Send Orders for Reprints to reprints@benthamscience.ae

Current Neuropharmacology, **2018**, *16*, **993-1003**

REVIEW ARTICLE

Nanoparticle-Mediated Delivery of Neuroprotective Substances for the Treatment of Diabetic Retinopathy

Rosario Amato[a], Massimo Dal Monte[a,b], Matteo Lulli[c], Vittoria Raffa[a,d] and Giovanni Casini[a,b,*]

[a]*Department of Biology, University of Pisa, Pisa, Italy;* [b]*Interdepartmental Research Center Nutrafood "Nutraceuticals and Food for Health", University of Pisa, Pisa, Italy;* [c]*Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy;* [d]*Institute of Life Science, Scuola Superiore Sant'Anna, Pisa, Italy*

Abstract: ***Background*:** Diabetic retinopathy (DR) is a major complication of diabetes, characterized by extensive vascular pathology leading to vision loss. Neuronal suffering and death are also present in the diabetic retina as a result of different molecular mechanisms that are compromised or modified in response to high glucose. The aim of this paper is to highlight recent data indicating that neurodegeneration is likely to play a primary role in the development of DR and that strategies based on nanomedicine may be exploited to deliver neuroprotection to the retina.

***Methods*:** An extensive analysis of the publications dealing with the role of neuroprotection in DR and with nanoparticle-mediated drug delivery to the retina has been conducted using PubMed, with particular attention to the most recent papers.

***Results*:** There are important limitations related to possible systemic side effects of neuroprotective substances and to drug bioavailability in the retina such as, for instance, the amount of drug reaching the retina, the need of keeping to a minimum the number of administrations (especially, for example, in the case of intraocular injections) and the need of assuring a long-lasting, graded intraocular drug delivery. In recent years, a variety of investigations have been aimed at the exploitation of approaches of nanomedicine to enhance the pharmacokinetics and pharmacodynamic activity of intraocularly delivered drugs. In particular, we provide some preliminary results that we have obtained about the feasibility of delivering magnetic nanoparticles functionalized with a neuroprotectant to mouse eyes through intraocular injections.

***Conclusion*:** We propose that nanoparticles functionalized with neuroprotective substances may be used to protect the diabetic retina, thus causing an impact in the design of future pharmacologic treatments for DR.

ARTICLE HISTORY

Received: May 04, 2017
Revised: July 03, 2017
Accepted: July 14, 2017

DOI:
10.2174/1570159X15666170717115654

Keywords: Retina, magnetic nanoparticles, ocular disease, somatostatin, octreotide, topical administration.

1. INTRODUCTION

Diabetic retinopathy (DR) is one of the most common complications occurring in type 1 and type 2 diabetes, characterized by high social impact and by an extremely complex pathogenesis that involves a variety of different cells, molecules, and factors. Metabolic changes in the diabetic retina result in altered expression patterns of a number of mediators, including growth factors, neurotrophic factors, cytokines/chemokines, vasoactive agents, and inflammatory as well as adhesion molecules, resulting in vascular lesions and cell death [1-4].

*Address correspondence to this author at the Department of Biology, University of Pisa, *via* S. Zeno 31, I-56127 Pisa, Italy; Tel: ++39-050-2211423; E-mail: giovanni.casini@unipi.it

DR has long been regarded exclusively as a vascular disorder characterized by microangiopathy and abnormalities in the retinal vasculature. Several studies have indicated that the vascular lesions are likely to be due to alterations of the physiological levels of factors controlling vascular homeostasis. Among them, vascular endothelial growth factor (VEGF) has been proven to play a central role in the pathophysiology of DR. Indeed, it is mainly an increase of VEGF levels in the retina that leads to the disruption of the blood-retinal barrier and determines the aberrant vascular proliferation that characterizes belated stages of DR [5]. However, the functional efficiency of retinal vascularization is known to be closely linked to the functionality of retinal neurons because of the existence of complex homeostatic mechanisms defining the neurovascular unit [6]. Therefore, considering the pathophysiological features of DR as exclusively

1570-159X/18 $58.00+.00

dependent on vascular dysfunctions might be not exhaustive. For this reason, the research has been recently focused on the responses of retinal neurons to the different types of stress caused by diabetes and several reports have described a conspicuous amount of neuronal death in the retinas of different animal models of DR as well as in diabetic patients. The causes of neuronal suffering are probably due to several early biochemical derangements, such as oxidative-stress, formation of advanced glycation-end products (AGEs), neuroinflammation, and excitotoxicity, initiated by increased glucose levels (Fig. **1**) [2, 4].

Interestingly, in the last years, numerous reports have established that functional alterations and degeneration of retinal neurons precede and possibly activate the typical microangiopathic processes and disruption of the blood-retinal barrier observed in DR [7-12], suggesting that DR displays enough pathophysiological traits to be considered a neurodegenerative eye disease [13].

Therefore, a neuroprotective strategy may be proposed as a basis for the design of novel DR treatments. However, although promising, the neuroprotective treatments may have important limitations related to drug bioavailability in the retina. For instance, systemic administrations could cause serious adverse effects due to general effects of the drug in the endocrine and/or the central nervous systems. In opposite, drug topical administrations (by intravitreous injection or as eye drops, when possible) need a relatively high frequency of treatment to maintain an effective drug concentration in the retina for a long time. Thus, the increase of administration frequency could lead to eye tissue damage and iatrogenic infections [14]. The use of various polymeric nanoparticles (NPs) to increase drug bioavailability and improve the delivery kinetics has been investigated in recent years. NPs as nanocarriers have been widely exploited in nanomedicine to provide sustained and long-term release of molecules at the desired sites [15].

In the present review, we summarize the evidence supporting a central role of neurodegeneration in DR and the therapeutic effects that may be obtained using neuroprotective substances. Then, in view of the limitations of the delivery methods used for intraocular drug administrations, possible NP-based approaches are reviewed. Finally, a proposal for the use of NP-mediated intraocular delivery of neuroprotectants to treat DR is formulated, together with the presentation of some preliminary results that we have recently obtained on this matter.

2. NEURODEGENERATION IN DIABETIC RETINOPATHY

The research is increasingly focusing on the analysis of the pathological mechanisms leading to degeneration of retinal neurons in DR, with the aim of finding novel therapeutic

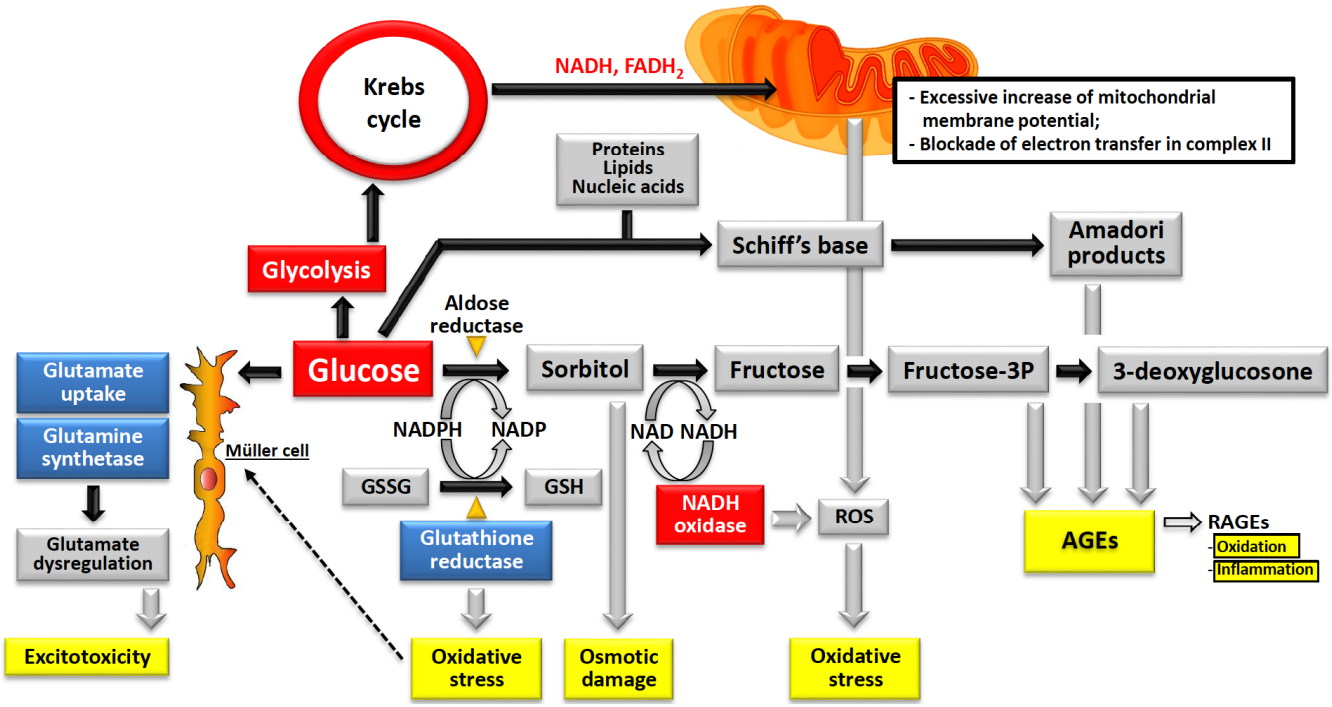

Fig. (1). Schematic reconstruction of the alterations induced by high glucose in the retina. Although the details of these mechanisms are beyond the scope of this review, it should be noted that main outcomes are represented by increased oxidative stress, excitotoxicity, and AGE formation, in addition to osmotic damage due to increased sorbitol levels and inflammation caused by the interaction of AGEs with their receptors (RAGEs) (yellow boxes). Oxidative stress is mainly caused by decreased glutathione reductase induced by high glucose, altered mitochondrial activity due to increased activity of the Krebs cycle, and RAGE activation by AGEs. AGE formation is due to biochemical pathways that are altered or incremented by high glucose and that include the formation of Amadori products or of 3-deoxyglucosone. Excitotoxicity is mainly secondary to dysregulation of glutamate homeostasis in Müller cells, which may be caused, at least in part, by increased levels of oxidation. Red color indicates increase; blue color indicates decrease.

approaches to reach high efficacy treatments. Neuronal suffering in the diabetic retina is the result of many different molecular mechanisms that are compromised or modified in response to high glucose conditions.

For instance, neurotransmitter homeostasis is profoundly affected in DR. Glutamate is the major excitatory neurotransmitter in the retina, and the stress condition caused by increased glucose levels brings to its accumulation in the retinal extracellular microenvironment. Consequently, the overstimulation of NMDA and AMPA receptors produces excessive calcium influx leading to neuronal death by excitotoxicity. Elevated retinal glutamate levels have been observed in animal models of diabetes as well as in diabetic patients [16-18]. In addition, alterations of the expression of glutamate receptors and calcium binding proteins have also been reported [19-21]. The reasons for extracellular accumulation of glutamate in DR may involve different mechanisms. For instance, Müller cells may become less efficient in performing glutamate uptake [22] or in converting glutamate to glutamine due to reduced levels of the enzyme glutamine synthetase [17, 18], while a decreased oxidation of glutamate to alpha-ketoglutarate has also been reported in diabetic retinas [16].

Non-enzymatic glycation is another pathological feature of glucose excess. Indeed, the production of AGEs and the presence of their receptors (RAGEs) have been detected in the diabetic retina [23], where they are known to play a central role in promoting inflammation and microvascular dysfunction [24]. RAGE activation may also induce retinal neuronal apoptosis through activation of nitric oxide synthase [25] or through activation of astrocytes and Müller glia resulting in enhanced expression of proinflammatory cytokines [26] that would contribute to neuronal death. A direct evidence of the detrimental effects of AGEs on retinal neurons is provided by data showing that AGEs induce neuronal death in nondiabetic rat [27, 28] or mouse [29] retinal explants.

Oxidative stress is likely to be one of the most important damaging factors in DR, as it may be considered as a sort of final common pathway for the mechanisms activated by high glucose. Oxidative stress may result from nitrative stress [30, 31], activation of polyol or hexosamine pathways, or uncoupling of endothelial nitric oxide synthase [32] in high glucose conditions. In addition, oxidative stress and AGE production appear to be interrelated mechanism [33, 34] and relationships between oxidative stress and glutamate release have also been reported [16].

3. ENDOGENOUS NEUROPROTECTIVE SUBSTANCES

In addition to pathologic changes leading to disruption of glutamate homeostasis, AGE formation, oxidative stress and other dysfunctions, DR has also been found to cause profound alterations in the expression of endogenous neuroprotective substances that are normally expressed in the retina. For instance, neurotrophins such as brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are known to play a crucial role in growth, differentiation, and survival of several cell types including retinal neurons, glia, and endothelial cells. A reduction in the expression of BDNF, probably caused by increased oxidative stress, has been reported in the retinas of mice with experimentally-induced diabetes [35]. Similar to BDNF, NGF expression is also decreased in DR. This effect seems to be secondary to impaired maturation of the NGF precursor, proNGF, caused by the oxidative milieu of the diabetic retina, resulting in increased expression of proNGF with a corresponding decrease in NGF. The dysregulation of proNGF maturation in the diabetic retina may determine ganglion cell damage due either to reduced trophic support caused by decreased NGF expression, or to direct activation of proapoptotic pathways caused by proNGF interaction with the p75NTR receptor [36-38]. Another pathway by which proNGF may cause ganglion cell death involves paracrine effects of proNGF/p75NTR-mediated secretion of tumor necrosis factor-α by Müller cells [39].

Insulin-like growth factors (IGFs) and pigment epithelium-derived factor (PEDF) are other neurotrophic factors that may be involved in the pathogenesis of DR. Indeed, reduced levels of IGF-1 mRNA have been found in the eye in early stages of clinical and experimental diabetes [40], although it should be noted that increased levels of IGF-1 have been detected in the vitreous fluid of diabetic subjects with DR, where IGF-1 may accelerate neovascular changes [41]. PEDF has been described as an endogenous multifunctional protein with neuroprotective functions [42]; intraocular levels of PEDF in diabetic patients are decreased, suggesting reduced PEDF expression may contribute to the development of DR [43].

The neuropeptide somatostatin (SST), which, together with its receptors, is known to be expressed in the retina [44], is likely to represent one of the most important factors affected by DR, in view of the potent protective effects observed upon somatostatinergic activation in diseased retinas (discussed below). The retinal expression of SST is markedly down-regulated in the vitreous of patients with proliferative DR [45, 46] or with diabetic macular edema [47] and this SST reduction is associated with retinal neurodegeneration [48].

An additional endogenous neuroprotective factor that is worth mentioning is coenzyme Q10 (CoQ10), an essential cofactor of the electron transport chain. Its protective effect mainly depends on its free radical scavenger ability and on the regulation of mitochondrial permeability transition pore [49]. Choroid/retina levels of endogenous CoQ10 have been found to decrease with aging concomitant with progression of apoptosis-related retinal diseases [50].

4. EFFECTS OF THE ADMINISTRATION OF NEUROPROTECTIVE SUBSTANCES IN MODELS OF DIABETIC RETINOPATHY

In the last few years, a large body of experimental evidence is being gathered to exploit the potential of neuroprotection delivered in the early stages of DR as an efficacious strategy for the treatment of the disease. At present, there are a number of reports showing that administration of different neuroprotective substances of endogenous origin results in significant amelioration of the pathological findings in different experimental models of DR.

Rescuing decreased BDNF retinal levels in diabetic rats has been shown to significantly decrease cell death of amacrine cells [51]. Similarly, neuroprotection of retinal ganglion cells has been obtained by inducing BDNF overexpression in rats with experimental diabetes [52]. Although it is not clear whether NGF is produced by retinal neurons, its administration in diabetic rats prevents ganglion cell and Müller cell apoptosis, pericyte loss and acellular capillary formation [53].

The disruption of insulin homeostasis is known to be one of the main pathological features of diabetes. Subconjunctival administrations of insulin have been demonstrated to reduce the rate of retinal cell death in rats with experimental diabetes [54]. Similarly, treatment with IGF-1 analogs has been observed to prevent early retinal biochemical abnormalities implicated in the progression of DR [55].

The administration of PEDF represents another neuroprotective and antiangiogenic treatment obtained with molecules of endogenous origin. PEDF has been observed to possess antioxidant, anti-inflammatory, and neuroprotective properties that may be at the basis of its protective actions in DR [56]. Indeed, systemic administration of PEDF to diabetic rats prevents Müller cell activation and retinal dysfunction [57], while intravitreal PEDF effectively reduces VEGF-induced vascular permeability in a mouse model of nonproliferative DR [58]. Intravitreal PEDF also upregulates glutamine synthetase and glutamate transporter expression, and decreases retinal glutamate levels in hypoxia [59]. Finally, the topical administration (through eye drops) of PEDF derivatives (the antiangiogenic PEDF60–77 and the neuroprotective PEDF78–121) has been reported to reduce neurodegeneration and microvascular leakage in the diabetic Ins2(Akita) mouse [60].

SST, acting at somatostatin subtype receptor 2 (sst2) expressed in the retina, may control glutamate release [61-65], suggesting that SST is likely to protect neurons from apoptosis caused by excessive extracellular glutamate levels in DR. Indeed, topical administration of SST has been proven to prevent retinal neurodegeneration in diabetic rats [64], while administration of SST or of some of its analogs to ischemic retinas has been found to reduce ischemia-induced VEGF up-regulation [66, 67]. In particular, the specific sst2 analogue octreotide (OCT) is effective in counteracting oxidative stress and cell death in an ischemic *ex vivo* retina model [62, 65, 68]. OCT has also been shown to be very efficient in reducing high glucose-induced cell death as well as VEGF over-production in an *ex-vivo* model of early DR [29].

Similar to SST, another peptide, pituitary adenylate cyclase-activating peptide (PACAP), is raising interest because of its recognized, strong neuroprotective effects exerted in mammalian retinas [69]. PACAP efficacy in counteracting oxidative stress-induced neuronal suffering in ischemic retinas has been reported both *in vivo* and *in vitro* [65, 70], while potent neuroprotective effects have been demonstrated in an *ex-vivo* model of early DR, where PACAP treatment has also been found to reduce high glucose-induced VEGF production [29].

CoQ10 may also constitute an interesting candidate to confer neuroprotection to the retina in DR. Indeed, CoQ10 is currently used as neuroprotective agent in a series of neurodegenerative diseases [71], and CoQ10 topical administrations (*via* eye drops) have been shown to efficiently protect retinal ganglion cells commissioned to apoptosis by a variety of noxious stimuli [72, 73].

5. LIMITATIONS IN THE ADMINISTRATION OF NEUROPROTECTIVE SUBSTANCES

Drug administration for the treatment of ocular pathologies is a crucial point for the transition from experimental work in the laboratory to drug testing in clinical trials. The majority of the drugs tested for proliferative retinopathies show several limitations, in terms of ocular drug bioavailability, depending on the administration modalities [74]. The presence of melanin in the choroid/retinal pigment epithelium (RPE) [75] should also be considered, since melanin interactions with basic and lipophilic drugs may alter ocular drug disposition [76]. This may significantly reduce the availability of free drug at the target site and lower drug activity [77]. In addition, as a consequence of melanin binding, chronic dosing may induce accumulation of drugs in the choroid/RPE and may cause toxicity, as reported, for instance, in the case of melanin binding to chloroquine [78].

The systemic administration represents a non-invasive and simple treatment modality. However, several restrictions may apply. In particular, systemic administration of neuroprotective substances of endogenous origin could affect the normal homeostatic processes in other organs resulting in severe side effects. For instance, in diabetic patients the systemic administration of NGF to ameliorate neuropathic symptoms provoked hyperalgesia at the injection site, myalgia and arthralgia [79]. Similarly, long-term systemic administration of OCT has been established to provoke gastritis, damage of the gastric mucosa, and focal atrophy in acromegalic patients [80]. In addition, important limitations in ocular drug bioavailability are also detected. Indeed, systemically administered drugs may find serious problems in reaching the posterior segment of the eye because of the low permeability of the sclera or the cornea, and the presence of the blood-retinal barrier [81].

Therefore, for these reasons topical administration of drugs in ocular diseases is preferred. However, topical administration also shows many restrictions that may limit the efficacy of treatment. Typically, topical drug administration is performed by eye drops or intraocular injection. The use of eye drops is a noninvasive way to make drugs to reach ocular tissues. Nevertheless, the presence of several layers of permeation barriers starting from the tear film till the inner layers of the cornea makes it difficult to achieve the therapeutic concentrations in the target tissue within the eye [82]. In addition, the preservatives contained in the eye drop solution to prevent contamination, together with the high frequency of the treatment in ocular chronic diseases, have been reported to determine corneal dryness, inflammation, and damage [83].

The intraocular injection represents the most frequently used drug administration modality in both clinical and ex-

perimental treatments of ocular diseases. The intraocular direct injection of drugs ensures the achievement of effective concentrations in ocular tissues. However, the long-term maintenance of the effective drug levels can only be obtained with relatively frequent administrations, which represents the main limitation of this modality of drug delivery. Indeed, many severe adverse effects may occur after long-term and high frequency intraocular injections, like retinal detachment, endophthalmitis, cataract formation, ocular hypertension, submacular hemorrhage [84, 85]. Intravitreal implants allowing slow drug release may represent a solution to these problems. For instance, intravitreal implants for the delivery of corticosteroids, which are known to suppress inflammation, reduce vascular leakage, and inhibit vascular proliferation, have been approved by the FDA for the treatment of diabetic macular edema [86].

6. NANOPARTICLE TECHNOLOGY

A considerable amount of studies centered on the improvement of treatment efficacy have been performed in different areas of research. Indeed, with the development of novel therapeutic approaches for several diseases, the real limitation is represented by the administration, the carriage, and the delivery of drugs. Therefore, new systems for drug delivery have been developed to avoid adverse effects and to obtain high efficacy treatments. Drug delivery and related pharmaceutical development in the context of nanomedicine should be viewed as science and technology of complex systems at the nanometer scale (10 - 1,000 nm). These systems consist of at least two components, one of which is a pharmaceutically active ingredient and the other is the nanocarrier [87, 88], although NP formulations of the drug itself are also possible.

Among the various systems that have been proposed, biodegradable polymeric NPs have emerged as potential candidates for the development of carriers to target drugs toward specific sites in the body. The polymeric materials used for NP preparations are usually biocompatible, non-antigenic and highly hydrophilic in nature. The preparation of NPs from these biodegradable polymers has become a major area of interest in many fields, as these NPs can carry a variety of molecules, including proteins and nucleic acids in the naked form or in the form of pro-drugs, thereby conferring protection against degrading enzymes that are present in the extracellular milieu [89]. Several polymers can be used for NP preparation, such as, for instance, chitosan, gelatin hydrogels, sodium alginate, Poly(D,L-lactic-co-glycolic)acid (PLGA), polylactide (PLA), polyacrylamide, polyamidoamine dendrimers, or solid lipid NPs. Also inorganic nanoparticles such as gold and iron oxide NPs are popular drug carriers. The use of NP technology allows specific drug entrapment and enhances its long-term delivery and/or uptake by target cells reducing the time-dependent biodegradation and the general toxicity in non-targeted organs [90].

Pre-clinical studies demonstrated that nanocarriers can be successfully employed to improve drug bioavailability and decrease drug dosage, and several nanoformulations have been proposed for the treatment of glaucoma, corneal diseases, uveitis and retinal diseases [91].

7. APPLICATIONS OF NANOPARTICLES IN OCULAR DISEASES

Considerable efforts are being made to design NPs for use in the therapy of ocular diseases. A large quantity of studies searching for the appropriate formulations of functionalized NPs is focused on DR [92]. In particular, chitosan-coated PLGA nanoparticles functionalized with the anti-VEGF drug bevacizumab have been characterized recently [93]. Most studies are concerned with the design and the characterization of NPs in *in vitro* or in animal models, and, to our knowledge, there are no clinical trials at the moment for therapies based on NP-mediated intraocular drug delivery. The routes of administration of functionalized NPs may vary, although most of the studies are concerned with topical delivery, which is through eye drops or *via* intraocular injections.

Parenteral delivery of betamethasone phosphate encapsulated in biocompatible and biodegradable blended NPs of PLA homopolymers and polyethylene glycol (PEG)-block-PLA copolymers (stealth nanosteroids) reduced the clinical scores of rats with experimental autoimmune uveoretinitis and decreased the inflammatory cytokines in their retinas [94]. In other studies, a method of drug delivery to the tissues of the eye posterior segment has been established, in which aerosolized NPs were added to the gas during the gas exchange phase of vitrectomy surgery in porcine eyes. This method may have implications for improved delivery of a variety of pharmacotherapeutic drugs in the treatment of various ocular/retinal disorders [95].

Superficial barriers impede direct and systemic drug access through the eye to the specific site of action. NPs can be used to help drug delivery through eye drops, as they may improve topical passage of large, poorly water-soluble molecules through the barriers of the eye [96]. Favorable biological properties of functionalized NPs include elongation of the residence time of the drug within eye drops, decreased toxicity, and easier drug penetration that minimizes precorneal drug loss due to the rapid tear fluid turnover [97]. Drug delivery systems through eye drops based on hybrid polyamidoamine dendrimer hydrogel NPs functionalized for the codelivery of the two traditional anti-glaucoma drugs brimonidine and timolol maleate have been tested *in vitro* in human corneal epithelial cells, in isolated bovine corneas, and in rabbit eyes. These NPs showed no cytotoxic effects, improved drug transcorneal transport and extended drug release, thus promising enhanced drug bioavailability and suggesting they have the potential to improve patient compliance, reduce side effects, increase efficacy, and preserve sight in glaucoma patients [98, 99]. Moreover, formulation of dorzolamide hydrochloride and methazolamide-loaded solid lipid NPs in a nanoemulsion form have been tested in rabbits and the results showed that they may represent a new approach for a more intensive treatment of glaucoma, a decrease in the number of applications per day, and a better patient compliance compared to conventional eye drops [100].

Functionalized NPs may provide significant improvements for the delivery of drugs through intraocular injections. For instance, intravitreal nanogold injections in rabbits

showed no signs of retinal or optic nerve toxicity during histologic examination by light microscopy [101], suggesting the possibility of using NP technology to treat diseases of the posterior ocular segment. PEGylated liposome-protamine-hyaluronic acid NPs loaded with siRNA have been investigated for the treatment of choroidal neovascularization [102], while NP-mediated delivery of plasminogen kringle 5 has been found to reduce vascular permeability as well as the expression of different angiogenic and inflammatory cytokines with an inhibitory effect on choroidal neovascularization, thus indicating therapeutic potential for age-related macular degeneration (AMD) [103]. Furthermore, intravitreal injections of cerium oxide NPs have been found to inhibit the increase of reactive oxygen species and the formation of intraretinal and subretinal neovascular lesions in a mouse model of AMD [104], while intravitreal injections of gelatin NPs functionalized with basic fibroblast growth factor have been shown to prevent photoreceptor degeneration by inhibiting apoptosis in rat retinas [105].

Recently, it has been suggested that the binding of different drugs (functionalization) to magnetic NPs (MNPs) may represent a promising perspective for intraocular drug delivery [106]. The class of MNPs include metallic, bimetallic, and superparamagnetic iron oxide NPs [107]. This type of NP is particularly attractive due to its reactive surface that can be functionalized with biocompatible coatings, bioactive molecules or targeting-moieties, thus increasing their specificity toward cellular targets and preventing possible damaging interactions with healthy tissues [108-112]. In general, the magnetic properties of these NPs may reveal useful in several biomedical applications, as, for instance, in magnetic resonance imaging as contrast agents, in external induction of hyperthermia to treat neoplastic disease, or in drug delivery with the possibility to guide the functionalized MNPs using an external magnetic field [113]. MNPs are biodegradable (entering in the normal iron metabolism) [114], and therefore they are intrinsically safe. Different MNPs have FDA approval for clinical use, *e.g.*, Combidex® (Advanced Magnetic Inc., Cambridge, USA, MRI contrast agent for differentiation of metastatic and non-metastatic lymph nodes), Endorem® (Amag Pharmaceutical Inc., Cambridge, USA, MRI contrast agent for diagnosis of liver tumors), Resovist® (Bayer Schering Pharma AG, Berlin, Germany, MRI contrast agent for diagnosis of liver metastases and colon cancer), Feraheme® (Amag Pharmaceutical Inc., Cambridge, USA, indicated for the treatment of iron deficiency anemia in adult patients with chronic kidney disease), and others.

MNPs have been tested recently for their potential to function as nanocarriers in intraocular drug delivery. Although MNPs have not yet been tested on humans for ocular applications, their usage for treatment of eye diseases has been proposed (U.S. Patent 20130225906) [115] and there is evidence from studies in experimental animals that the iron oxide MNPs are non-toxic to the eye tissues [116]. In addition, studies in Xenopus and zebrafish have shown that intraocularly injected MNPs enter the retina rapidly and persistently localize within the RPE [117]. When functionalized with recombinant VEGF, the MNPs have been found to localize to the choroid [106], suggesting that the combination of MNPs with different bioactive molecules may induce cell- or tissue-specific targeting in the posterior segment of the eye.

8. FUNCTIONALIZED NANOPARTICLES FOR THE TREATMENT OF DIABETIC RETINOPATHY WITH NEUROPROTECTANTS

In the treatment of retinal diseases, time-related bioavailability of the drug may represent a serious limitation. In particular, despite their excellent efficacy in counteracting several pathological mechanisms of DR, the effects of neuroprotectants of endogenous origin are limited in time and repeated administrations would be necessary. Matching the efficacy of neuroprotectants with NP administration technology could be a way to overcome these limitations and significantly improve the treatment outcomes. Supporting this perspective, substantial evidence is available reporting the efficacy of NP carriages for intraocular administration of different pharmacologic agents, as reviewed above.

Several studies have been specifically addressed to the design and characterization of NP-based approaches for the treatment of DR [92]; however neuroprotection in the diabetic retina does not seem to be the primary goal of these investigations. To test the possibility of using NPs to deliver neuroprotection in the diabetic retina, we are currently investigating the efficacy of the administration of MNPs functionalized with OCT in different models of experimental DR. Our preliminary data indicate that the functionalization process does not affect OCT bioactivity *in vitro*, as evidenced in standard assays using human retinal endothelial cells. In these tests, the OCT-functionalized MNPs displayed the same efficacy as free OCT in inhibiting VEGF-induced cell proliferation, migration, and tube formation. In addition, in

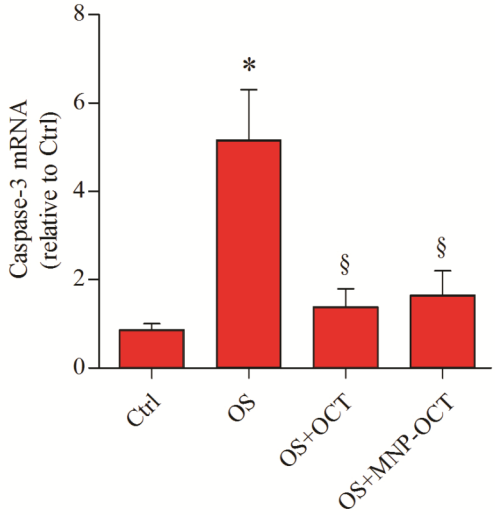

Fig. (2). Mouse retinal explants were subjected to oxidative stress (OS) using 100 μM H_2O_2 in the incubation medium, as previously described [29], for 3 days. The protective effects of 1 μM OCT or of 1μM OCT-functionalized MNPs (MNP-OCT) were evaluated using caspase-3 mRNA as an apoptotic marker. Caspase-3 mRNA expression was measured with qPCR. Values are expressed as mean ± SEM. *$p<0.05$ vs control (Ctrl); §$p<0.05$ vs OS.

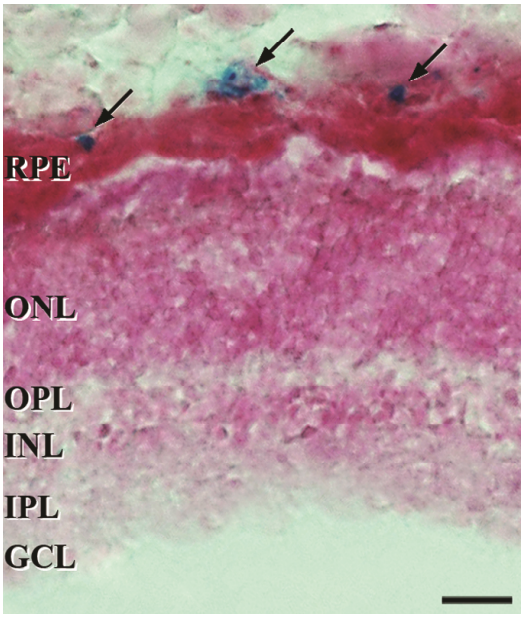

Fig. (3). Coronal section of a mouse retina 24 hours after intraocular injection (1µl) of 1 mM MNP-OCT. Arrows point to MNP-OCT localized to the RPE. Whole mouse eyes were fixed in 4% paraformaldehyde for 2 h, cryoprotected with 20% sucrose and sectioned (10 µm) with a cryostat. The sections were stained with Prussian Blue according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, USA), after a treatment of pigment bleaching in 5% formamide-1% hydrogen peroxide in the presence of cold light. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer. Scale bar, 20 µm.

ex vivo retinal explants, OCT-functionalized MNPs have been observed to reduce retinal cell apoptosis induced by oxidative stress as efficiently as free OCT (Fig. **2**). We also found that, after intraocular injection in mice, OCT is released from MNPs in a graded, time-dependent manner presumably in relation with the biodegradation of the carrier. Finally, we have observed that OCT-functionalized MNPs intraocularly injected in mice localize to the RPE after 24 hours from injection (Fig. **3**). Similar investigations are programmed involving the neurotrophin NGF and the neuropeptide PACAP.

CONCLUSION

In summary, a review of the literature, together with our preliminary findings showing unaltered bioactivity of OCT-functionalized MNPs and their specific localization in the retina after intraocular injection, suggests that the application of NP technology to intraocular administration of neuroprotective drugs for the treatment of DR could be a relevant reply to actual treatment restrictions. The matching of novel neuroprotective drugs with the improvement of nanocarriers could represent an innovation toward a next-generation treatment of DR.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by grants from the Italian Ministry of Education, from the University of Pisa (PRA-2016; Pisa, Italy), and from the Italian Space Agency (ASI).

REFERENCES

[1] Abcouwer, S.F.; Gardner, T.W. Diabetic retinopathy: loss of neuroretinal adaptation to the diabetic metabolic environment. *Ann. N. Y. Acad. Sci.,* **2014**, *1311*, 174-190. [http://dx.doi.org/10.1111/nyas.12412] [PMID: 24673341]

[2] Hernández, C.; Dal Monte, M.; Simó, R.; Casini, G. Neuroprotection as a therapeutic target for diabetic retinopathy. *J. Diabetes Res.,* **2016**, *2016*, 9508541. [http://dx.doi.org/10.1155/2016/9508541] [PMID: 27123463]

[3] Ola, M.S.; Nawaz, M.I.; Siddiquei, M.M.; Al-Amro, S.; Abu El-Asrar, A.M. Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. *J. Diabetes Complications,* **2012**, *26*(1), 56-64. [http://dx.doi.org/10.1016/j.jdiacomp.2011.11.004] [PMID: 22226482]

[4] Tarr, J.M.; Kaul, K.; Chopra, M.; Kohner, E.M.; Chibber, R. Pathophysiology of diabetic retinopathy. *ISRN Ophthalmol.,* **2013**, *2013*, 343560. [http://dx.doi.org/10.1155/2013/343560] [PMID: 24563789]

[5] Ajlan, R.S.; Silva, P.S.; Sun, J.K. Vascular endothelial growth factor and diabetic retinal disease. *Semin. Ophthalmol.,* **2016**, *31*(1-2), 40-48. [http://dx.doi.org/10.3109/08820538.2015.1114833] [PMID: 26959128]

[6] Newman, E.A. Glial cell regulation of neuronal activity and blood flow in the retina by release of gliotransmitters. *Philos. Trans. R. Soc. Lond. B Biol. Sci.,* **2015**, *370*(1672), 370. [http://dx.doi.org/10.1098/rstb.2014.0195] [PMID: 26009774]

[7] Adams, A.J.; Bearse, M.A., Jr Retinal neuropathy precedes vasculopathy in diabetes: a function-based opportunity for early treatment intervention? *Clin. Exp. Optom.,* **2012**, *95*(3), 256-265. [http://dx.doi.org/10.1111/j.1444-0938.2012.00733.x] [PMID: 22497728]

[8] Jindal, V. Neurodegeneration as a primary change and role of neuroprotection in diabetic retinopathy. *Mol. Neurobiol.,* **2015**, *51*(3), 878-884. [http://dx.doi.org/10.1007/s12035-014-8732-7] [PMID: 24826918]

[9] Jonsson, K.B.; Frydkjaer-Olsen, U.; Grauslund, J. Vascular changes and neurodegeneration in the early stages of diabetic retinopathy: Which comes first? *Ophthalmic Res.,* **2016**, *56*(1), 1-9. [http://dx.doi.org/10.1159/000444498] [PMID: 27035578]

[10] Simó, R.; Hernández, C. Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence. *Prog. Retin. Eye Res.,* **2015**, *48*, 160-180. [http://dx.doi.org/10.1016/j.preteyeres.2015.04.003] [PMID: 25936649]

[11] Sohn, E.H.; van Dijk, H.W.; Jiao, C.; Kok, P.H.; Jeong, W.; Demirkaya, N.; Garmager, A.; Wit, F.; Kucukevcilioglu, M.; van Velthoven, M.E.; DeVries, J.H.; Mullins, R.F.; Kuehn, M.H.; Schlingemann, R.O.; Sonka, M.; Verbraak, F.D.; Abràmoff, M.D. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. *Proc. Natl. Acad. Sci. USA,* **2016**, *113*(19), E2655-E2664. [http://dx.doi.org/10.1073/pnas.1522014113] [PMID: 27114552]

[12] Zhang, X.; Wang, N.; Barile, G.R.; Bao, S.; Gillies, M. Diabetic retinopathy: neuron protection as a therapeutic target. *Int. J. Biochem. Cell Biol.,* **2013**, *45*(7), 1525-1529. [http://dx.doi.org/10.1016/j.biocel.2013.03.002] [PMID: 23506699]

[13] Barber, A.J. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2003**, *27*(2), 283-290. [http://dx.doi.org/10.1016/S0278-5846(03)00023-X] [PMID: 12657367]

[14] Peyman, G.A.; Lad, E.M.; Moshfeghi, D.M. Intravitreal injection of therapeutic agents. *Retina,* **2009**, *29*(7), 875-912. [http://dx.doi.org/10.1097/IAE.0b013e3181a94f01] [PMID: 19584648]

[15] Wilczewska, A.Z.; Niemirowicz, K.; Markiewicz, K.H.; Car, H. Nanoparticles as drug delivery systems. *Pharmacol. Rep.,* **2012**, *64*(5), 1020-1037. [http://dx.doi.org/10.1016/S1734-1140(12)70901-5] [PMID: 23238461]

[16] Kowluru, R.A.; Engerman, R.L.; Case, G.L.; Kern, T.S. Retinal glutamate in diabetes and effect of antioxidants. *Neurochem. Int.,* **2001**, *38*(5), 385-390. [http://dx.doi.org/10.1016/S0197-0186(00)00112-1] [PMID: 11222918]

[17] Lieth, E.; Barber, A.J.; Xu, B.; Dice, C.; Ratz, M.J.; Tanase, D.; Strother, J.M. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. *Diabetes,* **1998**, *47*(5), 815-820. [http://dx.doi.org/10.2337/diabetes.47.5.815] [PMID: 9588455]

[18] Lieth, E.; LaNoue, K.F.; Antonetti, D.A.; Ratz, M. Diabetes reduces glutamate oxidation and glutamine synthesis in the retina. *Exp. Eye Res.,* **2000**, *70*(6), 723-730. [http://dx.doi.org/10.1006/exer.2000.0840] [PMID: 10843776]

[19] Castilho, A.F.; Liberal, J.T.; Baptista, F.I.; Gaspar, J.M.; Carvalho, A.L.; Ambrósio, A.F. Elevated glucose concentration changes the content and cellular localization of AMPA receptors in the retina but not in the hippocampus. *Neuroscience,* **2012**, *219*, 23-32. [http://dx.doi.org/10.1016/j.neuroscience.2012.05.056] [PMID: 22659015]

[20] Santiago, A.R.; Gaspar, J.M.; Baptista, F.I.; Cristóvão, A.J.; Santos, P.F.; Kamphuis, W.; Ambrósio, A.F. Diabetes changes the levels of ionotropic glutamate receptors in the rat retina. *Mol. Vis.,* **2009**, *15*, 1620-1630. [PMID: 19693289]

[21] Santiago, A.R.; Rosa, S.C.; Santos, P.F.; Cristóvão, A.J.; Barber, A.J.; Ambrósio, A.F. Elevated glucose changes the expression of ionotropic glutamate receptor subunits and impairs calcium homeostasis in retinal neural cells. *Invest. Ophthalmol. Vis. Sci.,* **2006**, *47*(9), 4130-4137. [http://dx.doi.org/10.1167/iovs.06-0085] [PMID: 16936133]

[22] Li, Q.; Puro, D.G. Diabetes-induced dysfunction of the glutamate transporter in retinal Müller cells. *Invest. Ophthalmol. Vis. Sci.,* **2002**, *43*(9), 3109-3116. [PMID: 12202536]

[23] Chen, M.; Curtis, T.M.; Stitt, A.W. Advanced glycation end products and diabetic retinopathy. *Curr. Med. Chem.,* **2013**, *20*(26), 3234-3240. [http://dx.doi.org/10.2174/09298673113209990025] [PMID: 23745547]

[24] Zong, H.; Ward, M.; Stitt, A.W. AGEs, RAGE, and diabetic retinopathy. *Curr. Diab. Rep.,* **2011**, *11*(4), 244-252. [http://dx.doi.org/10.1007/s11892-011-0198-7] [PMID: 21590515]

[25] Nitti, M.; Furfaro, A.L.; Traverso, N.; Odetti, P.; Storace, D.; Cottalasso, D.; Pronzato, M.A.; Marinari, U.M.; Domenicotti, C. PKC delta and NADPH oxidase in AGE-induced neuronal death. *Neurosci. Lett.,* **2007**, *416*(3), 261-265. [http://dx.doi.org/10.1016/j.neulet.2007.02.013] [PMID: 17317001]

[26] Zong, H.; Ward, M.; Madden, A.; Yong, P.H.; Limb, G.A.; Curtis, T.M.; Stitt, A.W. Hyperglycaemia-induced pro-inflammatory responses by retinal Müller glia are regulated by the receptor for advanced glycation end-products (RAGE). *Diabetologia,* **2010**, *53*(12), 2656-2666. [http://dx.doi.org/10.1007/s00125-010-1900-z] [PMID: 20835858]

[27] Lecleire-Collet, A.; Tessier, L.H.; Massin, P.; Forster, V.; Brasseur, G.; Sahel, J.A.; Picaud, S. Advanced glycation end products can induce glial reaction and neuronal degeneration in retinal explants. *Br. J. Ophthalmol.,* **2005**, *89*(12), 1631-1633. [http://dx.doi.org/10.1136/bjo.2005.079491] [PMID: 16299145]

[28] Reber, F.; Geffarth, R.; Kasper, M.; Reichenbach, A.; Schleicher, E.D.; Siegner, A.; Funk, R.H. Graded sensitiveness of the various retinal neuron populations on the glyoxal-mediated formation of advanced glycation end products and ways of protection. *Graefes Arch. Clin. Exp. Ophthalmol.,* **2003**, *241*(3), 213-225. [http://dx.doi.org/10.1007/s00417-002-0528-1] [PMID: 12644946]

[29] Amato, R.; Biagioni, M.; Cammalleri, M.; Dal Monte, M.; Casini, G. VEGF as a Survival Factor in Ex Vivo Models of Early Diabetic Retinopathy. *Invest. Ophthalmol. Vis. Sci.,* **2016**, *57*(7), 3066-3076. [http://dx.doi.org/10.1167/iovs.16-19285] [PMID: 27286364]

[30] Kowluru, R.A. Diabetes-induced elevations in retinal oxidative stress, protein kinase C and nitric oxide are interrelated. *Acta Diabetol.,* **2001**, *38*(4), 179-185. [http://dx.doi.org/10.1007/s592-001-8076-6] [PMID: 11855796]

[31] Zheng, L.; Kern, T.S. Role of nitric oxide, superoxide, peroxynitrite and PARP in diabetic retinopathy. *Front. Biosci.,* **2009**, *14*, 3974-3987. [http://dx.doi.org/10.2741/3505] [PMID: 19273327]

[32] Coucha, M.; Elshaer, S.L.; Eldahshan, W.S.; Mysona, B.A.; El-Remessy, A.B. Molecular mechanisms of diabetic retinopathy: potential therapeutic targets. *Middle East Afr. J. Ophthalmol.,* **2015**, *22*(2), 135-144. [http://dx.doi.org/10.4103/0974-9233.154386] [PMID: 25949069]

[33] Behl, T.; Kaur, I.; Kotwani, A. Implication of oxidative stress in progression of diabetic retinopathy. *Surv. Ophthalmol.,* **2015**. [PMID: 26074354]

[34] Piperi, C.; Goumenos, A.; Adamopoulos, C.; Papavassiliou, A.G. AGE/RAGE signalling regulation by miRNAs: associations with diabetic complications and therapeutic potential. *Int. J. Biochem. Cell Biol.,* **2015**, *60*, 197-201. [http://dx.doi.org/10.1016/j.biocel.2015.01.009] [PMID: 25603271]

[35] Sasaki, M.; Ozawa, Y.; Kurihara, T.; Kubota, S.; Yuki, K.; Noda, K.; Kobayashi, S.; Ishida, S.; Tsubota, K. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia,* **2010**, *53*(5), 971-979. [http://dx.doi.org/10.1007/s00125-009-1655-6] [PMID: 20162412]

[36] Al-Gayyar, M.M.; Matragoon, S.; Pillai, B.A.; Ali, T.K.; Abdelsaid, M.A.; El-Remessy, A.B. Epicatechin blocks pro-nerve growth factor (proNGF)-mediated retinal neurodegeneration *via* inhibition of p75 neurotrophin receptor expression in a rat model of diabetes. *Diabetologia,* **2011**, *54*(3), 669-680. [corrected]. [http://dx.doi.org/10.1007/s00125-010-1994-3] [PMID: 21136036]

[37] Ali, T.K.; Matragoon, S.; Pillai, B.A.; Liou, G.I.; El-Remessy, A.B. Peroxynitrite mediates retinal neurodegeneration by inhibiting nerve growth factor survival signaling in experimental and human diabetes. *Diabetes,* **2008**, *57*(4), 889-898. [http://dx.doi.org/10.2337/db07-1669] [PMID: 18285558]

[38] Mysona, B.A.; Al-Gayyar, M.M.; Matragoon, S.; Abdelsaid, M.A.; El-Azab, M.F.; Saragovi, H.U.; El-Remessy, A.B. Modulation of p75(NTR) prevents diabetes- and proNGF-induced retinal inflammation and blood-retina barrier breakdown in mice and rats. *Diabetologia,* **2013**, *56*(10), 2329-2339. [http://dx.doi.org/10.1007/s00125-013-2998-6] [PMID: 23918145]

[39] Mysona, B.A.; Shanab, A.Y.; Elshaer, S.L.; El-Remessy, A.B. Nerve growth factor in diabetic retinopathy: beyond neurons. *Expert Rev. Ophthalmol.,* **2014**, *9*(2), 99-107. [http://dx.doi.org/10.1586/17469899.2014.903157] [PMID: 25031607]

[40] Gerhardinger, C.; McClure, K.D.; Romeo, G.; Podestà, F.; Lorenzi, M. IGF-I mRNA and signaling in the diabetic retina. *Diabetes,* **2001**, *50*(1), 175-183. [http://dx.doi.org/10.2337/diabetes.50.1.175] [PMID: 11147784]

[41] Iwona, B.S. Growth factors in the pathogenesis of retinal neurodegeneration in diabetes mellitus. *Curr. Neuropharmacol.,* **2016**, *14*(8), 792-804. [http://dx.doi.org/10.2174/1570159X14666160813182009] [PMID: 27528260]

[42] Barnstable, C.J.; Tombran-Tink, J. Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Prog. Retin. Eye Res.,* **2004**, *23*(5), 561-577. [http://dx.doi.org/10.1016/j.preteyeres.2004.05.002] [PMID: 15302351]

[43] Zheng, B.; Li, T.; Chen, H.; Xu, X.; Zheng, Z. Correlation between ficolin-3 and vascular endothelial growth factor-to-pigment epithelium-derived factor ratio in the vitreous of eyes with proliferative diabetic retinopathy. *Am. J. Ophthalmol.,* **2011**, *152*(6), 1039-1043. [http://dx.doi.org/10.1016/j.ajo.2011.05.022] [PMID: 21851923]

[44] Casini, G.; Catalani, E.; Dal Monte, M.; Bagnoli, P. Functional aspects of the somatostatinergic system in the retina and the potential therapeutic role of somatostatin in retinal disease. *Histol. Histopathol.,* **2005**, *20*(2), 615-632. [PMID: 15736065]

[45] Hernández, C.; Carrasco, E.; Casamitjana, R.; Deulofeu, R.; García-Arumí, J.; Simó, R. Somatostatin molecular variants in the vitreous fluid: a comparative study between diabetic patients with proliferative diabetic retinopathy and nondiabetic control subjects. *Diabetes Care,* **2005**, *28*(8), 1941-1947. [http://dx.doi.org/10.2337/diacare.28.8.1941] [PMID: 16043736]

[46] Simó, R.; Lecube, A.; Sararols, L.; García-Arumí, J.; Segura, R.M.; Casamitjana, R.; Hernández, C. Deficit of somatostatin-like immunoreactivity in the vitreous fluid of diabetic patients: possible role in the development of proliferative diabetic retinopathy. *Diabetes Care,* **2002**, *25*(12), 2282-2286. [http://dx.doi.org/10.2337/diacare.25.12.2282] [PMID: 12453974]

[47] Simó, R.; Carrasco, E.; Fonollosa, A.; García-Arumí, J.; Casamitjana, R.; Hernández, C. Deficit of somatostatin in the vitreous fluid of patients with diabetic macular edema. *Diabetes Care,* **2007**,

30(3), 725-727. [http://dx.doi.org/10.2337/dc06-1345] [PMID: 17327350]

[48] Carrasco, E.; Hernández, C.; Miralles, A.; Huguet, P.; Farrés, J.; Simó, R. Lower somatostatin expression is an early event in diabetic retinopathy and is associated with retinal neurodegeneration. *Diabetes Care,* **2007**, *30*(11), 2902-2908. [http://dx.doi.org/10.2337/ dc07-0332] [PMID: 17704349]

[49] Papucci, L.; Schiavone, N.; Witort, E.; Donnini, M.; Lapucci, A.; Tempestini, A.; Formigli, L.; Zecchi-Orlandini, S.; Orlandini, G.; Carella, G.; Brancato, R.; Capaccioli, S. Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property. *J. Biol. Chem.,* **2003**, *278*(30), 28220-28228. [http://dx.doi.org/10.1074/jbc.M302297200] [PMID: 12736273]

[50] Qu, J.; Kaufman, Y.; Washington, I. Coenzyme Q10 in the human retina. *Invest. Ophthalmol. Vis. Sci.,* **2009**, *50*(4), 1814-1818. [http://dx.doi.org/10.1167/iovs.08-2656] [PMID: 19060288]

[51] Seki, M.; Tanaka, T.; Nawa, H.; Usui, T.; Fukuchi, T.; Ikeda, K.; Abe, H.; Takei, N. Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factor for dopaminergic amacrine cells. *Diabetes,* **2004**, *53*(9), 2412-2419. [http://dx.doi.org/10.2337/diabetes.53.9.2412] [PMID: 15331553]

[52] Gong, Y.; Chang, Z.P.; Ren, R.T.; Wei, S.H.; Zhou, H.F.; Chen, X.F.; Hou, B.K.; Jin, X.; Zhang, M.N. Protective effects of adeno-associated virus mediated brain-derived neurotrophic factor expression on retinal ganglion cells in diabetic rats. *Cell. Mol. Neurobiol.,* **2012**, *32*(3), 467-475. [http://dx.doi.org/10.1007/s10571-011-9779-x] [PMID: 22297541]

[53] Hammes, H.P.; Federoff, H.J.; Brownlee, M. Nerve growth factor prevents both neuroretinal programmed cell death and capillary pathology in experimental diabetes. *Mol. Med.,* **1995**, *1*(5), 527-534. [PMID: 8529118]

[54] Fort, P.E.; Losiewicz, M.K.; Reiter, C.E.; Singh, R.S.; Nakamura, M.; Abcouwer, S.F.; Barber, A.J.; Gardner, T.W. Differential roles of hyperglycemia and hypoinsulinemia in diabetes induced retinal cell death: evidence for retinal insulin resistance. *PLoS One,* **2011**, *6*(10), e26498. [http://dx.doi.org/10.1371/journal.pone.0026498] [PMID: 22046295]

[55] Kummer, A.; Pulford, B.E.; Ishii, D.N.; Seigel, G.M. Des(1-3)IGF-1 treatment normalizes type 1 IGF receptor and phospho-Akt (Thr 308) immunoreactivity in predegenerative retina of diabetic rats. *Int J Exp Diabesity Res,* **2003**, *4*(1), 45-57. [http://dx.doi.org/10.1080/15438600303729] [PMID: 12745670]

[56] Elahy, M.; Baindur-Hudson, S.; Cruzat, V.F.; Newsholme, P.; Dass, C.R. Mechanisms of PEDF-mediated protection against reactive oxygen species damage in diabetic retinopathy and neuropathy. *J. Endocrinol.,* **2014**, *222*(3), R129-R139. [http://dx.doi.org/10.1530/JOE-14-0065] [PMID: 24928938]

[57] Yoshida, Y.; Yamagishi, S.; Matsui, T.; Jinnouchi, Y.; Fukami, K.; Imaizumi, T.; Yamakawa, R. Protective role of pigment epithelium-derived factor (PEDF) in early phase of experimental diabetic retinopathy. *Diabetes Metab. Res. Rev.,* **2009**, *25*(7), 678-686. [http://dx.doi.org/10.1002/dmrr.1007] [PMID: 19685553]

[58] Liu, H.; Ren, J.G.; Cooper, W.L.; Hawkins, C.E.; Cowan, M.R.; Tong, P.Y. Identification of the antivasopermeability effect of pigment epithelium-derived factor and its active site. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(17), 6605-6610. [http://dx.doi.org/10.1073/pnas.0308342101] [PMID: 15096582]

[59] Wang, Y.; Lu, Q.; Gao, S.; Zhu, Y.; Gao, Y.; Xie, B.; Shen, X. Pigment epithelium-derived factor regulates glutamine synthetase and l-glutamate/l-aspartate transporter in retinas with oxygen-induced retinopathy. *Curr. Eye Res.,* **2015**, *40*(12), 1232-1244. [http://dx.doi.org/10.3109/02713683.2014.990639] [PMID: 25548969]

[60] Liu, Y.; Leo, L.F.; McGregor, C.; Grivitishvili, A.; Barnstable, C.J.; Tombran-Tink, J. Pigment epithelium-derived factor (PEDF) peptide eye drops reduce inflammation, cell death and vascular leakage in diabetic retinopathy in Ins2(Akita) mice. *Mol. Med.,* **2012**, *18*, 1387-1401. [http://dx.doi.org/10.2119/molmed.2012.00008] [PMID: 23019073]

[61] Catalani, E.; Cervia, D.; Martini, D.; Bagnoli, P.; Simonetti, E.; Timperio, A.M.; Casini, G. Changes in neuronal response to ischemia in retinas with genetic alterations of somatostatin receptor expression. *Eur. J. Neurosci.,* **2007**, *25*(5), 1447-1459. [http://dx.doi.org/10.1111/j.1460-9568.2007.05419.x] [PMID: 17425570]

[62] Cervia, D.; Martini, D.; Ristori, C.; Catalani, E.; Timperio, A.M.; Bagnoli, P.; Casini, G. Modulation of the neuronal response to ischaemia by somatostatin analogues in wild-type and knock-out mouse retinas. *J. Neurochem.,* **2008**, *106*(5), 2224-2235. [http://dx.doi.org/10.1111/j.1471-4159.2008.05556.x] [PMID: 18624922]

[63] Dal Monte, M.; Petrucci, C.; Cozzi, A.; Allen, J.P.; Bagnoli, P. Somatostatin inhibits potassium-evoked glutamate release by activation of the sst(2) somatostatin receptor in the mouse retina. *Naunyn Schmiedebergs Arch. Pharmacol.,* **2003**, *367*(2), 188-192. [http://dx.doi.org/10.1007/s00210-002-0662-7] [PMID: 12595961]

[64] Hernández, C.; García-Ramírez, M.; Corraliza, L.; Fernández-Carneado, J.; Farrera-Sinfreu, J.; Ponsati, B.; González-Rodríguez, A.; Valverde, A.M.; Simó, R. Topical administration of somatostatin prevents retinal neurodegeneration in experimental diabetes. *Diabetes,* **2013**, *62*(7), 2569-2578. [http://dx.doi.org/10.2337/db12-0926] [PMID: 23474487]

[65] D'Alessandro, A.; Cervia, D.; Catalani, E.; Gevi, F.; Zolla, L.; Casini, G. Protective effects of the neuropeptides PACAP, substance P and the somatostatin analogue octreotide in retinal ischemia: a metabolomic analysis. *Mol. Biosyst.,* **2014**, *10*(6), 1290-1304. [http://dx.doi.org/10.1039/c3mb70362b] [PMID: 24514073]

[66] Cervia, D.; Catalani, E.; Dal Monte, M.; Casini, G. Vascular endothelial growth factor in the ischemic retina and its regulation by somatostatin. *J. Neurochem.,* **2012**, *120*(5), 818-829. [http://dx.doi.org/10.1111/j.1471-4159.2011.07622.x] [PMID: 22168912]

[67] Mei, S.; Cammalleri, M.; Azara, D.; Casini, G.; Bagnoli, P.; Dal Monte, M. Mechanisms underlying somatostatin receptor 2 down-regulation of vascular endothelial growth factor expression in response to hypoxia in mouse retinal explants. *J. Pathol.,* **2012**, *226*(3), 519-533. [http://dx.doi.org/10.1002/path.3006] [PMID: 21960021]

[68] Wang, J.; Sun, Z.; Shen, J.; Wu, D.; Liu, F.; Yang, R.; Ji, S.; Ji, A.; Li, Y. Octreotide protects the mouse retina against ischemic reperfusion injury through regulation of antioxidation and activation of NF-κB. *Oxid. Med. Cell. Longev.,* **2015**, *2015*, 970156. [http://dx.doi.org/10.1155/2015/970156] [PMID: 26175842]

[69] Atlasz, T.; Szabadfi, K.; Kiss, P.; Racz, B.; Gallyas, F.; Tamas, A.; Gaal, V.; Marton, Z.; Gabriel, R.; Reglodi, D. Pituitary adenylate cyclase activating polypeptide in the retina: focus on the retinoprotective effects. *Ann. N. Y. Acad. Sci.,* **2010**, *1200*, 128-139. [http://dx.doi.org/10.1111/j.1749-6632.2010.05512.x] [PMID: 20633141]

[70] Werling, D.; Reglodi, D.; Banks, W.A.; Salameh, T.S.; Kovacs, K.; Kvarik, T.; Vaczy, A.; Kovacs, L.; Mayer, F.; Danyadi, B.; Lokos, E.; Tamas, A.; Toth, G.; Biro, Z.; Atlasz, T. Ocular Delivery of PACAP1-27 Protects the Retina From Ischemic Damage in Rodents. *Invest. Ophthalmol. Vis. Sci.,* **2016**, *57*(15), 6683-6691. [http://dx.doi.org/10.1167/iovs.16-20630] [PMID: 27936470]

[71] Yang, X.; Zhang, Y.; Xu, H.; Luo, X.; Yu, J.; Liu, J.; Chang, R.C. Neuroprotection of coenzyme Q10 in neurodegenerative diseases. *Curr. Top. Med. Chem.,* **2016**, *16*(8), 858-866. [http://dx.doi.org/10.2174/1568026615666150827095252] [PMID: 26311425]

[72] Lulli, M.; Witort, E.; Papucci, L.; Torre, E.; Schiavone, N.; Dal Monte, M.; Capaccioli, S. Coenzyme Q10 protects retinal cells from apoptosis induced by radiation *in vitro* and *in vivo*. *J. Radiat. Res. (Tokyo),* **2012**, *53*(5), 695-703. [http://dx.doi.org/10.1093/jrr/rrs025] [PMID: 22843363]

[73] Lulli, M.; Witort, E.; Papucci, L.; Torre, E.; Schipani, C.; Bergamini, C.; Dal Monte, M.; Capaccioli, S. Coenzyme Q10 instilled as eye drops on the cornea reaches the retina and protects retinal layers from apoptosis in a mouse model of kainate-induced retinal damage. *Invest. Ophthalmol. Vis. Sci.,* **2012**, *53*(13), 8295-8302. [http://dx.doi.org/10.1167/iovs.12-10374] [PMID: 23154463]

[74] Yavuz, B.; Kompella, U.B. Ocular Drug Delivery. *Handb. Exp. Pharmacol.,* **2016**, *26*, 26. [PMID: 27783270]

[75] Durairaj, C.; Chastain, J.E.; Kompella, U.B. Intraocular distribution of melanin in human, monkey, rabbit, minipig and dog eyes. *Exp. Eye Res.,* **2012**, *98*, 23-27. [http://dx.doi.org/10.1016/j.exer.2012.03.004] [PMID: 22440812]

[76] Leblanc, B.; Jezequel, S.; Davies, T.; Hanton, G.; Taradach, C. Binding of drugs to eye melanin is not predictive of ocular toxicity. *Regul. Toxicol. Pharmacol.,* **1998**, *28*(2), 124-132. [http://dx.doi.org/10.1006/rtph.1998.1243] [PMID: 9927562]

[77] Schoenwald, R.D.; Tandon, V.; Wurster, D.E.; Barfknecht, C.F. Significance of melanin binding and metabolism in the activity of 5-acetoxyacetylimino-4-methyl-delta2-1,3,4,-thiadiazolin e-2-

sulfonamide. *Eur. J. Pharm. Biopharm.,* **1998**, *46*(1), 39-50. [http://dx.doi.org/10.1016/S0939-6411(97)00166-5] [PMID: 9700021]

[78] Hobbs, H.E.; Sorsby, A.; Freedman, A. Retinopathy following chloroquine therapy. *Lancet,* **1959**, *2*(7101), 478-480. [http://dx.doi.org/10.1016/S0140-6736(59)90604-X] [PMID: 14402143]

[79] Zhao, M.; Li, X.Y.; Xu, C.Y.; Zou, L.P. Efficacy and safety of nerve growth factor for the treatment of neurological diseases: a meta-analysis of 64 randomized controlled trials involving 6,297 patients. *Neural Regen. Res.,* **2015**, *10*(5), 819-828. [http://dx.doi.org/10.4103/1673-5374.156989] [PMID: 26109961]

[80] Plöckinger, U.; Dienemann, D.; Quabbe, H.J. Gastrointestinal side-effects of octreotide during long-term treatment of acromegaly. *J. Clin. Endocrinol. Metab.,* **1990**, *71*(6), 1658-1662. [http://dx.doi.org/10.1210/jcem-71-6-1658] [PMID: 2229321]

[81] Gaudana, R.; Ananthula, H.K.; Parenky, A.; Mitra, A.K. Ocular drug delivery. *AAPS J.,* **2010**, *12*(3), 348-360. [http://dx.doi.org/10.1208/s12248-010-9183-3] [PMID: 20437123]

[82] Agarwal, R.; Iezhitsa, I.; Agarwal, P.; Abdul, N.N.A.; Razali, N.; Alyautdin, R.; Ismail, N.M. Liposomes in topical ophthalmic drug delivery: an update. *Drug Deliv.,* **2016**, *23*(4), 1075-1091. [PMID: 25116511]

[83] Baudouin, C.; Labbé, A.; Liang, H.; Pauly, A.; Brignole-Baudouin, F. Preservatives in eyedrops: the good, the bad and the ugly. *Prog. Retin. Eye Res.,* **2010**, *29*(4), 312-334. [http://dx.doi.org/10.1016/j.preteyeres.2010.03.001] [PMID: 20302969]

[84] Adelman, R.A.; Zheng, Q.; Mayer, H.R. Persistent ocular hypertension following intravitreal bevacizumab and ranibizumab injections. *J. Ocul. Pharmacol. Ther.,* **2010**, *26*(1), 105-110. [http://dx.doi.org/10.1089/jop.2009.0076] [PMID: 20187807]

[85] Krishnan, R.; Goverdhan, S.; Lochhead, J. Submacular haemorrhage after intravitreal bevacizumab compared with intravitreal ranibizumab in large occult choroidal neovascularization. *Clin. Experiment. Ophthalmol.,* **2009**, *37*(4), 384-388. [http://dx.doi.org/10.1111/j.1442-9071.2009.02043.x] [PMID: 19594565]

[86] Schwartz, S.G.; Scott, I.U.; Stewart, M.W.; Flynn, H.W, Jr. Update on corticosteroids for diabetic macular edema. *Clin. Ophthalmol.,* **2016**, *10*, 1723-1730. [http://dx.doi.org/10.2147/OPTH.S115546] [PMID: 27660409]

[87] Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.,* **2003**, *2*(5), 347-360. [http://dx.doi.org/10.1038/nrd1088] [PMID: 12750738]

[88] Ferrari, M. Nanovector therapeutics. *Curr. Opin. Chem. Biol.,* **2005**, *9*(4), 343-346. [http://dx.doi.org/10.1016/j.cbpa.2005.06.001] [PMID: 15967706]

[89] Singh, R.; Lillard, J.W., Jr Nanoparticle-based targeted drug delivery. *Exp. Mol. Pathol.,* **2009**, *86*(3), 215-223. [http://dx.doi.org/10.1016/j.yexmp.2008.12.004] [PMID: 19186176]

[90] De Jong, W.H.; Borm, P.J. Drug delivery and nanoparticles: applications and hazards. *Int. J. Nanomedicine,* **2008**, *3*(2), 133-149. [http://dx.doi.org/10.2147/IJN.S596] [PMID: 18686775]

[91] Zhou, H.Y.; Hao, J.L.; Wang, S.; Zheng, Y.; Zhang, W.S. Nanoparticles in the ocular drug delivery. *Int. J. Ophthalmol.,* **2013**, *6*(3), 390-396. [PMID: 23826539]

[92] Fangueiro, J.F.; Silva, A.M.; Garcia, M.L.; Souto, E.B. Current nanotechnology approaches for the treatment and management of diabetic retinopathy. *Eur. J. Pharm. Biopharm.,* **2015**, *95*(Pt B), 307-322. [http://dx.doi.org/10.1016/j.ejpb.2014.12.023]

[93] Pandit, J.; Sultana, Y.; Aqil, M. Chitosan-coated PLGA nanoparticles of bevacizumab as novel drug delivery to target retina: optimization, characterization, and *in vitro* toxicity evaluation. *Artif. Cells Nanomed. Biotechnol.,* **2016**, *18*, 1-11. [PMID: 27855494]

[94] Sakai, T.; Ishihara, T.; Higaki, M.; Akiyama, G.; Tsuneoka, H. Therapeutic effect of stealth-type polymeric nanoparticles with encapsulated betamethasone phosphate on experimental autoimmune uveoretinitis. *Invest. Ophthalmol. Vis. Sci.,* **2011**, *52*(3), 1516-1521. [http://dx.doi.org/10.1167/iovs.10-5676] [PMID: 21178146]

[95] Zhang, G.; Feng, X.; Wabner, K.; Fandrey, C.; Naqwi, A.; Wiedmann, T.; Olsen, T.W. Intraocular nanoparticle drug delivery: a pilot study using an aerosol during pars plana vitrectomy. *Invest. Ophthalmol. Vis. Sci.,* **2007**, *48*(11), 5243-5249. [http://dx.doi.org/10.1167/iovs.07-0323] [PMID: 17962479]

[96] Diebold, Y.; Calonge, M. Applications of nanoparticles in ophthalmology. *Prog. Retin. Eye Res.,* **2010**, *29*(6), 596-609. [http://dx.doi.org/10.1016/j.preteyeres.2010.08.002] [PMID: 20826225]

[97] Kesavan, K.; Balasubramaniam, J.; Kant, S.; Singh, P.N.; Pandit, J.K. Newer approaches for optimal bioavailability of ocularly delivered drugs: review. *Curr. Drug Deliv.,* **2011**, *8*(2), 172-193. [review]. [http://dx.doi.org/10.2174/156720111794479925] [PMID: 21235476]

[98] Yang, H.; Tyagi, P.; Kadam, R.S.; Holden, C.A.; Kompella, U.B. Hybrid dendrimer hydrogel/PLGA nanoparticle platform sustains drug delivery for one week and antiglaucoma effects for four days following one-time topical administration. *ACS Nano,* **2012**, *6*(9), 7595-7606. [http://dx.doi.org/10.1021/nn301873v] [PMID: 22876910]

[99] Holden, C.A.; Tyagi, P.; Thakur, A.; Kadam, R.; Jadhav, G.; Kompella, U.B.; Yang, H. Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs. *Nanomedicine (Lond.),* **2012**, *8*(5), 776-783. [http://dx.doi.org/10.1016/j.nano.2011.08.018] [PMID: 21930109]

[100] Li, R.; Jiang, S.; Liu, D.; Bi, X.; Wang, F.; Zhang, Q.; Xu, Q. A potential new therapeutic system for glaucoma: solid lipid nanoparticles containing methazolamide. *J. Microencapsul.,* **2011**, *28*(2), 134-141. [http://dx.doi.org/10.3109/02652048.2010.539304] [PMID: 21142697]

[101] Bakri, S.J.; Pulido, J.S.; Mukherjee, P.; Marler, R.J.; Mukhopadhyay, D. Absence of histologic retinal toxicity of intravitreal nanogold in a rabbit model. *Retina,* **2008**, *28*(1), 147-149. [http://dx.doi.org/10.1097/IAE.0b013e3180dc9360] [PMID: 18185152]

[102] Liu, H.A.; Liu, Y.L.; Ma, Z.Z.; Wang, J.C.; Zhang, Q. A lipid nanoparticle system improves siRNA efficacy in RPE cells and a laser-induced murine CNV model. *Invest. Ophthalmol. Vis. Sci.,* **2011**, *52*(7), 4789-4794. [http://dx.doi.org/10.1167/iovs.10-5891] [PMID: 21519028]

[103] Park, K.; Chen, Y.; Hu, Y.; Mayo, A.S.; Kompella, U.B.; Longeras, R.; Ma, J.X. Nanoparticle-mediated expression of an angiogenic inhibitor ameliorates ischemia-induced retinal neovascularization and diabetes-induced retinal vascular leakage. *Diabetes,* **2009**, *58*(8), 1902-1913. [http://dx.doi.org/10.2337/db08-1327] [PMID: 19491211]

[104] Cai, X.; McGinnis, J.F. Nanoceria: a Potential Therapeutic for Dry AMD. *Adv. Exp. Med. Biol.,* **2016**, *854*, 111-118. [http://dx.doi.org/10.1007/978-3-319-17121-0_16] [PMID: 26427401]

[105] Sakai, T.; Kuno, N.; Takamatsu, F.; Kimura, E.; Kohno, H.; Okano, K.; Kitahara, K. Prolonged protective effect of basic fibroblast growth factor-impregnated nanoparticles in royal college of surgeons rats. *Invest. Ophthalmol. Vis. Sci.,* **2007**, *48*(7), 3381-3387. [http://dx.doi.org/10.1167/iovs.06-1242] [PMID: 17591912]

[106] Giannaccini, M.; Pedicini, L.; De Matienzo, G.; Chiellini, F.; Dente, L.; Raffa, V. Magnetic nanoparticles: a strategy to target the choroidal layer in the posterior segment of the eye. *Sci. Rep.,* **2017**, *7*(43092), 43092. [http://dx.doi.org/10.1038/srep43092] [PMID: 28256525]

[107] Sun, C.; Lee, J.S.; Zhang, M. Magnetic nanoparticles in MR imaging and drug delivery. *Adv. Drug Deliv. Rev.,* **2008**, *60*(11), 1252-1265. [http://dx.doi.org/10.1016/j.addr.2008.03.018] [PMID: 18558452]

[108] Gupta, A.K.; Gupta, M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials,* **2005**, *26*(18), 3995-4021. [http://dx.doi.org/10.1016/j.biomaterials.2004.10.012] [PMID: 15626447]

[109] Gupta, A.K.; Naregalkar, R.R.; Vaidya, V.D.; Gupta, M. Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. *Nanomedicine (Lond.),* **2007**, *2*(1), 23-39. [http://dx.doi.org/10.2217/17435889.2.1.23] [PMID: 17716188]

[110] Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller, R.N. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chem. Rev.,* **2008**, *108*(6), 2064-2110. [http://dx.doi.org/10.1021/cr068445e] [PMID: 18543879]

[111] McCarthy, J.R.; Kelly, K.A.; Sun, E.Y.; Weissleder, R. Targeted delivery of multifunctional magnetic nanoparticles. *Nanomedicine (Lond.),* **2007**, *2*(2), 153-167. [http://dx.doi.org/10.2217/17435889.2.2.153] [PMID: 17716118]

[112] Misra, R.D.K. Magnetic nanoparticle carrier for targeted drug delivery: Perspective, outlook and design. *Mater. Sci. Technol.,* **2008**, *24*, 1011-1019. [http://dx.doi.org/10.1179/174328408X341690]

[113] Ito, A.; Shinkai, M.; Honda, H.; Kobayashi, T. Medical application of functionalized magnetic nanoparticles. *J. Biosci. Bioeng.,* **2005**, *100*(1), 1-11. [http://dx.doi.org/10.1263/jbb.100.1] [PMID: 16233845]

[114] Bulte, J.W. *In vivo* MRI cell tracking: clinical studies. *Am. J. Roentgenol.,* **2009**, *193*(2), 314-325. [http://dx.doi.org/10.2214/AJR.09.3107] [PMID: 19620426]

[115] Zysler, R.B. Material for Medical Use Comprising Nanoparticles with Superparamagnetic Properties and Its Utilization in Surgery. U.S. Patent 20130225906. 29 August. **2013**.

[116] Raju, H.B.; Hu, Y.; Vedula, A.; Dubovy, S.R.; Goldberg, J.L. Evaluation of magnetic micro- and nanoparticle toxicity to ocular tissues. *PLoS One,* **2011**, *6*(5), e17452. [http://dx.doi.org/10.1371/journal.pone.0017452] [PMID: 21637340]

[117] Giannaccini, M.; Giannini, M.; Calatayud, M.P.; Goya, G.F.; Cuschieri, A.; Dente, L.; Raffa, V. Magnetic nanoparticles as intraocular drug delivery system to target retinal pigmented epithelium (RPE). *Int. J. Mol. Sci.,* **2014**, *15*(1), 1590-1605. [http://dx.doi.org/10.3390/ijms15011590] [PMID: 24451140]