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# Optimization of a Vaginal Suppository Formulation to Deliver SHetA2 as a Novel Treatment for Cervical Dysplasia

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ABSTRACT

Cervical dysplasia induced by the human papilloma virus unpredictably progresses to cervical cancer. Therapeutic options are invasive and affect the patient's quality of life. SHetA2 has demonstrated therapeutic efficacy against human and murine human papilloma virus–induced tumors, but its oral bioavailability is <1%. An optimized vaginal suppository formulation can deliver SHetA2 in sufficient doses to prevent cervical dysplasia. The quality by design approach was employed to optimize the suppository formulation consisting of cocoa butter as base with 5% Kolliphor and 40% SHetA2. The suppository had a content uniformity of 105.44 ± 0.42%, melted in <8 min, and had a complete release of SHetA2 in water. Administration of the suppository to mice-achieved cervix concentrations that were significantly higher than the SHetA2 therapeutic concentration, with the maximum concentration ($C_{max\text{-}cervix}$ = 336.78 μg/g) being more than 100-fold the therapeutic SHetA2 concentration. Furthermore, the levels of cyclin D1 protein decreased 9-fold indicating a correlation of drug concentrations with the pharmacodynamic endpoint. These proof-of-concept studies suggest that the SHetA2 optimized vaginal suppository formulation may have a potential use in the prevention of cervical dysplasia, but detailed efficacy studies are required to confirm this assumption.



## Introduction

Cervical cancer is the second most common form of gynecologic cancer worldwide affecting about 528,000 women and caused approximately 266,000 deaths in 2012.[1] Infection of the cervix with the human papilloma virus (HPV) is widespread in women under 25 years of age.[2] Epidemiological and experimental data suggest a strong association between cervical HPV infection and cervical cancer.[3] High risk HPV infection causes premalignant lesions in the cervical epithelium, which are known as cervical dysplasia. If untreated, cervical dysplasia can progress to cervical cancer through a series of cellular mechanisms. In the United States, there are 330,000 new cases of high grade dysplasia[4] compared with only 12,000 cases of cervical cancer every year.[2,3] Therefore, treatment of cervical dysplasia may be a practical way to prevent cervical cancer and has the potential of benefiting more patients.

Current treatment for high-grade cervical dysplasia involves cryogenic destruction of tissue or surgical removal of tissue.[5] While this treatment is effective, many women are unnecessarily treated because many cases will resolve on their own, but there is no way to know which dysplasia patients will progress to cancer. Moreover, because these therapeutic measures are invasive, they can cause infertility and poor quality of life for the patient.[5] The use of a chemopreventive drug instead of these intrusive measures could reduce cost, discomfort, and potential loss of fertility.

SHetA2 (NSC726189) is a novel, nontoxic, flexible heteroarotinoid compound that has demonstrated therapeutic and preventive efficacy in human and murine HPV-induced tumors by causing G1 cell cycle arrest by suppressing cyclin D1 levels and ultimately inducing apoptosis.[6] In association with cyclin-dependent kinase 4 or 6, cyclin D1 forms a complex that phosphorylates retinoblastoma protein, which in turn can act as tumor suppressor.[7,8] Previous studies demonstrated that SHetA2 induced G1 arrest by suppressing cyclin D1 levels *in vivo*.[9] Given that SHetA2 can counteract the influence of HPV on cell cycle

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progression by suppression of cyclin D1 cellular levels, a reduction in cyclin D1 levels can be considered as pharmacodynamic endpoint in preclinical studies. However, the potential efficacy of SHetA2 is limited by its low oral bioavailability caused by its poor aqueous solubility.[10] Kabirov et al.[11] reported that the oral bioavailability of SHetA2 is null and that this bioavailability increases to approximate 10% when Kolliphor, a self-emulsifying drug delivery system is added to the suspension. Kolliphor was selected to enhance bioavailability because of its lack of toxicity at lower concentrations on epithelial cells[12] and its enhancement of tumor-inhibitory effect observed with other chemotherapeutic agents.[13]

Vaginal suppositories are commonly employed to treat urogenital infections and other local diseases[14-17] due to the ease of drug administration. Direct delivery of SHetA2 at the site of dysplasia would overcome its limited oral bioavailability and circumvent the need for systemic absorption. Previous studies demonstrated that topical application of SHetA2 did not irritate mice skin,[18] therefore, we hypothesized that a vaginal suppository would be able to deliver an effective dose of SHetA2 that in turn, may be capable to treat and prevent cervical dysplasia.

The present study developed and optimized a suppository formulation to deliver SHetA2 by the vaginal route to achieve therapeutic drug concentrations at the cervix. Preliminary *in vivo* studies were performed as proof-of-concept to evaluate the drug tissue concentrations and cyclin D1 protein levels that administration of the optimized SHetA2 suppository would be able to achieve.

## Materials and Methods

### *Materials*

SHetA2 was synthesized by Cayman Chemical company, Inc. under a contract from the Rapid Access to Preventive Intervention Development National Cancer Institute program. Cocoa butter was purchased from Nature's Oils (Streetsboro, OH). Polyethylene glycols (PEGs) (molecular weight 400, 3350), acetonitrile (high performance liquid chromatography [HPLC] grade $\geq$99.5%), sodium chloride, lactic acid, acetic acid, potassium hydroxide (KOH), bovine serum albumin, glucose were purchased from Sigma-Aldrich (St. Louis, MO). Glycerol United States Pharmacopeia (USP) and mineral oil USP were purchased from VWR International (Radnor, PA). Kolliphor was obtained from BASF (Ludwigshafen, Germany). Captiva® filtration plates were purchased from Agilent Technologies Inc. Anti-cyclin D1 antibody was purchased from Cell Signaling Technology (Boston, MA), and anti-β-actin peroxidase/FITC conjugated secondary antibodies were purchased from Santa Cruz (Santa Cruz, CA) for Western blot experiments. Mammalian Protein Extraction Reagent (m-PER), Pierce BCA protein assay kits were purchased from Thermo Fisher Scientific (Grand Island, NY). Protease inhibitor cocktail and phosphatase inhibitor cocktail were purchased from Roche (New York, NY). Polyvinylidene difluoride membrane and enhanced chemiluminescence reagent were purchased from Bio-Rad (Hercules, CA).

### *HPLC Determination of SHetA2 in Solutions*

Determination of SHetA2 in solutions was performed using a Waters Alliance HPLC System with a $V_{ydac}$ 201 TP $C_{18}$ 5 μ (250 mm × 2.1 mm) column equipped with a guard column ($V_{ydac}$ 201 TP, Grace), and a UV detector set at 341 nm. The mobile phase consisted of acetonitrile:water (80:20, v/v). The flow rate was 0.3 mL/min with a retention time of 3.65 min.

### *Determination of the Partition Coefficient (Log* p*) and pKa of SHetA2*

Given that SHetA2 is a new drug, the Log *p* was estimated using the interactive Log *p* calculator on Molinspiration® website (http://www.molinspiration.com/). Log *p* was also determined experimentally by the shake flask method[19] using 3 different octanol:-water volume ratios: 1:2, 1:1 and 1:4.

The p*Ka* of SHetA2 was determined using the potentiometric titration method described by Benet and Goyan[20] using KOH 0.1 N. Ethanol was used as co-solvent, as SHetA2 is poorly soluble in water. The sp*Ka* values (defined as the p*Ka* obtained from semi aqueous titration) were determined from each titration curve at half equivalence point. The average sp*Ka* + Log (water%) was plotted against 1/D (D = dielectric constant of the semi aqueous solvent).[21]

### *Human Size Suppositories*

#### *Formulation and Optimization of SHetA2 Suppository Formulation*

Quality by design (QbD) methodology was employed to optimize the formulation of SHetA2 into vaginal suppositories by first considering the material attributes (Fig. 1). The desired characteristics for the SHetA2 vaginal suppository formulation were: (1) ease of insertion, (2) fast drug release of from the base, and (3) meet quality control specifications outlined by the USP.[22] Thus, the critical quality attributes (CQAs) in the optimum formulation were defined as to: (1) be solid at room temperature and maintain physical integrity during administration, (2) disintegrate/melt within 5-10 min, (3) have content uniformity (85%-115%), and weight variation within 7.8%.

Suppositories were manufactured using the fusion molding method[23] and USP stainless steel suppository molds. The formulation ingredients consisted of a hydrophilic base (PEG mixture) or a lipophilic base (cocoa butter), drug, and Kolliphor. These ingredients were entered at different proportions into the Design of Experiments (DoE) software (Design-Expert, version 8.0.1, Stat-ease®) to evaluate statistically the effects of the formulation components on the characteristics of the product.[24] The final formulation composition was determined using 2 sequential DoEs as follows.

The first DoE (DoE1, 16 formulations) was an "optimum mixture" design to identify the proportions of PEGs and Kolliphor that would yield a fully formed suppository that would remain solid and stable at room temperature. Combinations of PEG 400 (10%-40%), PEG 3350 (40%-60%), and Kolliphor (5%-30%) were entered as factors and the responses evaluated were: (A) is the suppository fully formed? and (B) does the suppository remain solid at room temperature?

In the second DoE (DoE2), the type of base, the proportion of drug, and the proportion of Kolliphor were optimized. DoE2 was a $2^3$ factorial design, with each of the 3 factors having 2 levels: (1) Percentage of drug (20% and 40% w/v), (2) types of base, (cocoa butter as the lipophilic base and the best combination of PEG 400 and PEG 3350 that was identified in DoE1 as the hydrophilic base), (3) percentage of Kolliphor (5% and 30%). To save SHetA2, suppositories in DoE2 were prepared using rifampicin as model drug because, like SHetA2, it has low solubility in water and a similar Log $p = 3.71$.[25,26] The responses evaluated in DoE2 were: is the suppository fully formed? (B) Does the suppository remain solid at room temperature? and (C) What is the disintegration time/softening time for PEG suppositories and cocoa butter suppositories, respectively. The dissolution profile was also determined for the 3 formulations that had the shortest disintegration/softening time.