A Thermosensitive Glycol Chitin Gel For The Vaginal Delivery of Progesterone: In vitro and In vivo Evaluation

Aliyah A. Almomen1, Sungpil Cho1, Zhengzheng Li2, Kang Moo Huh2, C. Matthew Peterson1 and Margit M. Janát-Amsbury1

1University of Utah, Salt Lake City, 84132, USA; 2Chungnam National University, Daejeon, South Korea
Aliyah.Almomen@utah.edu

ABSTRACT SUMMARY

In this study, we evaluated newly developed vaginal progesterone (P4) Glycol-chitin (GC) gel delivery system based upon an amphiphilic chitosan-based polymer. This polymer exhibits thermosensitive and mucoadhesive properties, both most desirable for vaginal delivery. Here, we report the systems release kinetics, as well as its tissue biocompatibility in vitro and in vivo as continuous work based on previous results presented during CRS Meeting 2013, Poster #139. Developing a novel, Glycol-Chitin based Thermo-sensitive Gel for the Vaginal Delivery of Progesterone: Synthesis and Sol-Gel Transition evaluation.

INTRODUCTION

Vaginal P4 administration poses a viable alternative and has proven to be as effective or even superior to intramuscular P4 with the ability to provide a more site-specific treatment. Currently commercially available vaginal P4 formulations (i.e. suppositories, creams) almost always liquefy immediately once applied at body temperature. This causes messiness but foremost loss of active drug, which negatively impacts patient compliance and therapeutic outcomes.

Recent studies using thermosensitive hydrogels demonstrated an increase in local residence times as well as the controlled release of other hydrophobic drugs, providing steady drug levels. Further, such gels are easy to administer and can reduce the frequency of application while minimizing formulation leakage.

In this study, Glycol-Chitin (GC), an amphiphilic chitosan-based polymer with thermosensitive and mucoadhesive characteristics, delivers P4 vaginally. Here, we demonstrated that GC released its cargo, P4 in a controlled manner and was safe to use in a vaginal environment in vitro as well as in vivo.

EXPERIMENTAL METHODS

GC-P4 gel was prepared according to the methods described in Poster #139, CRS 2013. Briefly, GC was slowly added to cold citrate phosphate buffer (4 °C, 0.1 M, pH 4.2) under gentle mixing and allowed to dissolve completely for 24 hr. Then P4 was added into the GC solution and stirred for 24 hr at 4 °C. The final gel contained 5 wt% GC and 0.1 wt% P4.

To further study the mechanism of P4 release from GC-P4 gel, we exposed the gel to vaginal fluid simulant (VFS) at 37 °C. The change of gel weight was then measured as a function of time. The amounts of VFS removed at each time interval was used to determine the percentage of P4 released. Released amounts of P4 were measured at 250 nm on a Varian Cary 400 Bio UV-visible spectrophotometer (BioTech, MD, USA).

Cytotoxicity of GC to human VK2/E6E7 vaginal epithelial cells was determined in vitro using MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells were exposed for 24 hr to GC solutions with various concentrations. In addition a clonogenic assay was conducted. Cells were seeded in 6 well plates in a density of 2000 cells/well and incubated in 2 ml Keratinocyte-Serum Free medium. Cells were then treated either with GC, GC-P4, P4, Gynol II®, 8% Crinone® (as positive controls for cell toxicity) or no treatment. Treatments were added to a 0.4 μm transwell membrane (Transwell®, Costar, NY, USA) and cells were exposed for 24 hr. Cells were cultured for additional 7 days and visible colonies were then fixed and stained. Visible colonies were quantified with ImageJ 1.44o software (N.I.H., Bethesda, MD, USA) (size range of 2 ~ 200μm).

To further assess the effect of GC on the sustainability of an intact, physiological and healthy vaginal flora, the viability of Lactobacillus crispatus was investigated. Bacteria were seeded and incubated with 100 μl serial dilutions of GC at 37 °C for 24 hr. After 24 hr bacterial viability was evaluated by MTS [3-(4,5-dimethyl thiazole-2-yl)-5-(3-carboxymethoxymethyl)-2-(4-sulfanyl) 2H-tetrazolium (Promega, Madison, WI, USA) and absorbance was measured at OD490 using Spectramax 250 microplate reader (Molecular device, CA, USA). All experiments were conducted in triplicates and results were expressed as mean ± SD with P<0.05 being considered as significant.

To determine that GC-P4 gel is safe for use on vaginal epithelium, in vivo tissue biocompatibility studies were conducted in 6-8 week old, female Nu/Nu mice (Jackson Lab.ME USA). Animals were divided into three groups all vaginally receiving either GC-P4 gel, Gynol II®, or PBS once daily for three consecutive days. Twenty-four hours after the last treatment, animals were sacrificed and vaginal tissues were harvested, sectioned and analyzed.

RESULTS AND DISCUSSION

When exposed to VFS in a vaginal like environment, GC-P4 gel showed a twenty percent weight increase within the first 30 minutes of exposure. GC-P4 gel lost up to fifty percent of its weight within the next 3.5 hours.
indicating that swelling and erosion mechanisms may be responsible for P4 release.

Figure 1. Release kinetics of GC-P4 gel, A. Gel weight change over time. B. P4 cumulative release from GC-P4 gel over time.

Within Figure 2A we demonstrated the lack of cytotoxicity and therefore the safety of GC to vaginal epithelium in vitro. Human VK2/E6E7 cells were exposed to several dilutions of GC, which did not impact viability compared to an untreated control.

We also found that the number of colonies following treatment with GC and GC-P4 were comparable to number of colonies formed in the non-treated group. However, the formation of colonies was found to be significantly reduced after treatment with either P4 alone, Crinone® or Gynol II® (Figure 2B).

Figure 2. In vitro Cytotoxicity of GC and GC-P4. A. MTT assay using VK2/E6E7 cells. B. Clonogenic assay using VK2/E6E7 cells. C. MTS assay using Lactobacillus crispatus.

We further evaluated GC-P4’s impact on maintaining a healthy, undisturbed, physiological bacterial vaginal flora, which is characterized by the presence of Lactobacillus crispatus. Results shown in Figure 2C indicated that there was no significant decrease in % viability between GC treated and non-treated bacteria, therefore rendering GC safe to the vaginal environment.

Histolopathological examination of murine vaginal tissues following in vivo exposure to GC-P4 revealed, mostly intact squamous cells (Keratinocytes) with only a slight, transient increase of neutrophils after repeated exposure (3x) to GC-P4 gel (Figure 3C). Only minimal signs of transient, fully reversible acute inflammation were identified. Whereas massive cell death of squamous cells and a significant increase in neutrophils indicative of non-resolving, persisting inflammatory processes occurred after exposing tissues to Gynol II® (Figure 3B).

CONCLUSION

According to the presented findings, we conclude that our gel is capable to reside in the vaginal environment for at least four hours enabling the continued, controlled release of P4. GC-P4 was found to be safe and biocompatible to the vaginal environment in vitro and in vivo, indicating its suitability for repeated applications. Currently, additional in vivo studies are ongoing to determine the efficacy of GC-P4 in murine gynecologic disease models.

REFERENCES


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