Biodegradable PLGA Microspheres Loaded with Ganciclovir for Intraocular Administration. Encapsulation Technique, *In Vitro* Release Profiles, and Sterilization Process

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Purpose. The purpose of this work was to obtain a sterilized formulation consisting of biodegradable microspheres of poly (DL-lactide-co-glycolide) (PLGA) for intraocular sustained release of ganciclovir.
Methods. Microspheres were prepared using a dispersion of ganciclovir in fluorosilicone oil (FSiO) that was further dispersed in an acetone solution of PLGA [50/50 and inherent viscosity 0.41 dl/g], and emulsified in silicone oil with a surfactant. Once prepared, the formulation was exposed with an effective γ radiation dose of 2.5 megarads. The release rate data of ganciclovir from the sterilized and nonsterilized batches were compared using the similarity factor (f_2).
Results. The dispersion of the drug in FSiO contributed to achieving a drug payload of up to 95% of the theoretical in the 300–500 μm microspheres. Ten mg released ganciclovir *in vitro* at 1.3 μg/h for the first 21 days, but decreased to ~ 0.2 μg/h from day 25 until the end of the release study (42 days). No significant differences in the amounts of encapsulated drug ($\alpha = 0.05$) were observed between the sterilized and nonsterilized microspheres. Furthermore, dissolution profiles of formulations behaved similarly before and after gamma radiation exposure.
Conclusions. The technique of microsphere preparation described resulted in high ganciclovir loading (95%) and prolonged drug release. The ganciclovir formulation behaved similarly before and after the sterilization process.

KEY WORDS: ganciclovir; microspheres; PLGA; intraocular; γ irradiation.

INTRODUCTION

Cytomegalovirus (CMV) retinitis is an ocular infection that occurs frequently in persons with acquired immunodeficiency syndrome (AIDS). The infection is progressive and results in blindness from retinal detachment associated with retinal necrosis (1). Ganciclovir and Foscarnet are used to treat CMV retinitis (2,3). In most cases, an intravenous dose (10 mg/kg daily, 7 to 21 days) of ganciclovir halts disease progression. Unfortunately, the disease recurs after discontinuation of the drug. Even on maintenance therapy, CMV recurs in 30 to 50% of patients (4–5). Dose-dependent myelosuppression prevents maintenance therapy in about 15% of patients. Sepsis related to permanent indwelling catheters is another problem associated with systemic ganciclovir administration (6)

Intravitreal ganciclovir injections provide higher intraocular drug concentrations than systemic therapy. The intravitreal half-life of ganciclovir (estimated to be approximately 13h) requires frequent injections (1 to 3 times/week) to maintain therapeutic ocular levels (7). Intravitreal ganciclovir injections of 200 to 400 μg, administered weekly, produce temporary remission of CMV retinitis (7,8). However, repeated intravitreal injections are poorly tolerated. The short intravitreal half-life of ganciclovir and its systemic toxicity make the drug a good candidate for using in a local sustained-delivery system. An intravitreous sustained-delivery ganciclovir device that releases ganciclovir for several months is currently used in human patients. This device is made of poly (ethylene vinyl acetate) and polyvinyl alcohol (PVA), is approximately 9 mm long and 3.5 mm wide, and has a depot filled with a pellet of several milligrams of ganciclovir. This nonbiodegradable device is implanted in the vitreous cavity through a scleral incision, where it is retained by a suture. The 1μg/h device released between 0.5 and 2.88 μg/h ganciclovir *in vivo* and was shown to be therapeutically effective in human CMV retinitis (9). The median effective inhibitory dose (ED_{50}) of ganciclovir for human CMV isolates ranged between 0.2 and 3.0 μg/ml, (10) which is equivalent to >0.9–13.5 μg per the 4.5 ml of the human vitreous (11). Despite the good performance of the available intraocular implant, a biodegradable injectable system could be a good alternative to this surgical implant, particularly for less-developed countries. Injectable drug-delivery devices are potentially less expensive than the surgical implants now available. Also, performing an intraocular injection requires a lower level of surgical sophistication than that needed to perform the delicate surgery for the placement of an intraocular implant. Experience has shown that poly (DL-lactide) (PLA) and poly (DL-lactide-co-glycolide) (PLGA) are biocompatible and degrade to metabolic products that are eliminated from the body (12,13). Microspheres loaded with retinoic acid, adriamycin, 5-Fluorouracil, and dexamethasone, as well as the ganciclovir formulation described here have been prepared to treat intraocular diseases such as proliferative vitreoretinopathy, (14,15) uveitis, (16) and cytomegalovirus retinitis.

The aim of this project, therefore, was to prepare microspheres of biodegradable polymers loaded with ganciclovir to obtain sustained release of the drug with a single intravitreous injection. However, a potential drawback of use of polymers for drug delivery could be the breakdown of their network after being exposed to ionizing radiation (17). Sterility is extremely important in drugs or devices to be used intravitreously, in which terminal sterilization procedures are preferred over aseptic processing. Consequently, we have developed a technique to prepare relatively hydrophobic microspheres with high drug payload and sustained ganciclovir release rates within the estimated therapeutical levels required for intravitreous therapy. Four diameter sizes of microspheres were tested (53–106μm, 106–212μm, 212–300μm, and 300–500μm) for drug loading and releasing. Of these, the most satisfactory microsphere formulation was size 300–500μm, and was used

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for the evaluation of the effect of 2.5 Mrad γ radiation dose on its stability.

MATERIALS AND METHODS

Materials

Ganciclovir sodium salt (BW B759 U, DHPG, dihydroxy propoxymethyl guanine) was obtained from Syntex Laboratories (Palo Alto, California). PLGA with a 50/50 ratio of DL-lactic to glycolic acids and an inherent viscosity of 0.41 dl/g in hexafluoroisopropanol was purchased from Birmingham Polymers Inc. (Birmingham, Alabama) (Molecular weight calculated by GPC; 0.4dl/g = 34,000 daltons; 0.5dl/g = 48,000 daltons). Methylene chloride and hexane were bought from Fisher Scientific (Pittsburgh, Pennsylvania). Silicone oil (SiO, polydimethylsiloxane, trimethylsiloxy terminated) viscosity 500 centistokes (cs), fluorosilicone oil (FSiO, polymethyl-3,3,3-trifluoropropyl siloxane) 1000 cs, and dimethylsiloxane ethylene oxide-propylene oxide copolymer (DMSiEPO) 1800 cs were obtained from Hüls America Inc. (Piscataway, New Jersey).

Methods

Preparation of Microspheres (18)

A suspension of ganciclovir sodium salt (equivalent to 25 mg of free ganciclovir) in FSiO (100 μl) was added to an acetone (0.8 ml) solution of PLGA (250 mg). The mixture was dispersed by agitation in vortex for 1 min and sonication (50 Sonic Dismembrator, Fisher Scientific) for 5 min at high frequency (intervals of 1 min of sonication and 10 sec of rest periods between sonications). This dispersion was immediately sonicated for 25 sec at high frequency (#18, outpower 80%) with a 2 ml aliquot of a solution of SiO (45 ml) in acetone (5 ml) and the surfactant DMSiEPO (100 μl). After the sonication, the mixture was added to the stirring remaining amount of the previously prepared SiO–acetone–surfactant solution. The stirring of the mixture, in the hood at room temperature, continued overnight until the acetone evaporated and the solid microspheres were formed. The microspheres were filtered and washed twice with hexane to remove residual SiO, and then separated in size fractions using sieves of 53, 106, 212, 300, and 500 μm (diameter aperture). Finally, the microspheres were dried over anhydrous $CaSO_4$ in a vacuum desiccator for at least 48 hours before they were used for the drug-release assays.

Analysis of Ganciclovir in the Microspheres

Replicate samples of microspheres from each batch and size were accurately weighed (10 mg) and dissolved in methylene chloride (2 ml). The ganciclovir was extracted from the methylene chloride solution twice into 3 ml of distilled water and measured spectrophotometrically at absorbance of 250.5 nm (Beckman DU-70 Spectrophotometer, Fullerton, California).

Microsphere Morphology

Microsphere samples were observed after sieve separation by light microscopy and by scanning electron microscopy (SEM, SAMR Model 1000A, Lico, Bedford, Massachussetts), before and at different stages of the release assay. For SEM the microspheres were attached to a specimen holder with a double-coated adhesive tape, and gold sputter-coated before observation at 20 Kv with a 12-mm working distance.

Ganciclovir Release from Microspheres

Replicate samples of microspheres (10 mg) from each batch and size were suspended in 1.5 ml of phosphate buffer (PBS 0.01 M, pH 7.4), and placed in a shaker with constant agitation (50 rpm IKA Bath, RT III, Germany) at 37°C. Samples for analysis (~1.5 ml) were taken with a syringe at 0h, 1h, 1, 4, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, and 42 days. The samples were filtered through a 0.45-μm filter (Gelman, Acrodisc LC 13 PVDF), and analyzed for ganciclovir by monitoring its absorbance at 250.5 nm. After sample removal for analysis, an identical volume of fresh phosphate-buffered saline (PBS) was added to the container with the microspheres to continue the release test.

When the release assay was concluded, the microspheres were dried for 2 days in a desiccator under vacuum, and the residual ganciclovir was then extracted from the polymer and analyzed as described above.

On the basis of the intravitreal pharmacokinetics of ganciclovir ($t_{1/2}$13h, and human vitreous volume 4.5 ml) and the CME_{50} range 0.2–3μg/ml, we calculated the theoretical release rate of ganciclovir to achieve therapeutical concentrations in the range of 1.15–17.26 μg/day. From all the microparticle sizes assayed, the microspheres of size 300–500μm were selected as the best suited to potentially deliver the required amount of drug intravitreously.

Gamma Irradiation of Ganciclovir Microspheres.

Five different batches of the selected formulation (300–500μm) were weighted and transferred to 5 ml glass screw cap vials.

The vials were labeled and packed surrounded with dry ice into a polyurethane container, assuring a low temperature during the irradiation process. Although γ irradiation causes only a minimal temperature rise, the low temperature avoids a possible acceleration of the hydrolytic degradation of the PLGA (17,19). The samples were shipped and treated at the Gamma Sterilization Unit of Aragogammma S.A. (Barcelona, Spain). Following the USP recommendations, an effective sterilizing dose of 2.5 megarad (Mrad) was used (20).

Before the sterilization, the mean values of ganciclovir payloads, and *in vitro* release rate from samples of 9 nonsterilized microsphere batches were determined in triplicate. The mean value of the dissolution profile was considered as the reference batch. On this way, the lot-to-lot variation in the release profile of the nonsterilized formulation was established.

Ganciclovir payload and release rate from the five sterilized batches of microspheres were determined in triplicate. The amounts of encapsulated drug before and after sterilization were compared by a mean t-test ($\alpha = 0.05$). A similarity factor (f_2) (21,22) was calculated to compare dissolution profiles of ganciclovir from the microspheres before and after sterilization. This factor allows characterization of the release profile of the drug. Conceptually, f_2 is a measure of the simi-

Table 1. Average Difference Between Two Dissolution Profiles of Reference Batches

	2%	5%	10%	15%	20%
f_2 Limit	83	65	50	41	36

larity in the percent dissolution between two curves. The value of similarity factor ranges between 0 to 100 with a higher f_2 value indicating more similarity between the two profiles. Table I provides the f_2 similarity limits for different average distances.

RESULTS

Ganciclovir Payloading and Release Rate from Microspheres

The 53–106 μm microspheres contained 681μg of ganciclovir per 10mg of microspheres (75.5% of theoretical); 106–212μm, 764μg/10mg (84.79%); 212–300μm, 845μg/10 mg (93.78%); and 300–500μm, 864 μg/10mg (95.89%), respectively.

Figure 1 compares the cumulative fractional release of ganciclovir from microspheres (M_t/M_∞) referring to the total encapsulated drug. Following the conditions described by Peppas (23) to describe the general solute release behavior from spheres (Fickian and non-Fickian) regardless of the release mechanism used, the fractional release of ganciclovir during the initial portion of the release curve were adjusted to the equation $M_t/M_\infty = K.t^n$; where M_t = percentage of released drug referred to M_∞; M_∞ = whole incorporated drug (100%); K = constant incorporating characteristics of the macromolecular network system and the drug; and n = diffusional exponent (indicative of the transport mechanism).

The rest of the release data were fitted to different kinetics (first or zero). Two sequential mechanisms of drug transport characterized the release kinetic of ganciclovir from the microspheres of size 53–106μm; 106–212μm and 212–300μm. Fickian diffusion is the predominant mechanism of drug release during the first 14 days. After that, phenomenologically the release rate behaves according to a zero-order release kinetic. Including the initial burst of the drug, the ganciclovir release rate never reached 100μg/day for any of the microspheres.

The total amount of ganciclovir released at 14 days, relative to drug payload was inversely proportional to the size of the microspheres (Figure 2).

Ten mg samples of the 300–500 μm microspheres with a total ganciclovir content of 864±30.71 μg released ganciclovir for at least 42 days (Figure 3). During the first 21 days, the microspheres released the drug following zero-order kinetics (32.76 μg/day) ($r = 0.9905$). From that time on, the microspheres released ganciclovir at a constant rate of 4.17 μg/day ($r = 0.9776$) until the conclusion of the experiments at day 44. The total amount of drug released averaged about 98% of the initial payload.

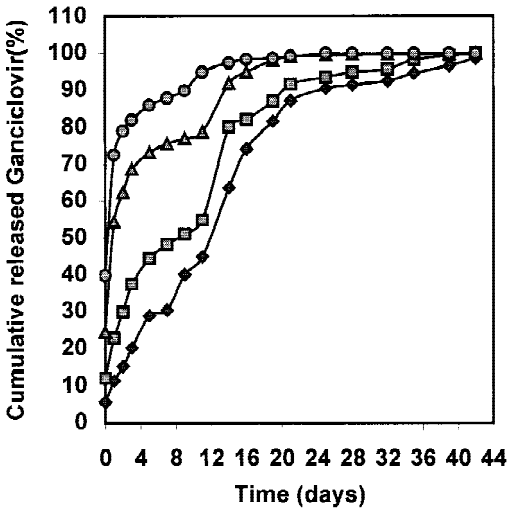

Fig. 1. Cumulative ganciclovir released in 1.5 ml PBS from 10-mg microspheres of different sizes (● 53–106 μm microspheres: 681 μg ganciclovir; ▲106–212 μm microspheres: 764 μg; ■ 212–300 μm microspheres: 845 μg; and ♦ 300–500 μm microspheres: 864 μg) prepared using the technique described in this work, with 250 mg of 0.41 dl/g polymer in 0.8 ml acetone, and 25 mg ganciclovir in 100 μl FSiO.

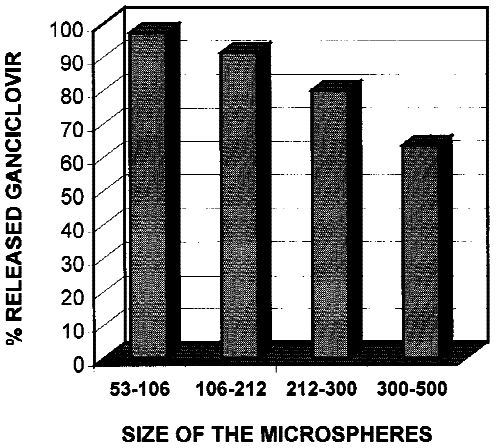

Fig. 2. Percentage of ganciclovir released by day 14 in 1.5 ml PBS the microparticles from Figure 1 as a function of microsphere size (μm).

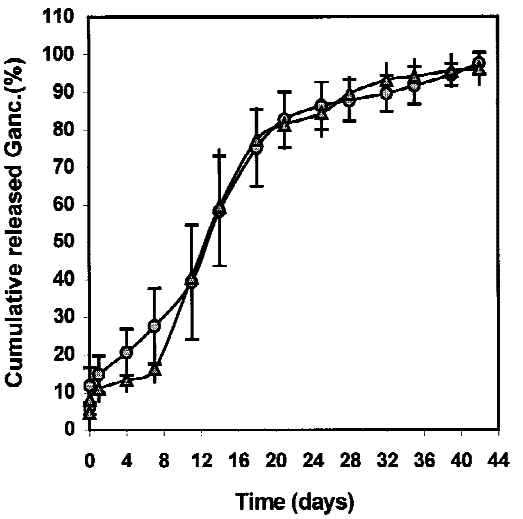

Fig. 3. Percentage of ganciclovir released in 1.5 ml PBS from 10-mg of microspheres (300–500μm). Data points (± standard deviations) from drug-release experiments before (● 9 batches) and after (▲ 5 batches) exposition to an effective dose (2.5 Mrad) of γ radiation.

Table 2. f_2 Values Calculated for the 9 Nonsterilized Batches from the Dissolution Profile Expressed in Percentage[a]

Batch #	I	II	III	IV	V	VI	VII	VIII	XIX
f_2	56.25	56.04	74.86	60.43	62.56	64.88	53.75	65.72	51.06

[a] Reference batch = mean dissolution data from the nine experiences.

Ten mg of 212–300μm and 300–500μm microspheres released ganciclovir at 1.15–17.26μg/day, which is within the therapeutic rate required per human vitreous (4.5 ml). However, not only was the drug release rate more controlled for these two sized microspheres than for the smaller sizes, but even 5 mg of the 300–500μm microspheres would be enough to achieve the therapeutic range of the drug required in the human vitreous. Therefore, the 300–500μm formulation was selected for the sterilization experiment.

Sterilization Effect

The maximum batch-to-batch variation for the 9 nonsterilized batches of microspheres prepared was 9%. Table III lists the mean (±SE) values for f_2, for the sterilized versus the non sterilized microsphere formulations. Both series—the pre- and post-sterilized batches—have similar drug release behavior, with f_2 values in the range 51–55. Furthermore, the difference between the release rate of ganciclovir from both sterilized and nonsterilized formulations was no larger than the maximum difference between any two batches of the presterilized microspheres (Tables II and III). Therefore, we can conclude that the γ irradiation dose of 2.5 Mrad did not produce significant changes on the ganciclovir release rate behavior of the selected microspheres.

Microsphere Morphology

Scanning electron microscopy (SEM) examination of the microspheres showed different structures at the surface and in the interior. Microspheres were round and homogeneous in shape for all sizes. The surface of the microspheres was smooth, but had some holes (Figure 4a). The interior of the fractured microspheres showed dark zones that were probably filled with the dispersion of ganciclovir in FSiO (Figure 4b). By day 28 of the release experiment, the surface of the microspheres was beginning to erode (Figure 5a) and had a highly porous interior. (Figure 5b).

DISCUSSION

The technique described here for preparing ganciclovir-loaded microspheres required mixing an acetone solution of PLGA with a fine dispersion of ganciclovir in FSiO. This dispersion was further dispersed in a small amount of SiO that contained 10% acetone to prevent premature polymer precipitation. The SiO does not dissolve the drug, the FSiO, or the polymer, although the more volatile acetone is slightly soluble in SiO. Therefore, when the mixture dispersed in a large volume of the SiO–acetone solution is stirred in an open container, in an aspirator hood, the acetone evaporates, yielding the solid microspheres. The relatively rapid solidification of the polymer at the interface with the SiO external phase resulted in an excellent yield of well-shaped microspheres that contained the entrapped suspension of ganciclovir in FSiO (24).

Most of the SiO in the microspheres is removed by washing it in hexane, which does not dissolve the drug, the FSiO, or the polymer. Because of the entrapped FSiO and any residual superficial SiO, the microspheres are fairly hydrophobic, which contributes to the high drug payload and the good ganciclovir release profile of the microspheres. The FSiO and the residual SiO retard but do not hinder the release of the drug from the microspheres to an aqueous environment. When the volume of FSiO was increased from 100μl to 150μl (18) we observed a slower release rate of the drug from the microspheres.

The mechanism of ganciclovir release from the microspheres in PBS starts with the typical burst effect that results from rapid dissolution of the drug at the surface of the microspheres. Then, the hydrophobic FSiO and SiO retard the penetration of water into the microspheres and consequently prolong the ganciclovir release. When the polymer is hydrolized and starts to disintegrate, water reaches the boundaries of FSiO globules into the pores of the microsphere, the oil flows out and the dispersed ganciclovir in the oil is partitioned into the surrounding water.

Depending on the size, microspheres have different constitution ratio of ganciclovir, FSiO, and PLGA. As expected, the smaller microspheres released the drug faster than the bigger microspheres as the result of the larger diffusion area and shorter diffusion path of the smaller microspheres. The smaller size also facilitates breakdown of the microspheres due to polymer hydrolysis. Consequently, the best ganciclovir loading and release profiles from microspheres among the sizes tested were obtained with the biggest (300–500 μm) microspheres. Five mg of these microspheres released ganciclovir *in vitro* at concentrations within the potential therapeutic range for at least 42 days. These *in vitro* results are in agreement with the ones obtained about the efficacy of the ganciclovir microspheres, evaluated ex vivo, against CMV infection in HS68 monolayers (25). Also, viral quantity inhibition assays in rabbit eyes indicated that 1 mg/ml of microspheres similar to the ones evaluated in this work inhibited more than two digits of the virus over 6 weeks (26).

In an earlier publication, no inflammatory signs were noted in the vitreous, retina, and choroid for up to 8 weeks after injection of up to 15 mg of ganciclovir microspheres prepared from a PLGA with similar inherent viscosity (0.39 dl/g) as used in the present study after ultraviolet light ster-

Table 3. f_2 Values Calculated for the 5 Sterilized Batches Using the Mean Dissolution Data of the Nonsterilized Lots as Reference

Batch #	S1	S2	S3	S4	S5
f_2 Limit	54.26	58.99	51.21	55.21	55.84

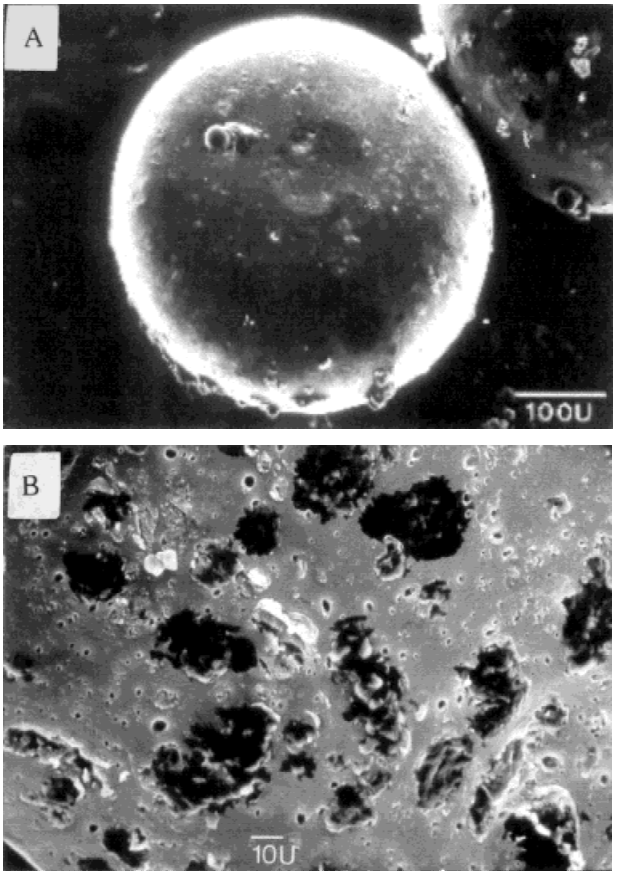

Fig. 4. (a) Microspheres (300–500 μm) prepared with 250 mg 0.41 dl/g PLGA, 25 mg ganciclovir, and 100 μl FSiO (SEM ×160). (b) Internal view of fragmented microsphere (×600).

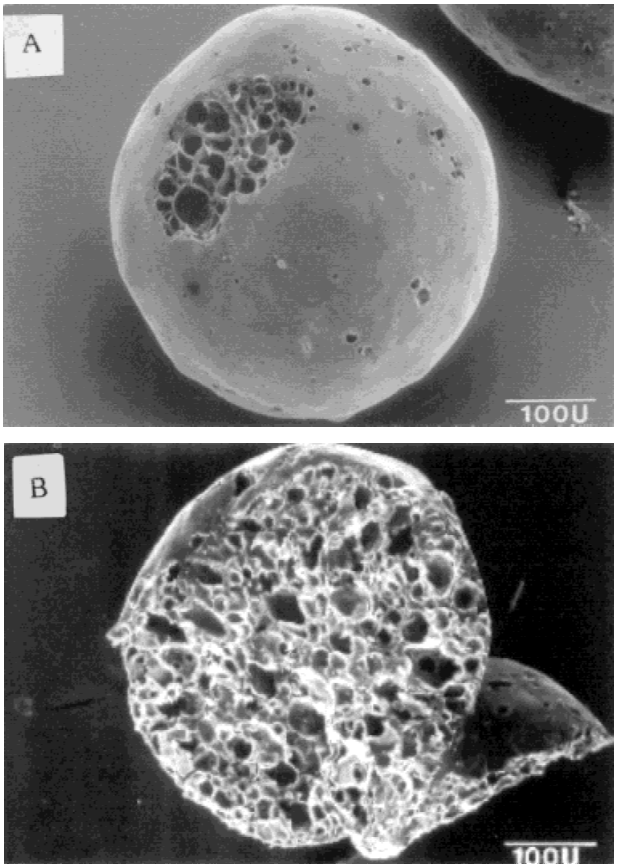

Fig. 5. (a) Microspheres from the same preparation as Figure 4 after 28 days *in vitro* ganciclovir release study in PBS (×160). (b). View of a fragmented microphere treated as (a) (×160).

ilization (26). Nevertheless, because intraocular drug delivery systems are required to be absolutely free from microorganisms, in this study we used the more effective γ irradiation procedure to sterilize the microspheres. Although γ irradiation causes a minimal temperature rise, alterations in the polymer network of PLGA by γ irradiation have been reported before (17,19). Therefore, we used care to avoid temperature increases during the γ irradiation time. If the polymer network was modified substantially by the ionizing irradiation, a variation in the drug release profile would be expected. However, the ganciclovir–microsphere formulation behaved similarly before and after the sterilization process.

CONCLUSIONS

The technique of microsphere preparation described in this paper resulted in high ganciclovir loading and prolonged drug release. The SiO in the microsphere solidification medium contributes to high drug loading by preventing the loss of the hydrophilic drug during the microsphere preparation. The FSiO in the microspheres retards but does not hinder the dissolution of the ganciclovir to the surrounding aqueous medium and prolongs the time of drug release. It is estimated that 5 mg of the 300–500μm ganciclovir microspheres could deliver an adequate amount of drug in a single injection into the human vitreous.

A potential drawback of FSiO and SiO in the microspheres is the fact that both oils are nonbiodegradable and would remain in the eye after the polymer disappeared. The theoretical FSiO calculated per 5 mg of ganciclovir microspheres described in this work is 1.30μl. However, for the potential intraocular injection of microspheres in AIDS patients with CMV retinitis, the residual ocular oils should not present an intolerance problem (27). Intravitreous injections of SiO are often used as internal retinal tamponade in AIDS patients who develop retinal detachments associated with retinal necrosis (28). FSiO also has been used in the eye, albeit less frequently due to earlier emulsification than SiO (29).

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REFERENCES

1. D. A. Jab, C. Enger, and J. G Bartlett. Cytomegalovirus retinitis and acquired immunodeficiency syndrome. *Arch. Ophthalmol.* **107**:75–80 (1989).
2. P. Chrisp and S. P. Clissold. Foscarnet. A review of its antiviral activity, pharmacokinetic properties and therapeutic use in immunocompromised patients with cytomegalovirus retinitis. *Drugs* **41**:104–129 (1991).
3. M. R. Robinson, B. W. Streeten, G. R. Hampton, E. C. Siebold, and C. Taylor-Findlay. Treatment of cytomegalovirus optic neuritis with dihydroxy propoxymethyl guanine. *Am. J. Ophthalmol.* **102**:533–534 (1986).

4. D. E. Henderly, W. R. Freeman, D. M. Causey, and N. A. Rao. Cytomegalovirus retinitis and response to therapy with ganciclovir. *Ophthalmology* **94**:425–434 (1987).
5. J. Orellana, S. A. Teich, A. H. Friedman, F. Lerebours, J.Winterkorn, and D. Mildvan. Combined short-and long therapy for the treatment of cytomegalovirus retinitis using ganciclovir (BW B759U). *Ophthalmology* **94**:831–838 (1987).
6. W. R. Freeman. Intraocular viral therapy. *Arch. Ophthalmol*. **107**: 1737–1739 (1989).
7. K. Henry, H. Cantrill, C. Fletcher, B. J. Chinnock, and H. H. Balfour. Use of intravitreal ganciclovir (dihydroxy propoxymethylguanine) for cytomegalovirus retinitis in a patient with AIDS. *Am. J. Ophthalmol*. **103**:17–23 (1987).
8. M. H.Heinemann. Long-term intravitreal ganciclovir therapy for cytomegalovirus retinopathy. *Arch. Ophthalmol.* **107**:1767–1772 (1989).
9. D. F. Martin, D. J. Parks, S. D. Mellow, F. L. Ferris, R. C. Walton, N. A. Remaley, E. Y. Chew, P. Ashton, M. D. Davis, and R. B. Nussenblatt. Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant. *Arch. Ophthalmol*. **112**:1531–1539 (1994).
10. Cytovene (ganciclovir sodium salt). In *Physicians' Desk Reference,* 49 Ed. Medical Economics Data Production, Montvale, New Jersey. 1995, pp 2471–2475.
11. J. S. Cumming. Relevant anatomy and physiology of the eye. In J. R. Robinson (ed.), *Ophthalmic Drug Delivery Systems,* American Pharmaceutical Association, Washington DC, 1980, pp. 1–27.
12. S. Cohen, T. Yoshioka, M. Lucarelli, L. H. Hwang, and R. Langer. Controlled delivery systems for proteins based on poly (lactic/glycolic acid) microspheres. *Pharm. Res.* **8**:713–720 (1991)
13. A. Zimmer and J. Kreuter. Microspheres and nanoparticles used in ocular delivery systems. *Adv. Drug Deliver. Rev*. **16:** 61–73 (1995).
14. T. Moritera, Y. Ogura, Y. Honda, R. Wada, S. H. Hyon, and Y. Ikada. Biodegradable microspheres containing adriamycin in the treatment of proliferative vitreoretinopathy. *Invest. Ophth. Vis. Sci*. **132**:1785–1790 (1991).
15. G. Giordano, M. F. Refojo, and M. H. Arroyo. Sustained release of retinoic acid from microspheres of biodegradable polymer in PVR. *Invest. Ophth. Vis. Sci*. **34**:2743–2751(1993).
16. R. Herrero-Vanrell, C. Alvarez-Santiago, and E. Barcia. Biodegradable microspheres loaded with dexamethasone for intravitreal administration. ARVO Abstracts. *Invest. Ophth. Vis. Sci.* **40**:S84 (1999).
17. A. G. Hausberger, R. A. Kenley, and P. P. De Luca. Gamma irradiation effects on molecular weight and in-vitro degradation of poly(D,L-lactide-glycolide) microparticles. *Pharm. Res*. **12**: 851–856 (1995).
18. R. Herrero Vanrell and M. F. Refojo, U.S.Patent # 5,718,922 (1998).
19. A. Merkli, J. Heller, C. Tabatabay, and R. Gurny. Gamma sterilization of a semi-solid (ortho ester) designed for controlled drug delivery- validation and radiation effects. *Pharm. Res*. **11**:1485–1491(1994).
20. USP Pharmacopoiea 24. Sterilization and Sterility Assurance.
21. V. P. Shah, Y. Tsong , P. Sathe, and P. Liu. In vitro dissolution profile comparison—statistic and analysis of the similarity factor, f_2. *Pharm. Res*. **15**:889–895 (1998).
22. Y. Tsong, T. Hammestrom, S. Pradeep, and P. S. Vinod. Statistical assesment of mean differences between two dissolution data sets. *Drug Inf. J.* **30**:1105–1112 (1996).
23. P. L. Ritger and N. A. Peppas. A simple equation for description of solute release I Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *J. Control. Release* **5:** 23–36 (1987).
24. T. R. Tice and R. M. Gilley. Preparation of injectable controlled release microcapsules by a solvent evaporation process. *J. Control. Release* **2**:343–350 (1985).
25. Q. Zhu, A. Veloso Jr., F. L. Leong, P. Geary, E. Dunkel, and M. F. Refojo. Efficacy of biodegradable microspheres loaded with ganciclovir during cytomegalovirus (CMV) infection on HS68 monolayers. Abstract. *Invest. Ophth.Vis. Sci.* **37**:S41 (1996).
26. A. A. S. Veloso Jr, Q. Zhu, R. Herrero-Vanrell, and M. F. Refojo. Ganciclovir-loaded polymer microspheres in rabbit eyes inoculated with human cytomegalovirus. *Invest. Ophth. Vis. Sci.* **38:**665–675(1997).
27. K. Miyamoto, M. F. Refojo, F. I. Tolentino, G. A. Fournier, and D. M. Albert. Fluorinated oils as experimental vitreous substitutes. *Arch. Ophthalmol*. **104**:1053–1056 (1986).
28. Y. Sidikaro, L. Silver, G. N. Holland, and A. E. Kreiger. Rhegmatogenous retinal detachments in patients with AIDS and necrotizing retinal infections. *Ophthalmology* **98** :129–135 (1991).
29. F. I. Tolentino, V. N. Cajita, H. Chung, J. Acosta, H. M. Freeman, and M. F. Refojo. High-density fluorosilicone oil in vitreous surgery. In H.M. Freeman and F.I. Tolentino (eds.), *Proliferative Vitreoretinopathy (PVR),* Springer-Verlag, New York, 1988, pp. 177–180.