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

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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.

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].

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
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.](image-url)
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 downregulated 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 neovascularization 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 neovascularization 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-
experimental treatments of ocular diseases. The intracocular 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 pharmacologically 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, nonantigenic 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 injection.

Parenteral delivery of betamethasone phosphate encapsulated in biocompatible and biodegradable blended NPs of PLA homopolymers and polyethylene glycol (PEG)-block-PLA copolymers (stealth nanostereoids) 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 preocular 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 properties 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., Combidx® (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)](image-url)

Fig. (2). Mouse retinal explants were subjected to oxidative stress (OS) using 100 µM H₂O₂ 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.
CONSENT FOR PUBLICATION

Not applicable.
Zheng, L.; Kern, T.S. Role of nitric oxide, superoxide, peroxynitrite, and advanced glycation end products (RAGE). Diabetes, 2016, 65(5), 183-197. [http://dx.doi.org/10.2337/db15-0951] [PMID: 26940691]

Behl, T.; Kau, I.; Kotwani, A. Implication of oxidative stress in progression of diabetic retinopathy. Surv. Ophthal., 2015, 60(7), 615-2017. [http://dx.doi.org/10.1097/IO.OFS.0000495501.15785.60] [PMID: 26047345]

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, 176-187. [http://dx.doi.org/10.1016/j.biocel.2015.01.009] [PMID: 25603271]

Sasaki, M.; Ozawa, Y.; Kurihara, T.; Kubota, S.; Yuki, K.; Noda, K.; Kobayashi, S.; Ishida, S.; Tsutoba, 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]

Al-Gayyar, M.M.; Matragona, S.; Pillai, B.A.; Ali, T.K.; Abdel-saad, M.A.; El-Remyess, 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. Diabetes, 2011, 60(3), 669-680. [corrected]. [http://dx.doi.org/10.2337/db10-0914] [PMID: 21136036]

Ali, T.K.; Matragona, S.; Pillai, B.A.; El-Remyess, 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/db07-1068] [PMID: 18285558]

Mysouna, B.A.; Al-Gayyar, M.M.; Matragona, S.; Abdel-saad, M.A.; El-Azab, M.F.; Saragovi, H.U.; El-Remyess, 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]

Mysouna, B.A.; Shanab, A.Y.; Elshaer, S.L.; El-Remyess, A.B. Nerve growth factor in diabetic retinopathy: beyond neurons. Expert Rev. Ophthalmol., 2012, 7(2), 99-107. [http://dx.doi.org/10.1586/17468999.2012.675101] [PMID: 23773101]

Gerhardinger, C.; McClure, K.D.; Romeo, G.; Podestà, F.; Lorenzo, 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]

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/10.2174/1570159114661608130] [PMID: 25728260]

Barnstumble, 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]

Zheng, B.; Li, T.; Chen, H.; Xu, X.; Zheng, Z. Correlation between ficollin-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]

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

Hernández, C.; Carrasco, E.; Casamitjana, R.; Deulofeu, R.; García-Arumí, J.; Simó, R. Somatostatin molecular variants in the vitreous fluid: a comparison 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]

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]

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

Dal Monte, M.; Petrucci, C.; Cozzi, A.; Allen, J.P.; Bagnoli, P. Somatostatin inhibits endothelin-1-evoked glutamate release by activation of the sst2 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]

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

D’Alessandro, A.; Cervi, 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/c3mb00736b] [PMID: 24514073]

Cervi, 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: 22169121]

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]

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]

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 retino-protective effects. Ann. N. Y. Acad. Sci., 2010, 1200, 128-139. [http://dx.doi.org/10.1111/j.1749-6632.2010.05512.x] [PMID: 20633141]

Werling, D.; Reglodi, D.; Banks, W.A.; Salameh, T.S.; Kovacs, K.; Kvarik, T.; Vazcy, 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(6), 6683-6691. [http://dx.doi.org/10.1167/iovs.16-20630] [PMID: 27936470]

Kummer, A.; Pulford, B.E.; Ishii, D.N.; Seigel, G.M. Des-(3)-GF-1 treatment normalizes type 1 IFG receptor and phospho-Akt (Thr 308) immunoreactivity in predegenerative retina of diabetic rats. Int J Exp Diabetes Res., 2003, 4(1), 45-57. [http://dx.doi.org/10.1080/15512900390178389] [PMID: 12360002]

Elahy, M.; Bainard-Hudson, S.; Cruzat, V.F.; Newsholme, P.; Dass, C.R. Mechanisms of PDE-mediated protection against reactive oxygen species damage in diabetic retinopathy and neuropathy. J. Endocrinol., 2014, 223(3), R129-R139. [http://dx.doi.org/10.1677/JOE-13-04665] [PMID: 24928938]

Yoshida, Y.; Yamagishi, S.; Matsui, T.; Jinnouchi, Y.; Fukami, K.; Imazumi, 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/dmr.1007] [PMID: 19685553]

Liu, H.; Ren, J.G.; Cooper, W.L.; Hawkins, C.E.; Cowan, M.R.; Tong, P.Y. Identification of the antivasopressin activity effectors pig- ment epithelium-derived factor and its active site. Proc. Natl. Acad. Sci. USA, 2004, 101(16), 6605-6610. [http://dx.doi.org/10.1073/pnas.0308342101] [PMID: 15096582]

Wang, Y.; Lu, Q.; Gao, S.; Zhu, Y.; Gao, Y.; Xie, B.; Shen, X. Pigment epithelium-derived factor regulates glutamine synthetase and l-glutamate/aspartate transporter in retinas with oxygen-induced retinopathy. Curr. Eye Res., 2015, 40(12), 1232-1244. [http://dx.doi.org/10.3109/02713683.2014.996639] [PMID: 25548969]

Liu, Y.; Leo, L.F.; McGregor, C.; Grivitshulv, 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 Ins2Akita mice. Mol. Med., 2011, 18, 1387-1401. [http://dx.doi.org/10.2119/mmol.2012.00008] [PMID: 23019073]

Catalani, E.; Cervia, D.; Martini, D.; Bagnoli, P.; Simonetti, E.; Timerio, 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]
[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.09.03107] [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]