Intravitreal Zero-order Release Levofloxacin Implant in an Experimental Model of Endophthalmitis

H. Zhang¹, F. Wang², L. Adams³, D. Yang¹, A. Donohue⁴, R. Pranker³, R. Robins-Browne⁴, R. Tait⁵

¹Centre for Eye Research Australia, The University of Melbourne, Melbourne, VIC, 3002, Australia; ²Eye Hospital, Harbin Medical University, Harbin, Heilongjiang Province, 150001, China; ³Monash University, Melbourne, VIC, 3806, Australia; ⁴Microbiology and Immunology, The University of Melbourne, VIC 3010, Australia; ⁵PolyActiva Pty. Ltd., Melbourne, VIC, 3000, Australia

presenting author email address: Hong.zhang@unimelb.edu.au

ABSTRACT SUMMARY

A novel intraocular Levofloxacin (LVX) monoglyceride-polyurethane (PU) conjugate was developed for the treatment of endophthalmitis, an infection inside the eyeball. Endophthalmitis is very serious and can lead to blindness and even loss of the eye itself. LVX release from the implant was evaluated under in vitro and in vivo conditions. A rabbit model of endophthalmitis was used to compare both the prophylactic and therapeutic efficacy of bioerodable polymers containing levofloxacin with the accepted treatment (vancomycin plus ceftazidime).

INTRODUCTION

Endophthalmitis (intraocular inflammation) can be catastrophic with irreversible damage to the retina and resultant visual loss [1]. Clinical intervention in the acute setting is required within hours of diagnosis to limit severe visual loss. By the time infection is clinically evident, initiation of antibiotic therapy may be too late to reverse the severe inflammatory response. Intravitreal injection of a bolus dose of antibiotics is the preferred method of treating bacterial endophthalmitis[2]. Unfortunately, because of the rate of clearance, the half-life of the drug in the eye is short and a single intravitreal injection of antibiotic(s) is often insufficient to cure endophthalmitis[3]. The use of an implant which would release an antibiotic at the appropriate dose as a substitute for multiple injections would remove the need for retreatment in severe cases.

In this study, LVX monoglyceride-PU rods were supplied by PolyActiva® chemists. LVX molecules pendant to the biodegradable PU backbone (ester link) is designed to be released on cleavage of the linkage bonds in aqueous media.

LVX release kinetics from the drug-polymer conjugate rods were evaluated both in vitro and in vivo. The efficacy of the implant was assessed in a rabbit model of endophthalmitis and the safety of the implant has also been proved in rabbits.

EXPERIMENTAL METHODS

The LVX monoglyceride-PU conjugate contained 48.8 %w/w LVX monoglyceride, equivalent to 40.3 %w/w LVX. Rods with dia. 0.5, 0.7, 1.0 and 1.5 mm were made in three groups: constant length, constant surface area and constant volume by cutting them to the appropriate lengths.

For in vitro release testing, each rod was suspended in isotonic phosphate buffer pH 7.4 at 37.0 °C with constant stirring. Aliquots (100 µL) of each release medium were taken with replacement at predetermined time points for HPLC.

In vivo experiments were performed in four adult New Zealand White rabbits. A preliminary in vivo investigation of drug release in the rabbit eye was performed by collecting vitreous humour samples from eyes with implanted rods at various times. The samples were diluted with PBS (1:1). An aliquot of this dilution was mixed with an equal volume of acetonitrile and then centrifuged to remove proteins or other macromolecules. The supernatant was analyzed by HPLC for LVX.

Treatment study: To demonstrate the efficacy of the conjugate in the treatment of endophthalmitis, Staphylococcus aureus strain...
29213 was inoculated into the vitreous cavity of one eye of twelve rabbits which were divided randomly into treatment group, negative and positive control group (n=4 respectively). On day 1 post-infection, the infected eyes in treatment group were treated with a levofloxacin-releasing implant, a bolus injection of 1% (w/v) vancomycin then 2.2% (w/v) ceftazidime (“gold standard”); left untreated as negative control or treated with vancomycin plus ceftazidime as positive control. On days 1, 3, 7, 10 and 14 after inoculation, samples of vitreous were collected for determination of the number of colony-forming units of S. aureus per ml. The Peyman classification was used to assess the severity of endophthalmitis.

Prophylaxis study: To demonstrate prophylaxis, the levofloxacin-releasing implant was inserted concurrently with the bacterial inoculum on day 0 in seven rabbits.

RESULTS AND DISCUSSION

All rods exhibited continuous release of LVX in vitro over 120 days without reaching a plateau. LVX release profiles indicated a mass-based release mechanism. In other words, there was little influence from the geometric characteristics of the rods. LVX concentrations in the vitreous humour matched the steady-state levels calculated from pharmacokinetic methoss that rely on the release rate measured in vitro and held well above the minimum inhibitory concentration (MIC) range for S. aureus (0.06 – 0.5 μg/ml) over a 10-day period.

Bacterial counts in vitreous samples collected on day 1 from rabbits in the treatment study increased in all cases. By day 3 the number of bacteria in the vitreous from all rabbits treated with the levofloxacin-eluting implant or standard antibiotics had fallen to below detectable levels demonstrating that both treatments had successfully cleared the infection. In contrast, bacterial counts in the vitreous from the untreated rabbit increased dramatically by day 3 and symptoms worsened to such an extent that euthanasia of the rabbit was required. For rabbits in the implant group, mild inflammation was observed on day 3 following inoculation. By the seventh day, the symptoms of endophthalmitis had subsided. Throughout the experiment, the Peyman scores for the implant group were similar to or better than rabbits in the standard treatment group.

The implant effectively prevented endophthalmitis in the prophylaxis study. In the safety study, the implants were visibly decreasing in size over time, and no inflammation or cell infiltration has been noticed.

CONCLUSION

The LVX conjugate rods exhibited a mass-based zero-order release profile in the in vitro release study which was not influenced by the geometric factors of the rods.

Implanted rods in the rabbit eye released LVX into the vitreous humour with concentrations well above that required for inhibition of Staphylococcus aureus.

The levofloxacin-releasing implant was at least as effective as the current standard therapy of vancomycin and ceftazidime in clearing S. aureus from the vitreous cavity of rabbits and is associated with a lower inflammatory response.

REFERENCES


ACKNOWLEDGMENTS

This project was supported by an NHMRC Development Grant and a Researcher in Business Grant.

CERA receives Operational Infrastructure Support from the Victorian Government