells from endo- and ecto-dermal lineages, a property known as cell plasticity (Krause et al. 2001; Phinney & Prockop 2007). Despite MSCs being scarce (<0.01% in the bone marrow), they appear as ideal candidates for cell therapy because (i) they can be obtained from donors without major complications; (ii) they can be easily expanded *ex vivo*; (iii) they are hypo-immunogenic. Therefore, receptor does not need to be conditioned before administration as in the case of total bone marrow or HSC transplantation (Kal et al. 2006); (iv) once administered intravenously, they are able to home into damaged organs (Francois et al. 2006) where they can differentiate into tissue-specific cells (Sasaki et al. 2008), orquestate endogenous regenerative mechanisms (Zhang et al. 2007), and/or protect the tissue from noxa (Fibbe et al. 2007; Valle-Prieto & Conget 2010) (Fig. 2). Furthermore, MSCs have been administered to more than 1000 human patients with no evidence of adverse events or tumour formation (Uccelli & Prockop 2010).

MSCs as a Tool for Eye-Related Disease Management

Within recent years, the putative use of MSCs for eye-related disease management has generated considerable interest. Most attention has been directed towards using MSCs to regenerate damaged retina. The hallmark of retinal diseases such as age-related macular degeneration and retinitis pigmentosa is the loss of neural cells due to the dysfunction of either the photoreceptor cells or the underlying retinal pigment epithelium that support them (Penfold et al. 2001). It has been shown that bone marrow- as well as adipose-derived MSCs injected locally (subretinal space) or systemically into animals with retinal diseases, differentiate into photoreceptor cells or retinal pigment epithelium (Kicic et al. 2003; Castanheira et al. 2008; Gong et al. 2008; Enzmann et al. 2009; Wang et al. 2010a,b). Also, it was proved that donor MSCs can survive, migrate, differentiate and integrate within the retina (Baker & Brown 2009). Interestingly, the engraftment of MSCs into damaged retina correlates with a delay in retinal degeneration and results in visual function preservation.

Mesenchymal stem cell transplantation also appears to be useful in the treatment of glaucoma (Johnson et al. 2010). Mesenchymal stem cells intravitreally administered into rats with experimental glaucoma induced by laser photocoagulation of the trabecular meshwork provide trophic support to the damaged tissue, and increased ganglion cell survival. Local delivery of MSCs also reduced retinal neurodegeneration in other disease models, such as ischaemia/reperfusion or light damage. Therefore, MSC transplantation appears as a promising tool to treat eye-related diseases.

MSCs as a Tool for Diabetic Retinopathy Management: Pros

Inflammation modulation

Mesenchymal stem cells have been recognized as immunomodulatory cells. *In vitro*, they inhibit the differentiation of monocytic precursors into activating dendritic cells (Zhang et al. 2004; Jiang et al. 2005). In mixed lymphocyte cultures, MSCs limit the expansion and the cytotoxic activity of NK and T cells (Maccario et al. 2005). Both *in vitro* and *in vivo*, MSCs down-regulate the expression of pro-inflammatory molecules (IL-1β, IL-12, TNF-α and INF-γ) $\alpha v \delta$ secrete anti-inflammatory factors (IL-4 and IL-10), establishing a tolerogenic microenvironment where activated T cells are unable to proliferate and die by apoptosis (Aggarwal & Pittenger 2005). Mesenchymal stem cells also promote the appearance of regulatory T cells, inducing antigen-specific

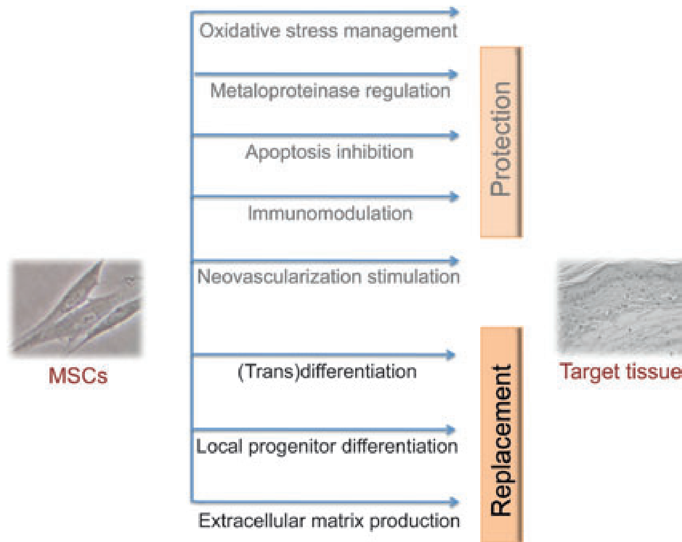

Fig. 2. Mesenchymal stem cell (MSC) contribution to tissue regeneration. Cellular and molecular mechanisms associated with MSC therapeutical effects.

tolerance. Mesenchymal stem cell immunomodulatory potential has been recognized as the mechanism underlying the therapeutic effect observed in autoimmune diseases such as graft-versus-host disease, experimental encephalomyelitis and diabetes (Le Blanc et al. 2004; Zhang et al. 2005; Ezquer et al. 2012). In these pathological conditions, systemic administration of MSCs prevents the destruction of old and newly generated tissue cells. Interestingly, it has been shown that the immunological properties of undifferentiated MSCs are retained when they differentiate into parenchymal cells (Le Blanc et al. 2003). Therefore, both undifferentiated and differentiated MSCs will contribute to the maintenance of a microenvironment that allows tissue regeneration. It has been shown that local administration of MSCs in animals with limbal stem cell deficiency greatly reduces the infiltration of inflammatory cells in the cornea (Oh et al. 2008). This was mainly mediated by the production of anti-inflammatory molecules including IL-4 and IL-10, and by the reduction of pro-inflammatory molecules including IL-2 and INF-γ in the cornea.

Whether donor MSCs are able to manage the chronic inflammation in the retina of individuals with DR, we hypothesize that their administration might protect vascular, perivascular and neural cells from dying, and thus prevent or delay the onset of DR.

Oxidative stress scavenging

Mesenchymal stem cells efficiently scavenge reactive species. It has been shown that MSCs are highly resistant to *ex vivo* culture and ionizing radiation, which are two conditions that generate a strong oxidative stress (Halliwell & Whiteman 2004; Chen et al. 2006). Recently, we have demonstrated that human MSCs have a high resistance to ROS and reactive nitrogen species due to the robust expression of SOD1, SOD2, CAT and GPX1 enzymes and high levels of glutathione (Valle-Prieto & Conget 2010). Furthermore, MSCs constitutively express methionine sulfoxide reductase A, an enzyme crucial for the repair of oxidized proteins (Salmon et al. 2009), and the enzymes required for the repair of damaged DNA (Silva et al. 2003). Therefore, MSCs possess the main enzymatic machinery to detoxify reactive species and to correct oxidative damage of proteome and genome. Accordingly, it has been shown that MSC transplantation is useful in the treatment of pathologies in which tissue damage is linked to oxidative stress including acute myocardial infarction (Chen et al. 2004), cerebral ischaemia (Kurozumi et al. 2005) and fulminant hepatic failure (Kuo et al. 2008). In these cases, the therapeutic effect observed has been attributed, amongst other mechanisms, to their potential to efficiently scavenge exogenous reactive species once homed into the niche of damaged tissues (Lanza et al. 2009). A widespread oxidative damage occurs in the retina of patients with DR and this plays a major role in the early loss of pericytes and neurosensory cells (Evans et al. 2002; Ejaz et al. 2008). Therefore, we hypothesize that donor MSCs, in the retina of diabetic individuals, will reduce the oxidative stress, and thus prevent or delay the onset of DR.

Endogenous regeneration induction

Mesenchymal stem cells are known to produce both *in vitro* and *in vivo,* a broad range of trophic factors including HGF, IFG, BDNF, GDNF, NGF, CNTF, VEGF and bFGF (Kinnaird et al. 2004; Caplan & Dennis 2006). The biological effects of these factors can be direct (triggering intracellular signalling) or indirect (inducing other neighbouring cells to secrete other bioactive factors). Hence, MSCs have been proposed to play a catalytic and pleiotropic role in tissue regeneration as once in the parenchyma, they modify the microenvironment by secreting trophic factors that would (i) prevent cells from dying (e.g., anti-apoptotic factors such as HGF and IGF, in models of acute myocardial infarction and pancreatic damage) (Chen et al. 2003; Izumida et al. 2005); (ii) induce the proliferation and differentiation of endogenous progenitors (e.g., secretion of neurogenic factors NGF and BDNF in models of neuronal damage) (Mahmood et al. 2004; Neuhuber et al. 2005); and (iii) induce neovascularization improving the irrigation of the damaged tissue through the secretion of angiogenic and vasculogenic factors (e.g., VEGF and bFGF in models of acute myocardial infarction and hypoxia) (Kinnaird et al. 2004; Nagaya et al. 2004; Tang et al. 2004). In the eye, local transplantation of MSCs delays the loss or preserves vision in animal models of inherited photoreceptor degeneration, retinal ischaemia and glaucoma (Yu et al. 2006; Lund et al. 2007; Li et al. 2009b; Johnson et al. 2010). In those cases, the survival of retinal neuronal cells is related to the local secretion by donor MSCs of anti-apoptotic factors such as BDNF, GDNF, NGF and CNTF.

As previously stated, in patients with DR, sustained hyperglycaemia adversely affects the entire neurosen-

sory retina with accelerated neuronal apoptosis that generally occurs before the onset of visible vascular lesions (Lieth et al. 2000; Antonetti et al. 2006). Therefore, we hypothesize that the production of neuroprotective factors by donor MSCs could reduce the apoptosis of neural cells inside the retina, helping to preserve visual acuity in individuals with DM.

Differentiation into pericytes and/or neural cells

Mesenchymal stem cell differentiation into mature cells has been considered as their main regenerative mechanism. It has been shown that donor MSCs can give rise to (i) osteoblast in patients with osteogenesis imperfecta (Horwitz et al. 2001); (ii) cardiomyocytes in animal models of acute myocardial infarction (Thiele et al. 2004); (iii) astrocytes, oligodendrocytes and neurons in experimental models of Parkinson's disease (Li et al. 2001), cerebral ischaemia (Chen et al. 2001) and mechanical brain damage (Lu et al. 2001). In ocular diseases, studies in animal models showed that donor MSCs survive and migrate to damaged areas of the retina after local or systemic administration. While some authors show that transplanted MSCs differentiate in both perivascular and neuronal retinal cells (Tomita et al. 2002; Kicic et al. 2003; Sengupta et al. 2003), others found that they are unable to do so (Yu et al. 2006; Hill et al. 2009). For instance, in an animal model of photoreceptor degeneration, it has been shown that once integrated into the retina, donor cells express rhodopsin, GFAP and pan-cytokeratin. This indicates that, in a damaged retina, MSCs can acquire the phenotype of photoreceptors, astrocytes and retinal pigment epithelium cells (Gong et al. 2008). These findings are in line with data showing that donor MSCs differentiated into (i) neural retinal cells in an ischaemia/reperfusion model (Li et al. 2009b); (ii) photoreceptors, amacrine cells and pericytes in a laser-injured model (Castanheira et al. 2008; Wang et al. 2010a) and (iii) microglial and endothelial cells in an inherited retinal degeneration model (Sasahara et al. 2008). Furthermore, due to striking similarities between MSCs and pericytes (Caplan 2008; Crisan et al. 2008), the former might replace the latter. Though MSCs have a broad differentiation potential, it has been suggested that they commit only to the lineage(s) triggered by the microenvironment they home (Caplan & Dennis 2006).

Thus, we hypothesize that, in the retina of individuals with DM, donor MSCs will counteract the loss of both pericytes and neural cells, preventing or delaying the onset of DR.

Ancillary support

It has been shown that human adipose-derived MSCs intravenously administered to animals with established DR results in an improvement of the blood–retinal barrier integrity (Yang et al. 2010). Into the retina of these rats, few donor cells differentiate into photoreceptor or astrocytes-like cells. In this murine model, MSC administration also led to a marked decrease in blood glucose levels. Thus, the observed beneficial effect in blood–retinal barrier breakdown could be secondary to the lowering of hyperglycaemia and not to a direct effect of donor MSCs in the damaged retina. Nonetheless, we hypothesize that donor MSCs would be eye-protective in diabetic individuals irrespective of hyperglycaemia correction, because we demonstrated recently that the intravenous administration of MSCs into mice with T1DM prevents diabetic nephropathy (Ezquer et al. 2009). While mice with T1DM treated with MSCs maintained low levels of albumin in urine and unaltered kidney structure, untreated diabetic mice presented marked albuminuria and significant structural renal alterations. In this animal model, donor MSCs were unable to reverse hyperglycaemia, suggesting that the observed renoprotection relates to a direct effect of MSCs over the kidney and not to a general improvement of DM condition. DR and diabetic nephropathy are microvascular complications of DM that share physiopathological mechanisms including inflammation and oxidative stress (Kaul et al. 2010; Romero-Aroca et al. 2010).

MSCs as a Tool for Diabetic Retinopathy Management: Cons

Vasculogenic potential

While the growth of collateral vessels is beneficial in ischaemic conditions (Sato et al. 2011), neovascularization in the eye associates with vision loss (Cheung et al. 2010). Proliferative retinopathy is characterized by the expression of high levels of VEGF and bFGF in ischaemic areas of the damaged retina inducing abnormal neovascularization that results in widespread haemorrhage (Cheung et al. 2010). It is well known that MSCs are able to secrete both *in vitro* and *in vivo* angiogenic factors, including VEGF and bFGF (Dharmasaroja 2009). Thus, this appears as the main limitation to use MSCs for the management of DR because MSC administration might worsen DR. Interestingly, it has been shown that MSCs modulate their regenerative mechanisms depending on the microenvironment they reach (Kinnaird et al. 2004; Caplan & Dennis 2006). In support of this idea, it has been shown that the local administration of MSCs in an animal model of corneal chemical injury characterized by an accelerated neovascularization process produced a rapid regression of the new vessels (Oh et al. 2008). This MSC anti-vascular effect was attributed to the production of TSP-1, a known anti-angiogenic factor. Thus, we hypothesize that donor MCSs will impair or block the retinal neoangiogenic process.

Animal models limitations

When comparing available animal models of DR regarding their degree of retinal injury, the general conclusion is that more complex lesions develop in larger, longer-lived (primates, dog) than in smaller, shorter-lived (rat, mice) models (Zheng & Kern 2010). This relates to the time that individuals are exposed to hyperglycaemia. Nevertheless, the possibility of genetic manipulation and the availability of reagents for molecular studies make mice the most used animal model to study DR (Kern et al. 2010). Amongst the models of T1DM, the most widely used to study DR are those in which mice receive one or more doses of streptozotocin (STZ) (Muranaka et al. 2006; Leal et al. 2007; Zheng et al. 2007). This drug is incorporated by the pancreatic beta-cells, but not by eye cells. As a powerful methyl agent for DNA, once in the cell, it leads to apoptosis and triggers the immune system (Konrad et al. 2001). Thus, mice exposed to STZ become highly hyperglycaemic and develop the long-term complications associated with

Table 1. Mouse models of diabetes mellitus that develop diabetic retinopathy.

Mouse model ID	Diabetes type	Diabetes etiology	Retinal lesions	References
C57BL/6 + STZ	1	Chemically-induced autoimmune destruction of pancreatic beta-cells	Pericyte loss Acellular capillaries Vascular cell apoptosis Blood-retinal barrier breakdown Ganglion cell loss Retina thinning	Joussen et al. (2003) Feit-Leichman et al. (2005) Leal et al. (2007) Zheng et al. (2007) Kern et al. (2010)
C57BL/6 + alloxan	1	Chemical destruction of pancreatic beta-cells	Pericyte loss Microglial cell morphology changes	Naeser & Andersson (1983) Gaucher et al. (2007)
NOD	1	Genetically-induced autoimmune destruction of pancreatic beta-cells	Pericyte loss Retinal microvessel loss	Shaw et al. (2006) Lee & Harris (2008)
*Ins2^Akita^*	1	Genetically-induced low insulin levels	Acellular capillaries Blood-retinal barrier breakdown Ganglion cell loss Retina thinning	Barber et al. (2005) Huang et al. (2011) Wright et al. (2012)
db/db	2	Obesity-induced insulin resistance	Pericyte loss Acellular capillaries Blood-retinal barrier breakdown Capillary basement membrane thickness	Cheung et al. (2005) Li et al. (2009a) Li et al. (2010)

STZ, streptozotocin.

DM, including DR. Other animal models of T1DM that develop DR are: the non-obese diabetic (NOD) mice, in whom the immune system spontaneously destroys pancreatic beta-cells (Shaw et al. 2006; Lee & Harris 2008) and the *Ins2*^{Akita} mice, which carried a dominant point mutation in the gene *insulin-2* (Gastinger et al. 2008). For T2DM, DR has been widely studied in C57BLKS-Leprdb/db (db/db) mice, an animal model of obesity-induced diabetes (Cheung et al. 2005; Li et al. 2009a,b, 2010). Due to the mutation of the leptin receptor gene, animals are hyperphagic, develop insulin-resistance and hyperglycaemia from the 5th week of age (Chua et al. 1996). In the mouse models of T1DM and T2DM, sustained hyperglycaemia results in structural retinal abnormalities similar to those observed at the nonproliferative stage of DR in human patients with DM (Kern et al. 2010) (Fig. 1 and Table 1). That is: reduction of pericytes in retinal capillaries, increased number of acellular-occluded capillaries, microaneurysms, increased leucostasis, increased vascular permeability resulting from breakdown of the blood–retinal barrier and thickening of the vascular basement membrane (Hammes et al. 2002; Cheung et al. 2005; Zheng et al. 2007; Li et al. 2009a). In addition, hyperglycaemic mice also develop functional retinal alterations due to neuronal cell death evidenced as changes in the retinal electrophysiological activity (Kern & Barber 2008). Nevertheless, due to limited rodent lifespan and relative short exposure to hyperglycaemia, all DM rodent models currently available do not develop preretinal neovascularization or other advanced lesions observed at the proliferative stage of DR in human patients with DM. Therefore, mouse models of DR are not useful to evaluate the therapeutic strategies aimed at managing advanced stage of DR. However, these models do offer insight into the early changes, which may determine the more advanced stages of the disease, and are useful for the assessment of therapeutic interventions aimed to prevent or delay the onset of DR.

Conclusion

Stem cell-based therapy represents a newly emerging therapeutic approach to treat eye degenerative diseases. MSCs are an attractive tool for this because, in preclinical and clinical studies, they have proved to trigger the regeneration of damaged tissue, with no evidence of adverse events. Due to the matching of pathological events that occur at the initial steps of DR, and the cellular and molecular mechanisms associated to MSC therapeutic effects, we hypothesize that, in an individuals with DM, the administration of undifferentiated MSCs will (i) reduce chronic inflammation by the secretion of anti-inflammatory cytokines, (ii) reduce oxidative damage by scavenging reactive species, (iii) be vascular- and neuroprotective due to the secretion of trophic factors, (iv) differentiate into pericytes and neuronal cells replacing the dead ones, thus preventing or delaying the onset of DR. This eye-protective potential of MSCs should be tested in relevant animal models of DR. If it does not result in abnormal neovascularization, it should be further proved in clinical trials and finally translated into clinical practise.

Acknowledgements

Supported by FONDECYT Grant # 11085033 to F.E. We thank Mrs. Carolina Larrain for English editing of the manuscript.

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Received on November 15th, 2011.
Accepted on January 15th, 2013.

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