In vitro properties of Gantrez Nanoparticles (NP´s) loaded with memantine. Ocular tolerance after subtenon and intravitreal administration of unloaded Gantrez NP´s in albino rabbits.

E. Prieto [a] B. Puente [b] A. Uixera [c] J.A. Garcia de Jalon [c] S. Perez [a] L. Pablo [a] J.M. Irache [d] M.A. Garcia [b] and M.A. Bregante [e]

[a]Department of Ophtalmology, Miguel Servet University Hospital, University of Zaragoza Spain, [b]Department of Analytical Chemistry, Veterinary Faculty University of Zaragoza Spain, [c]Department of Animal Pathology, Veterinary Faculty, University of Zaragoza Spain, [d]Department of Pharmacy and Pharmaceutical Technology, University of Navarra Spain, [e]Department of Pharmacology and Physiology, Veterinary Faculty University of Zaragoza (Spain).

Short Title, Properties of NP´s of memantine.

Address:

M.A.Bregante, Department of Pharmacology and Physiology, Veterinary Faculty. Miguel Servet 177, (50013) Zaragoza (Spain)
Phone number, 0034976761627
Fax number, 0034976761612
e-mail address, bregante@unizar.es

Key Words: memantine, retina, nanoparticles, Gantrez

Abstract

Purpose: We have prepared and have evaluated "in vitro" release of memantine–loaded poly (anhydride) (Gantrez®) nanoparticles. Clinical safety and retinal toxicity induced by these nanoparticles unloaded after subtenon and intravitreal ocular injections had also been evaluated. **Methods:** This paper describes the preparation and characterization of this type of nanoparticles and as well as "in vitro" release study. Twenty three healthy New Zealand rabbits were used for clinical and histological evaluation after subtenon and intravitreal ocular injections of nanoparticles unloaded. **Results:** The amount of drug associated to nanoparticles was 55 μg of memantine/mg of nanoparticles. The release profile of memantine from this type of nanoparticles was characterized by a burst initial effect followed by a continuous release of the drug for at least 15 days. No relevant complications were found during the clinical follow up. The histological evaluation suggested that Gantrez® nanoparticles are well tolerated after subtenon ocular injection and that inflammation signs at the first days after intravitreal ocular injections can be considered a normal reaction of the defence mechanism of the eye.

Introduction

Glaucoma affects to 65 million persons in the world and is the second main cause of blindness. Nearly 10% of ophthalmologic assistants are related to this pathology.

This ocular disease is a neurodegenerative disease characterized by progressive degeneration of retinal ganglion cells (RGCs) [1]. It is characterized by structural changes in the optic disk and retinal fiber layer, as well as irreversible visual field loss. Reducing intraocular pressure help to prevent the appearance of glaucoma and its progression in some patients, but only reducing IOP is not always effective.

Neuroprotection refers to the use of any therapeutic modality that prevents, retards, or reverses neuronal cell death resulting from primary neuronal lesion [2-3]. The concept of ocular neuroprotection has been advanced to treat the primary problem in glaucoma, neuronal death and is the name given to treatments directed at the loss of neurons and address the mechanism by which the cellular injury results in cell death or dysfunction, while lowering IOP address the process responsible for the injure of cells.

Excessive activity of NMDA-type (N-methyl-d-aspartate) glutamatergic channels has been implicated as a mechanism for neuronal injury in neurologic disorders, including glaucoma and retinal disease. So memantine, an NMDA-type glutamatergic channel blocker, could be effective in reversing experimental excitotoxicity [4]

Memantine (1-amino-3, 5-dimethyladamantane) is a low-affinity voltage-dependent uncompetitive antagonist at glutamatergic NMDA receptors. This drug is able to inhibit the prolonged flow of Ca^{2+} ions which represents the basis of neuronal excitotoxicity. Therefore, memantine may rescue neurons through its blockade of excessive glutamate receptor activation and can prevent ganglion cell death mediated by activation of NMDA. Currently, studies have shown that treatment with memantine protected retinal

ganglion cells in monkey models of glaucoma, reducing ganglion cell loss during the treatment with this drug as it was measured by multifocal electroretinogram and visual evoked responses. [5]

So far, clinical applications of these neuroprotective therapies have been conditioned, by the highly systemic doses needed to achieve therapeutic concentrations at the target tissue as the retinal ganglion cell layer.

An important goal is the development of a safe and effective means of chronic delivery of memantine to the posterior pole. [6-7-8].

At present we administered ocular medications by four routes: topical, systemic, intraocular and periocular ways. When we administer topical medication, just 5% of dose remains at corneal surface able to penetrate into eye, so it´s very difficult to achieve effective concentration at vitreous, choroidal and retinal tissues. [9]. Necessary doses by systemic way are too high, causing many secondary effects

Specifically, nanoparticles (NP´s) are proven to come in useful for increasing the bioavailability of some active molecules in ophthalmology [11]. Gantrez® AN is a copolymer of methyl vinyl ether and maleic anhydride widely used for pharmaceutical and medical purposes as a thickening and suspending agent in aqueous solutions and adhesive bases in denture preparations, transdermal patches and oral tablets. In addition, it was considered as a nanoparticle carrier taking into account its low toxicity and excellent biocompatibility[12-13-14] Moreover, this copolymer can easily react with amino groups, which makes possible to load or link different types of proteins and other drugs which have these functional groups.

The aims of this work were the preparation, characterization and in vitro evaluation of memantine-loaded poly(anhydride) (Gantrez®) nanoparticles. In addition, clinical

safety and retinal toxic response induced by these nanoparticles unloaded after their subtenon and intravitreal administration were also evaluated.

Materials and Methods

Chemicals

Gantrez® AN 119 [poly(methyl vinyl ether-co-maleic anhydride) or poly(anhydride); MW 200,000] was kindly gifted by ISP (Barcelona, Spain). Memantine hydrocloride (grade >98% GC) was provided by Sigma–Aldrich (Barcelona, Spain). Acetone and ethanol were obtained from VWR Prolabo (Fantenay sous Bois, France) and phosphate buffer saline from Gibco (Spain). O-phthalalhyde (OPA) was supplied from Molecular Probes (USA). All other chemicals used were of analytical grade and obtained from Merck (Madrid, Spain). Methanol,2-mercaptoethanol, boric acid, potassium hydroxide.

Preparation of poly(anhydride) nanoparticles

Poly(anhydride) nanoparticles were prepared by a solvent displacement method [15]. Then, memantine was associated to the nanoparticles by simple incubation in an aqueous medium at room temperature.

Briefly, 10 mL of an ethanol-water mixture (1:1, v/v) were dripped slowly into 5 mL of acetone containing 100 mg poly(anhydride) previously dissolved. The organic solvents were eliminated under reduce pressure (Büchi R-144, Switzerland) and 10 mg of memantine hydrochloride, previously dissolved in 1 mL of water, were dispersed in the previously formed nanoparticles and incubated for 30 minutes under magnetic stirring at room temperature. After this time, the resulting carriers were purified by centrifugation at 20,000 rpm for 20 min (sigma 3K30, Germany). The supernatants resulted in the purification step were recovered and stored at -20 ºC in order to quantify the unloaded memantine. Finally, the pellets were resuspended in water and the formulations were frozen and freeze-dried (Genesis 12EL, Virtis, USA) using sucrose (5% w/v) as cryoprotector.

Control NP´s were prepared in the same way, in the absence of memantine and all batches of lyophilized nanoparticles were stored at room temperature until use.

Characterization of Np´s

The particle size and the zeta potential of nanoparticles were determined by photon correlation spectroscopy (PCS) and electrophoretic laser Doppler anemometry, respectively, using a Zetamaster analyzer system (Malvern Instruments, UK). Samples were diluted with double distilled water and measured at room temperature with a scattering angle of 90 ºC. All measurements were performed in triplicate.

The morphological characteristics of the nanoparticles were examined by scanning electron microscopy (Zeiss DSM 940A SEM; Oberkochen, Germany) with a digital imaging capture system (DISS, Point Electronic GmBh; Halle, Germany). For this purpose, freeze-dried nanoparticles were resuspended in ultrapure water and centrifuged at 27,000×g for 20 min at 4 ºC. Then, supernatants were rejected and the pellets were mounted on a glass plate adhered with a double-sided adhesive tape onto metal stubs and dried under hot flow air. Finally, the particles were coated with a thin layer of 12 nm of gold using an Emitech K550 sputtering device (Emitech, UK).

Yield

The yield of the nanoparticles preparation process was determined by gravimetry as described previously [16]. Briefly, Gantrez® AN nanoparticles, freshly prepared, were freeze-dried. Then, the yield was calculated as the difference between the initial amount of the polymer used to prepare nanoparticles and the weight of the freeze-dried carriers.

Memantine quantification

The amount of memantine associated to the nanoparticles was estimated by quantification of free memantine in the supernatants obtained during the purification

step using a fluorometric method with *o*-phthaldialdehyde (OPA), previously described [17], with some minor modifications.

A stock standard solution of o-phthalalhyde (OPA) was prepared by dissolving it in methanol (125 mg in 1.5 mL), 200 μL of 2-mercaptoethanol were added and the mixture was taken up to a volume of 100 ml with borate buffer 0.4 M titrated to pH 10.4 with KOH. This solution was stored in absence of light at 4 °C during a time not more than one week. Stock standard solution of memantine was prepared by directly dissolving commercially available reagent in doubly distilled water at a concentration of 100 μg·mL^{-1}. Working solutions were prepared by diluting the stock solution in water in a range of 0–50 μg·mL^{-1}.

In a set of 96 microtiter plates (Thermo LabSystems, Vantaa, Finland) a volume of 50 μL of working solution of memantine or sample and 100 μL of stock standard solution of OPA were added per well. After the mixture was shaking for 60 seconds, fluorescence measurements were performed in a spectrophotometer at λex 340 nm and λem 450 nm (Labsystems iEMS Reader MF, Finland).

For analysis, the supernatants (obtained from control nanoparticles and memantine NP) were defrosted at room temperature and diluted with ultrapure water (1/60 v/v). Each sample was assayed in triplicate and no interferences in the analysis of memantine from the presence of poly(anhydride) were found.

The amount of memantine associated to the nanoparticles was calculated as the difference between the initial amount of memantine added and the amount of memantine determined in the supernatants. Thus, memantine loading was expressed as the amount of memantine (in μg) per mg of nanoparticles whereas the encapsulation efficiency (A.E.) was determined as follow:

A.E. (%) = ($Q_{associated}$ / $Q_{initial}$) x 100

where $Q_{associated}$ is the total weight of memantine entrapped in the batch of nanoparticles and $Q_{initial}$ is the initial weight of memantine added.

In vitro release study

In order to obtain the memantine release profile from poly (anhydride) nanoparticles, 10 mg of lyophilized memantine-loaded nanoparticles were dispersed in 1 mL phosphate-buffered saline (PBS from GIBCO™, pH 7.4) in eppendorf tubes. The samples were incubated at 37 ± 1ºC under agitation in a VorTemp 56™ Shaking Incubator (Labnet International, Inc) during specified periods of time. At each time interval, samples were assayed in triplicate and blank Gantrez® nanoparticles (NP) were used as control. After the incubation, formulations were centrifuged at 17000 rpm (Allegra ™ X-22R Centrifuge, Beckman Coulter), for 20 min at 4 ºC. The memantine released was quantified in the supernatants, which were frozen at -80ºC to be analysed later by the fluorometric method as described above. Release profile was expressed in terms of cumulative release in percentage, and plotted versus time.

Intravitreal and subtenon administration.

All injections were performed under general anaesthesia and aseptic conditions. Anaesthesia general was accomplished using an intramuscular injection of 20 mg/kg of ketamine and 2mg/kg of xilacine

Subtenon and intravitreal inyection of NP´s unloaded

The ocular subtenon injection was carried out into the posterior space; the ocular surface was anaesthetized using topical lidocaine 4% drops. Adequate exposure is obtained by placing an eyelid speculum. Entry is made into the episcleral using a trocar of 22 gauge, 0.85x25mm intravenous cannula made of polytetrafluorethylene. The

trocar is advanced for about 12-15mm within the episcleral space under visualization using a microscope Zeiss Omi 99 (Germany).

Intravitreous injections were administered into the approximate centre of the vitreous humour through a 27 gauge needle insert below the superior rectus muscle 2 to 3 mm from the corneal limbus. Previously, 0.1 ml of aqueous humour had been removed through a 27 gauge needle.

Eventually twenty three healthy New Zealand white rabbits, weighing 2-3 kg and aged 2-3 months were selected and divided in two lots (1 and 2):

Lot 1: Thirteen animals received by intravitreal route 1.6 mg of nanoparticles in 0.1 ml of saline solution. This group of animals underwent bilateral enucleation at 24 hours and 1, 2, 3 and 6 weeks, after the injection..

Lot 2: Ten animals received by subtenon route 4 mg of nanoparticles in 0.3 ml of saline solution. This group of animals underwent bilateral enucleation at 1, 2, and 3 weeks after the administration.

One animal of each lot, intravitreal and subtenon route, were used as control and only received 0.1 mL and 0.3 ml of saline solution respectively.

 Clinical Evaluation

Each rabbit, of the subtenon and intravitreal group, was examined by slit lamp inspection and by indirect ophthalmoscopy before injection and on each successive predetermined time points (24 hours, 1, 2, 3 and 6 weeks after the injection for the intravitreal group and 1, 2, 3 weeks after the injection for the subtenon group).

The visual inspection was carried out to looking for inflammatory signs at the anterior polo of the eye. The frequency and severity of conjunctive hyperaemia and chemosis, subconjunctival haemorrhage and iris vessel engorgement and cataract formation were evaluated and a scale of 0 to 3 was used for its gradation: grade 0 for

normal; 1 for slight conjunctive and iris congestion; 2 for moderate and 3 for severe congestion or hyperaemia. The cataract formation was analyzed, the type (nuclear, cortical or subcapsular), the extension (focal or diffuse) and the density (grade 1 corresponds to a slight opacity that let to a clear view of the fundus details, grade 2 for moderate opacity and grade 3 indicated a total opacity that retinal details observation was difficult) of the cataract were encoded.

Vitreous clarity was evaluated by indirect ophthalmoscopic examination and a scale of 0 to 4 was used for assessing this. Briefly, grade 0 indicated a clear view of the retinal details, grade 1 corresponds to a slight obscuration of retinal details, a mild obscuration of retinal details with clearer view of retinal small vessels was grade 2, a moderate obscuration of the retinal details with only visibility of large vessels corresponds to grade 3 and grade 4 indicated that a faint view of the retina was observed with an intuition of the papilla of optic nerve. Other findings like vitreous haemorrhage, areas of local retinal inflammation, retinal detachment, retinal fibrosis or retinal haemorrhages were analyzed in the fundus examination.

Histopathlogy

After the macroscopic analysis, all rabbits that were administered by subtenon and intravitreal route were euthanized by an intravenous injection of 20% sodium pentobarbital (30mg/Kg) and the eyes were immediately enucleated. Previously, the globes had been marked off on the twelve o´clock hour position with a scleral silk 6-0 suture. Then the globes were removed of periocular tissues (muscle, Tenon´s capsule and conjunctiva) and washed with 0.9% saline solution. An aqueous paracentesis with 27-gauge needle was made to decrease intraocular pressure before the injection of 0.1 ml of the fixative solution into the vitreous cavity of the eye. After the globes were

immersed in 10% formaldehyde fixative solution and sent to the Histology and Pathology Department of Veterinary Science University of Zaragoza for analyzing.

The samples were processed and dyed with hematoxiline-eosine for light microscopic examination and assessing the following parameters: preservation of the architecture and normal proportions of the different eye structures and specifically of the retinal layers (retinal pigment epithelium, inner and outer segments of photoreceptors, external nuclear layer, internal nuclear layer and ganglion cell layer). Presence of degeneration or necrotic processes, inflammatory signs and abnormal cell growths as atrophy, hyperplasia, neoplasia or neovascularization, were evaluated. Others parameters like optic disk oedema or retinal detachment were analyzed.

All procedures were carried out under Project License PI 07/07 approved by the in-house Ethics Committee for Animal Experiments from the University of Zaragoza (Spain). The care and use of animals was compliant with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 on the protection of animals used for experimental and other scientific purposes.

Results

Characterisation of Gantrez® Nanoparticles

The main physic-chemical properties of poly(anhydride) nanoparticle formulations are summarised in Table 1. Overall, memantine loaded nanoparticles displayed a similar mean size to control nanoparticles. Furthermore, in all cases, the batches were highly homogeneous with a very low polydispersity (PDI < 0.2). Similarly, all the nanoparticle formulations displayed a similar negative surface charge. The morphological analysis by scanning electron microscopy (fig. 1) showed that poly (anhydride) NPs consisted of a homogeneous population of spherical particles. Nevertheless, the surface of control nanoparticles was smooth whereas memantine-loaded nanoparticles appeared to show a rough surface. Finally, the amount of drug associated to nanoparticles was calculated to be about 55 µg memantine per mg nanoparticles, with an adsorption efficiency of about 40%.

In Vitro Release Study

Fig. 2 shows the release profile of memantine from poly(anhydride) nanoparticles under physiological conditions (PBS, pH 7.4) for 15 days. These nanoparticles displayed a biphasic release pattern characterized by a burst effect followed by a continuous release of the drug for at least 15 days. Initially, the amount of released memantine increased rapidly during the first two hours. At this time, about 35% of the loaded memantine was released from the nanoparticles. Then, the levels of released memantine were maintained constant for at least 7 days. At day 15th, the amount of released memantine was slightly lower than at day 7th probably due to its degradation.

<u>Clinical Safety</u>

Ophthalmoscopic and Clinical Observations

Tables 2 and 3 show the results of clinical examination after the intravitreal and subtenon administration of nanoparticles. One and two weeks post-administration, variable grade -slight or mild- of conjunctival hyperaemia and chemosis were found in all eyes of the subtenon group, which had disappeared at the three weeks control. In 10 of 18 subtenon injected eyes, a conjunctival haemorrhage was observed coinciding with the injection site, around the superior rectus muscle insertion. Only one eye of the subtenon group showed iris vascular engorgement at the second week after the injection.

Variable grade of cataract was observed in some eyes of the intravitreal group but it not was observed in the subtenon group. In most of cases, it was a focal lens opacity located in the lower quadrants at the posterior lens capsule, with higher density of the cataract in eyes of three weeks-control but without problems for fundus examination. There were only two cases of diffuse lens opacity affecting the posterior capsule and only one case of an anterior polar cataract corresponding with an eye in which paracentesis had been traumatic. Immediately after the intravitreal injection, the formulation was visible as a whitish film in the vitreous. During the six weeks of follow-up, this film remained floating in the anterior vitreous next to the posterior surface of the lens and in the inferior vitreous forming whitish lumps. Four eyes that received nanoparticles intravitreal injections showed a vitreous haemorrhage with a slight obscuration of view of retinal details, corresponding with eyes in which the injection had been more traumatic. Indirect ophthalmoscopy did not reveal any retinal lesion as retinal detachment or haemorrhages. None eye showed optic disc inflammation

or oedema. No signals of local retinal inflammation or retinal fibrosis were observed in any group.

Histopathology

The histological analysis of eyes after nanoparticles subtenon injection showed a certain degree of distortion of the cytoarchitecture of the outer retina, with disruption of the retinal epithelium and the outer segments of the photoreceptors, this fact was also observed in the control eyes. Fig. 3 (a, b, c) shows the retinal appearance after one, two and three weeks post administration of subtenon unloaded Gantrez nanoparticles. The architecture of the retinal layers was preserved in five of the six subtenon-eyes studied. Only one eye showed a focus of retinal distortion affecting mainly to the ganglion cells layer associated to oedema of the choroid, but this fact was also observed in one of the control eyes. Inflammatory signs were present at corneoscleral layer and cilliary body into the two first weeks post administration regressing to minimal grades, three weeks post administration.

None signal of degeneration were found in the retina after 24 hours 1, 3 and 6 weeks after NP´s Gantrez intravitreal injection. A focus of choroid oedema and swelling of the retinal nerve fiber layer was only detected in one eye after 24 hours of the intravitreal injection, and in this eye the cilliary body presented a mild inflammatory infiltration.

Unlike the subtenon group, intravitreal injected eyes show inflammatory cells in the vitreous cavity. These inflammatory cells are composed mainly by mono and polinuclear cells at 24 hours, macrophages and multinuclear giant cells at 1 and 3 weeks that practically disappeared at 6 weeks (fig. 4). In only one case was histologically confirmed the presence of cataract.

Discussion

Memantine (1-amino-3, 5-dimethyladamantane hydrochloride) is a novel, low-to-moderate affinity, noncompetitive N-methyl-D-aspartate receptor antagonist of the open channel blocker type. Memantine is used in the treatment of Parkinson's disease, some types of movement disorders [18], some dementia syndromes [19] and has recently been described as a potential treatment for glaucoma [20].

Different works are being carried out about memantine for our work group according to memantine determination in vitreous humor and plasma [21] and about bioavailability of memantine after subtenon and intravitreous administration (unpublished data).

Now, further experimental studies for determining the possibility of using a drug-delivery system to maintain an efficient intravitreal memantine level for a longer period of time to improve its therapeutic use are been carried out for our work group. We can point out our initial experimental studies using bioadhesive Gantrez® nanoparticles as carriers of memantine for different ocular ways.

Gantrez® nanoparticles have demonstrated the ability to load memantine (nearly 55μgr/mg of nanoparticle) and provide its release in a sustained way. In fact almost 40% of the drug loaded was released within 8 h and after 15 days increased up to 30%.

In this work Gantrez® nanoparticles unloaded were administered by intravitreous and subtenon routes and both clinical safety and histological evaluation were carried out.

The visual inspection of the anterior segment of the eyes revealed more inflammation, including conjunctival hyperaemia and quemosis, in the subtenon injected eyes, probably by the greater manipulation of the periocular tissues in this route of administration. Nevertheless, the inflammation signs were mild to moderate and at three weeks control had already disappeared. Cataract formation was observed in some eyes after intravitreal

nanoparticles injection. In most cases, we could observe focal lens opacity located in the lower quadrants at the posterior lens capsule. However in only one eye were found histological evidences of cataract with fragmentation of lens cortical fibres and globules of denatured proteins, suggesting that the clinically lens opacities observed and interpreted as cataracts are probably artefacts caused by the presence of formulation masses floating in the anterior vitreous in contact with the posterior surface of the lens, producing a false image of cataract. After intravitreal injections had been described the formation of cataracts suggesting a traumatic origin associated with the technique of injection. Moreover posterior subcapsular cataract has been suggested to be associated with elevated IOP post intravitreal injection of some drugs [22] more than consider that it be associated with an oxidative toxic damage induced by the nanoparticles similar to the mechanism described for steroid-induced cataract. The nanoparticles administrated as suspension by intravitreal route appeared floating as whitish films and lumps in the vitreous, whereas the eyes injected with saline solution or the subtenon injected eyes appeared clear. The position of precipitates in vitreous could be a key factor for the appearance of vitritis or local retinal damage [23] but in our case, indirect ophthalmoscopy did not reveal any sign of retinal damage like fibrosis hemorrhages, infiltrates or vascular proliferations. Only one of the eyes that received subtenon NP´s developed a focus of retinal distortion affecting to the ganglion cells layer that was associated to oedema of the choroids, and this was also observed in a control eye injected with saline solution, suggesting a mechanical damage more than a chemical insult.

The histological evaluation of the different ocular tissues suggested that Gantrez® nanoparticles are well tolerated after the subtenon administration. The injuries observed are typical of physical trauma and tend to disappear with the pass of the time. We

observed a few inflammatory cells (lymphocytes) in the periocular tissue at the injection site, but not in the retina or other ocular tissues. The intravitreal route could be also considered tolerable in the most of the cases since inflammation signs observed in the vitreous cavity and ciliary body at the first days tend to disappear 6 weeks later without affecting to the retina layers. This can be considered a normal reaction of self defence mechanism of the eye. The retina appeared normal without apparent structural changes. The disruption in the outer segments of the photoreceptors observed in the histopathologic section from some eyes is probably produced by the histological manipulation itself.

Our results suggest that rabbit eyes receiving intravitreal or subtenon Gantrez nanoparticles did not show notable structural changes. It should be kept in mind that after intravitreal injection, nanoparticles remain floating in the vitreous and so it may interfere with the visual clarity of patients. The advantage of subtenon administration is that nanoparticles are retained at the site of injection without penetrating into the vitreous [24].

References

1 Weinreb RN, Khaw PT: Primary open-angle glaucoma. The Lancet 2004; 363: 1711-1720.

2 Barkana Y, Belkin M. Neuroprotection in ophthalmology: A review. Brain Res Bull. 2004; 62: 447-453.

3 Levin LA, Peeples P: History of neuroprotection and rationale as a therapy for glaucoma. Am J Manag Care. 2008; 14:S11-S14)

4 Hare WA, Wheeler: Experimental glutamatergic excitatory in rabbit retinal ganglion cells: block by memantine. Invest Ophthalmol Vis Sci. 2009; 50(6):2940-2948.

5 Hare W.A., WoldeMussie E., Lai R.K., Ton H., Ruiz G., Chun T., Wheeler L: Efficacy and Safety of Memantine Treatment for Reduction of Changes Associated with Experimental Glaucoma in Monkey, I-II: Functional Meassures. Invest Ophthalmol Vis Sci. 2004; (45): 2625-2651.

6 Ghate D, Edelhauser H-F: Ocular Drug Delivery Expert Opin. Drug Deliv. 2006; 3(2): 275.

7 Koevary SB: Trends in the noncorneal delivery of drugs into eye Arch Soc. Esp Oftalmol. 2002; 77(7); 347-349.

8 Raghava S, Hammond M, Kompella U-B: Periocular routes for retinal drug delivery. Expert Opin. Drug Deliv. 2004; 1(1): 99-114.

9 Vanrell R: New trends in controlled drug delivery to posterior segment. Arch Soc Esp Oftalmol. 2007; 82(1):3-5.

10 Hughes PM, Olejnik O, Chang-Lin J, Wilson CG: Topical and systemic drug delivery to the posterior segments. Advanced Drug delivery reviews. 2005; 2010-2032.

11 Irache JM: Albumin nanoparticles for the intravitreal delivery of anticytomegaloviral drugs. Mini Reviews in medicinal Chemistry. 2005; 5:293-3051.

12 Kohane S: Microparticles and nanoparticles for drug delivery. Biotechnol.Bioeng. 2007; 96: 203-209.

13 Irache JM, Huici M, Konecny M, Espuelas S, Campanero MA, Arbós P: Bioadhesive properties of Gantrez nanoparticles, Molecules. 2005; 10:126-145.

14 Kompella UB, Bandi N, Ayalasomayajula SP: Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. Invest Ophthalmol Vis Sci. 2003; 44:1192-1201.

15 Arbos P, Wirth M, Arangoa MA, Gabor F, Irache JM: Corrigendum to: Gantrez ® AN as a new polymer for the preparation of ligand- nanoparticles conjugates. Journal of Controlled Release. 2003; 83: 321-330.

16 Arbós P, Wirth M, Arangoa MA, Gabor F, Irache JM: Gantrez® AN as a new polymer for the preparation of ligand-nanoparticle conjugates.J Control Release. 2002 Oct 30; 83(3):321-30.

17 Benson JR, Hare PE: O-phthalaldehyde: fluorogenic detection of primary

amines in the picomole range. Comparison with fluorescamine and ninhydrin. Proc Natl Acad Sci U S A. 1975 Feb; 72(2):619-22.

18 Schneider E, Fischer PA, Clemens R, Balzereit F, Fünfgeld EW. Haase H.J: Effects of oral memantine on symptoms of Parkinson's disease. Results of a placebo-controlled multicenter study. Dtsche Med Wochenschr 2003; 109: 987-990.

19 Ditzler K: Efficacy and tolerability of memantine in patients with dementia syndrome. A double-blind, placebo controlled trial. Arzneimittelforschung.1991; 41: 773-780.

20 Yucel YH, Gupta NG, Zhang S, Mizisin A.P, Kalichman MW, Weinreb RN: . Memantine protects neurons from shrinkage in the lateral geniculate nucleus in experimental glaucoma. Arch. Ophthamol 2006; 124: 217-225.

21 Puente B, Hernandez E, Perez S, Pablo L, Prieto E, Garcia MA, Bregante MA: Determination of memantine in plasma and vitreous humor by HPLC with precolumn derivatization and fuorescence detection. J. of Chromatographic Science, (accepted for publication).

22 Gillies MC, Kuzniarz M, Craig J, Ball M, Luo W, Simpson JM: Intravitreal triamcinolone-induced elevated intraocular pressure is associated with the development of posterior subcapsular cataract. Ophthalmology. 2005 Jan; 112(1):139-43.

23 Cheng L, Hostetler KY, Gardner MF, Avila CP, Jr, Bergeron-Lynn G, Keefe KS, Wiley CA, Freeman WR: Intravitreal Toxicology in rabbits of two preparations of 1-0-octadecyl-sn-glycerol-3-phosphonoformate, a sustained-delivery anti-CMV drug. Invest Ophthalmol and Vis Sci 1999; 40(7): 1487-1495.

24 Amrite AC, Kompella UB: Size dependent disposition of nanoparticles and microparticles following subconjuntival administration.. J Pharm Pharmacol 2005; 57: 1555-1563.
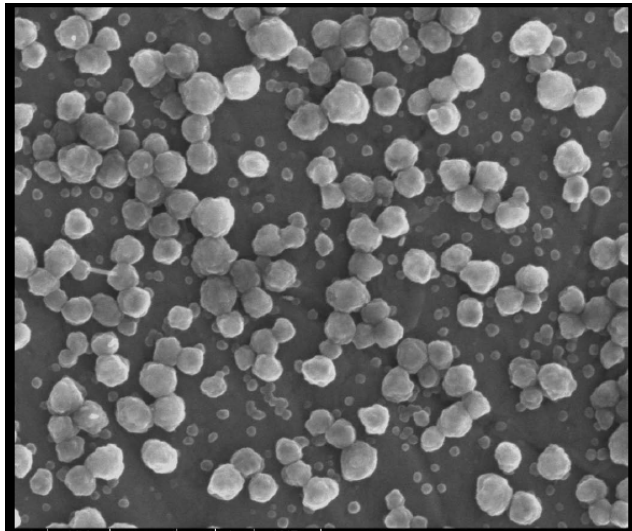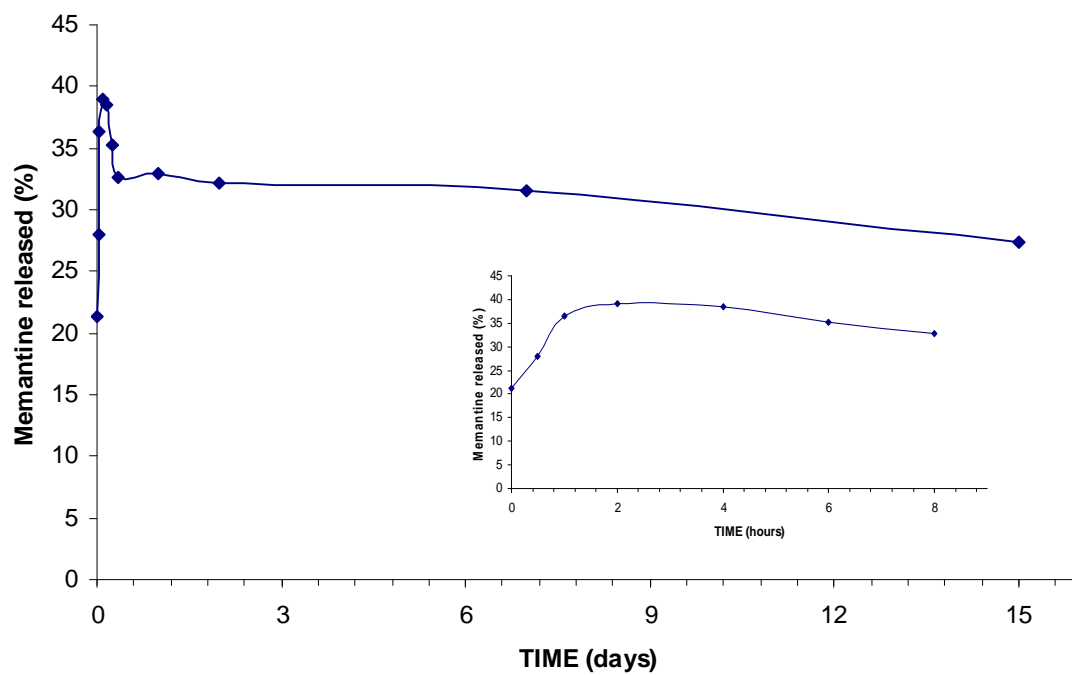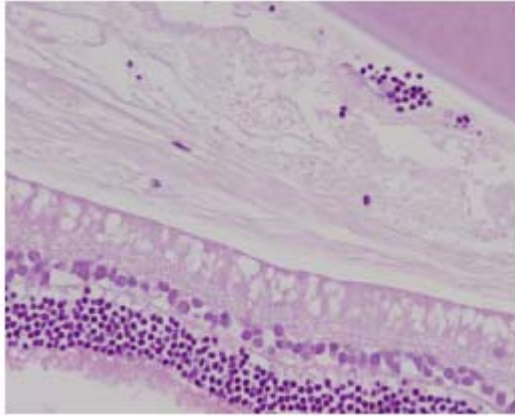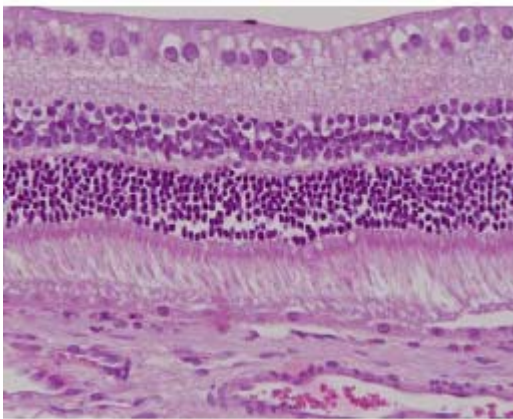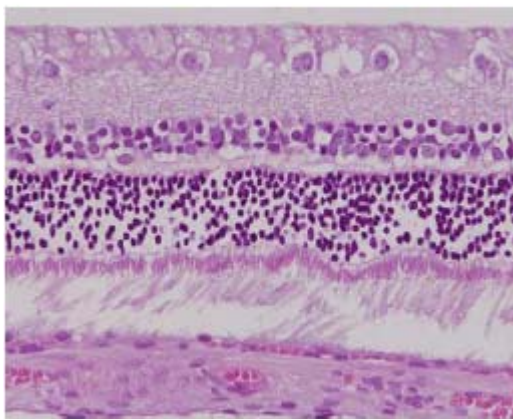
Fig. 1

Fig. 2

A

B

C

Fig. 3 (A B C)

Fig. 4