CONTROLLED DRUG RELEASING INTRAVITREAL IMPLANT USING BIODEGRADABLE PLGA

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ABSTRACT

The goal of this work is to compare the *in vitro* drug (Nimodipine) release rate from three different types (matrix, reservoir, and combination types) of intravitreal implants using a biodegradable polymer (PLGA). The matrix implants were prepared by a solvent cast method and the reservoir implants were fabricated by using a pellet press. The combination implants were a mixed type of matrix and reservoir implants. Each implant was placed in a vial with 7mL of Phosphate Buffered Saline (PBS) containing 0.4 g/L of Bovine Serum Albumin (BSA) and 0.5 mL aliquots were removed for the drug assay for 25 days. The sample was analyzed to determine the concentration using High Performance Liquid Chromatography (HPLC). Over 20 days, the average steady state release rate *in vitro* is 13.05±6.17, 2.66±1.98, 8.85± 6.36 µg/day for matrix, reservoir, and combination implants, respectively.

INTRODUCTION

Eye diseases have been treated with traditional medical approaches such as eye drops, oral medication, and systemic drug therapy. However, the problems with these methods are their limited effectiveness due to the blood-retinal barrier (Bleeker et al., 1968) and harmful side effects. The intravitreal injection is also restricted by pulsed drug concentration profile that may have limited therapeutic outcome. In contrast, a controlled drug releasing implant using polymer is a prospective candidate for treating retina diseases, since it could have a more uniform release rate at a desired drug concentration for a sustained period of time.

Yamazaki et al. demonstrated that a calcium channel blocker is beneficial for the preservation of photoreceptor cells in rats and can be used to treat Retinitis Pigmentosa (RP), which is characterized by progressive degeneration of the retina and often leads to blindness. Accordingly, in our report, Nimodipine (PDR 2000), a calcium channel blocker of the dihydropyridine class, is the test drug used.

The copolymer of lactide and glycolide, poly (lactide-co-glycolide) (PLGA) was used to make the implants, since it has excellent biocompatibility, biodegradability, and mechanical strength properties (Jain, 2000). In order to achieve a more sustained Nimodipine release rate for *in vitro* experiments, matrix, reservoir, and combination type implants were evaluated.

METHODS

Implant preparation

Films were prepared by dissolving 1.3 grams of PLGA 50:50 (latide:glycolide) (Birmingham Polymers, Inc.) in 10mL acetone under magnetic stirring. The solution was cast on Teflon dish and the solvent was evaporated under ambient condition for 4 days. The films obtained were 0.3 mm thick.

The matrices containing 10 w/w % Nimodipine were prepared with the same method as the fabrication of the films and were cut into rectangular pieces (7.15×7.15×0.3 mm) from the matrices. Each piece represented a matrix implant.

The reservoir implants, with a diameter of 4.3 mm, a thickness of 0.8 mm, and a suture stub that was 1.5 mm long and 0.6 mm thick, were constructed by compressing Nimodipine powder (Argenol Co.) into drug pellets (3 mm diameter) using a pellet press. Subsequently, the film was pressed into the Teflon mould to make a reservoir. The drug pellet was placed into reservoir and the film covered the top of reservoir by heating at 60°C.

The combination implants were created with the same method as the reservoir implant, using the matrices containing 10 w/w % Nimodipine instead of only PLGA films.

Measurement of Nimodipine release rate from different types of implants *In vitro*

In vitro release rates assays were performed for all the three different types of implants. For each type, three implants (n=3) with the similar dimensions were evaluated. Each implant was placed in a vial with 7 mL of PBS (pH 7.4) containing 0.4 g/L BSA at 37°C under micro-magnetic stirring. The PBS was changed every day to simulate sink conditions and 0.5 mL aliquots were removed for a drug assay every one to four days over 25 days. The release rates were determined by calculating the amount of Nimodipine released in a given volume over time. The cumulative Nimodipine released was calculated by intergrating the area under the release rate curve using the trapezoidal rule (Welling, 1986).

HPLC Condition

The mobile phase consisted of acetonitrile-methanol (79:21, v/v) and 0.015 M phosphate buffer containing 2.8 mM triethylamine (60:40, v/v). The mobile phase was filtered and degassed before use. The flow rate of the mobile phase was 1.2 mL/min. 5 mL volume of extract solvent, hexane-butanol, (12:1, containing 1 µM Nicadipine as an internal standard), was added in the aliquot of 0.5 mL and then vortex-mixed for 30 sec and centrifuged for 3 min at 3000 rpm. The 5 mL sample of the extract solvent layer was collected and evaporated until dry with nitrogen at 50°C and 150 µL of the mobile phase was added to dissolve the residue. After 30 sec of vortex mixing, 50 µL of the sample solution was injected into the HPLC (Waters system).

Chromatographic separations were carried out on a C18 column (Length: 250 mm, I.D.: 4.6 mm, particle size: 5 µm) at 25°C. Nimodipine and Nicardipine (internal standard) was detected at 236 nm with a retention time of 8.1 min and 14.8 min, respectively, as shown at Figure 1.

RESULTS AND DISCUSSION

Nimodipine release profile over 25 days *in vitro* from the three different types of implants are shown in Figure 2. The release profile for all types of implants followed a triphasic profile that is, in general, common for biodegradable polymer (Kunou et al., 2000). In Figure 2A, the release profile data represents an initial burst within the first couple of days. The second stage (from approximately day 2 to day 20) shows a sustained drug release phase that is caused by diffusional release of drug whereas the third stage (after day 20) shows a sudden burst resulting from swelling and disintegration of the polymeric matrix.

Figure 2B compares the release rates of three different types of implants. The release rate mainly depends on surface area of implant and the amount of drug loading on the polymer (Siepmann et al., 2001). The surface area of matrix implants was larger than the other implants. Therefore, the release rate during the 25 days for matrix implants was higher than that for the other implants. The reservoir implants released Nimodipine at very slow rate and there was insignificant release of drug during the second stage. The drug release from combination implants was intermediate between those of matrix and reservoir types, but more closely followed that of the reservoir type. The combination implants can reduce the initial burst compared to matrix implant and sustain a better release of drug during the second stage as compared to the reservoir implant. During the second stage, the average steady state release rate was 13.05±6.17, 2.66±1.98, 8.85±6.36 µg/day for matrices, reservoir and combination types of implants, respectively. During the third stage, there was sudden burst and a high drug concentration that could cause toxicity *in vivo*. Implant with sustained drug release rate over a period of time, without sudden burst, is desired for treating eye disease such as RP.

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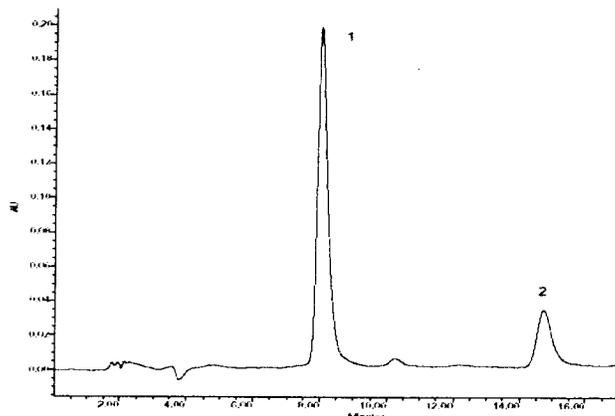Welling, P. G., 1986, "Pahrmacokinetics: Processes and Mathematics", ACS Monograph 185, pp.145-149

Figure 1: Sample Chromatograms of Nimodipine and Nicardipine (internal standard).
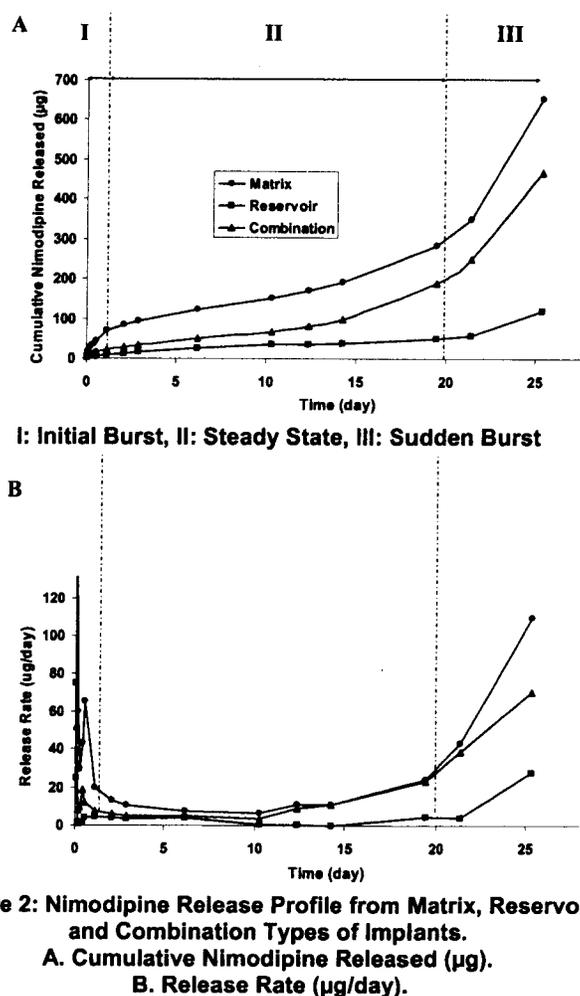**1: Nimodipine (8.1 min)   2: Nicardipine (14.8 min)**

I: Initial Burst, II: Steady State, III: Sudden Burst

Figure 2: Nimodipine Release Profile from Matrix, Reservoir and Combination Types of Implants.
A. Cumulative Nimodipine Released (µg).
B. Release Rate (µg/day).