Thiomers-Mediated Drug Delivery Systems for Vaginal Infections

A Thesis Submitted for the Partial Fulfillment of the Requirements for Master Degree of Pharmaceutical Sciences (pharmaceutics)

By

Doaa Hamdy Mohamed Shakshak
Bachelor of Pharmaceutical Sciences, May 2004, Ain Shams University
Demonstrator, Department of pharmaceutics
Faculty of Pharmacy, Ain Shams University

Under the Supervision of

Prof. Dr.
Abdel-Hamid El-Shamy

Professor of Pharmaceutics
and Industrial Pharmacy
Faculty of Pharmacy,
Ain Shams University

Prof. Dr.
Nahed Daoud Mortada

Professor of Pharmaceutics
and Dean of
Faculty of Pharmacy,
Ain Shams University

Dr.
Noha Mohamed zaki Rayad
Lecturer of Pharmaceutics
Faculty of Pharmacy,
Ain Shams University

Ain Shams University
Faculty of Pharmacy
Department of pharmaceutics
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نظم توصيل دوائية من البوليمرات المكبرتة ضد العدوي فى المهبل

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دعاء حمدي محمد شكشك

بكالوريوس العلوم الصيدلية 2004 ,جامعة عين شمس
معيدة بقسم الصيدلانيات ، كلية الصيدلة ، جامعة عين شمس

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(صيدلانيات)

تحت اشراف كل من]

ا.د عبد الحميدعبد الله الشامى
أستاذ الصيدلانيات و الصيدلة الصناعية
كلية الصيدلة، جامعة عين شمس

ا.د. ناهد داود مرتضي
أستاذ الصيدلانيات وعميد
كلية الصيدلة، جامعة عين شمس

د. نهى محمد زكى رياض
مدرس الصيدلانيات
كلية الصيدلة، جامعة عين شمس

جامعة عين شمس
كلية الصيدلة
قسم الصيدلانيات
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Dedication

To my dear husband, Ayman, who without his help and unlimited support, this thesis wouldn't have appeared; thank you.

Abstract

Thiomers -Mediated Drug Delivery Systems For Vaginal Infections

Doaa Hamdy Shakshak

Department of Pharmaceutics, Faculty of Pharmacy, Ain Shams University

Thiomers are highly potent mucoadhesives that extend the residence time of medicament on a mucosal site and allow therapeutic levels to be maintained locally, hence reduce the dosing frequency and quantity of drug administered. The aim of this study was to explore the potential of thiomers, the new generation of mucoadhesives, as a vaginal delivery system for the antifungal drug, fluconazole and to evaluate that clinically in female patients suffering from vulvovaginal candidiasis (VVC).

In chapter 1, different types of thiomers was synthesized using almost the whole panel of over–the-shelf (or currently existing) mucoadhesive polymers. Anionic polymers were used namely; carboxymethyl cellulose (CMC), polycarbophil (PC), carbopol (CBP), sodium alginate (ALG), gelatin (GEL) or hyaluronic acid (HA) in addition to the cationic polymer chitosan (CS). The synthesis was performed through carbodiimide chemistry using EDAC at different concentrations and pHs where the polymers were coupled to thiol-bearing amino acid namely cysteine or thioglycolic acid. A factorial design approach was applied to verify the optimum conditions for the synthesis and the prepared thiolated polymers were characterized by FTIR, H_1NMR, thiol content and mucoadhesive force. Progressively, the safety of the polymer conjugates was investigated by testing their biocompatibility with red blood cells.

Based on factorial analysis of the main effects and interaction plots, it was found that pH 5 and EDAC concentration of 125 mM were optimum for all thiomers except for thio-CMC where EDAC concentration of 50mM was optimal. This was revealed by a high thiol content and superior mucoadhesion. Moreover, Chitosan medium molecular weight coupled to thioglycolic acid showed a greater thiol content and bioadhesion than the thiolated chitosan prepared using cysteine as an amino acid. The in –vitro hemolytic test revealed that the safety of thio-CMC, thio-Gel and thio- PC whereas thio-CS was the least biocompatible.

In chapter two, more focus was given to prepare sustained release fluconazole vaginal tablets using the synthesized thiolated polymers while preserving the high mucoadhesion. To achieve this aim, the prepared tablets were evaluated regarding their physical properties, mucoadhesive force, swelling index, *in vitro* drug release and differential scanning calorimetric pattern (DSC). The results showed that all the thiolated tablets conformed to the pharmacopeal requirements regarding to weight variation, content uniformity, hardness, thickness and diameter. The thiolated tablets exhibited lower swelling index and higher mucoadhesive force than those obtained with the tablets formulated using unmodified polymers. The different thiolated medicated tablets could be arranged according to their mucoadhesive force in the following ascending order: Thio-CS< thio-GEL < thio-HA< thio-CBP< thio-ALG< thio-PC< thio-CMC.

In terms of release, thiolated CMC and thiolated PC showed an optimum release pattern affording drug release higher than 80% over 8 hours. Finally, DSC thermograms revealed no interaction between fluconazole and all the thiolated polymers. Hence, the two thiolated mucoadhesive fluconazole vaginal tablet based on thio-CMC and thio-PC (synthesized

by coupling CMC and PC with cysteine at pH 5 using EDAC at the concentrations of 50 and 125 mM respectively) were selected for further clinical study**.**

In chapter three, a randomized single-blind clinical trial was conducted on 144 female patient at Elgalla Maternity Teaching Hospital to evaluate the efficacy of the selected thiomer-based mucoadhesive fluconazole vaginal tablets in comparison to their unmodified conjugates tablets, Canesten® commercial tablets as well as placebo tablets.

The clinical study was performed by recording the severity of various symptoms of vaginal candidiasis namely leucorrhea, dysuria, dyspareunia, pruritis and burning sensation. These parameters were recorded on a scale basis from score 0 (no symptoms) to 5 (severe symptoms). Moreover microscopical examination of vaginal smear was conducted to detect presence of *candida*. Results revealed that 3 days post-treatment with the different vaginal tablets, revealed that the thiolated PC and thiolated CMC mucoadhesive vaginal tablet showed an early extremely significant reduction in median score ($P < 0.001$) after three days in contrast to unmodified PC , CMC and Canesten mucoadhesive vaginal tablets which showed significant reduction after 7 days. The microscopical examination, matching with the clinical signs and symptoms revealed absence of hyphae and spores after 3 days treatment of both thiolated PC and thiolated CMC mucoadhesive vaginal tablet. The study presented herein demonstrated that thiomers-based vaginal tablets of fluconazole were successful in controlling drug release and increasing vaginal residence time through improved mucoadhesion while in clinical research such tablets resulted in early curing all symptoms of VVC in female patients.

Key words: thiomers, fluconazole, vaginal tablets, candidiasis, mucoadhesion.

List of contents

List of abbreviations

ALG	Alginate
ANOVA	Analysis of variance
BP	British pharmacopeia
CBP	Carbopol
CS	Chitosan
CMC	Carboxy methyl cellulose
CYS	Cysteine
Conc.	Concentration
DSC	Differential scanning calorimetry
EDAC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA	Ethylene diamine tetra acetic acid
FTIR	Fourier transform infrared
FLZ	Fluconazole
GEL	Gelatin
gm	Gram
h.	Hour
HA	Hyaluronic acid
mM	Milli-mole
Mwt	Molecular weight
µM	Micromole
NaCl	Sodium chloride
N	Newton
PB	Phosphate Buffer
PC	Polycarbophil
rpm	Revolution per minute

S.D	Standard deviation
TGA	Thioglycolic acid
USP	United States Pharmacopoeia
UV	Ultraviolet
λ_{max}	Lambda maximum