ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ
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STABILITY AND SKIN PERMEATION OF ALL-TRANS RETINOIC ACID LOADED PEG-PE MICELLES FOR TOPICAL APPLICATION

ANGKANA WICHIT

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree
in Pharmaceutical Sciences (International Program)
May 2011

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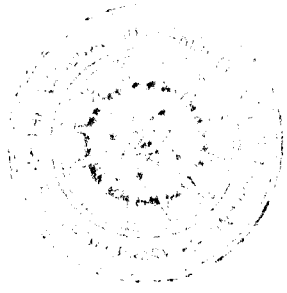

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This thesis entitled "Stability and skin permeation of all-*trans* retinoic acid loaded PEG-PE micelles for topical application" submitted by Angkana Wichit in partial fulfillment of the requirements for the Doctor of Philosophy in Pharmaceutical Sciences (International Program) in hereby approved.

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46901

ABSTRACT

The topical application of all-*trans* retinoic acid (ATRA) is an effective treatment for several skin disorders, including photo-aging. Unfortunately, ATRA is susceptible to light, heat, and oxidizing agents. However, isomerized forms of ATRA via light exposure have biological activity while oxidized forms of it do not present pharmacological effect on topical application. Thus, this study aimed to investigate the ability of polymeric micelles prepared from polyethylene glycol-conjugated phosphatidylethanolamine (PEG-PE) to stabilize ATRA under various storage conditions. *In vitro* skin permeation studies of ATRA-loaded PEG-PE micelles were performed using Franz diffusion cells. Toxicity of the polymeric carrier i.e. PEG-PE was also observed on cultured primary keratinocyte and fibroblast cells. ATRA-loaded polymeric micelles were prepared using various types of PEG and PE fragments. The critical micelle concentrations (CMCs) of the PEG-PE micelles were 97-243 μM, depending on the structures of the PEG and PE molecules. All of the micelles had particle diameters of 6-20 nm and neutral charges. The highest entrapment efficiency (82.7%) of the tested micelles was exhibited by ATRA in PEG with a molecular weight of 750 Da conjugated to dipalmitoyl phosphatidylethanolamine (PEG_{750}-DPPE) micelles and was selected to further studied. Result of stability

showed that the PEG_{750}-DPPE micelles could significantly retard ATRA oxidation compared to ATRA in 75% methanol/HBS solution. Up to 87% of ATRA also remained in the PEG_{750}-DPPE micelles after storage in ambient air with light protection for 28 days. For *in vitro* permeation study, ATRA-loaded PEG_{750}-DPPE micelles were effectively permeated through human skin under non-occlusive condition compared to ATRA in 50% ethanol/HBS solution. In addition, at low concentration of ATRA in the PEG_{750}-DPPE solution was not toxic on the cultured keratinocytes and fibroblasts. Moreover, ATRA in the PEG_{750}-DPPE solution could effective to improve cell survival both keratinocytes and fibroblasts in comparing with the empty PEG_{750}-DPPE solution. From this study it could be concluded that the PEG_{750}-DPPE micelles were able to improve chemical stability of ATRA and enhance permeation of ATRA through the human skin as well as reduce undesirable effects on the applied skin. Therefore, ATRA-loaded PEG_{750}-DPPE micelle is an interesting carrier. Efficacy and safety of ATRA-loaded PEG_{750}-DPPE micelles might be studied and further to development a new cosmeceutical formulation.

ABBREVIATIONS

ATRA	=	All-*trans* retinoic acid
Ca^{2+}	=	Calcium ion
CRABP-I	=	Cellular retinoic acid binding protein type I
CRABP-II	=	Cellular retinoic acid binding protein type II
13-*cis* RA	=	13-*cis* retinoic acid
9-*cis* RA	=	9-*cis* retinoic acid
CMC	=	Critical micelle concentration
cm^2	=	Square centimeter
DMSO	=	Dimethyl sulfoxide
DMEM	=	Dulbecco's minimum essential medium
DPPE	=	Dipalmitoyl phosphatidylethanolamine
ELISA	=	Enzyme linked immunosorbent assay
EtOH/HBS	=	Ethanol and HEPES buffer saline
FBS	=	Fetal bovine serum
G	=	Gram
HBS	=	HEPES buffer saline
HPLC	=	High performance liquid chromatography
h	=	Hour
KGM	=	Keratinocyte Growth Medium
kV	=	Kilovolt
LOD	=	Limit of detection
LOQ	=	Limit of quantitation
MTT	=	3(4,5-Dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide
µg	=	Microgram
µl	=	Microliter
µm	=	Micrometer
µM	=	Micromolar

ABBREVIATIONS (CONT.)

mg	=	Milligram
ml	=	Milliliter
mm	=	Millimeter
mM	=	Millimolar
min	=	Minute
M	=	Molar
mV	=	Millivolt
nm	=	Namometer
P-450	=	Cytochrome P-450 enzyme
PBS	=	Phosphate buffer saline
PE	=	Phosphatidylethanolamine
PEG_{750}-DMPE	=	Methoxy polyethylene glycol (PEG_{750}) conjugated dimyristoyl phosphatidylethanolamine
PEG_{750}-DOPE	=	Methoxy polyethylene glycol (PEG_{750}) conjugated dioleoyl phosphatidylethanolamine
PEG_{750}-DPPE	=	Methoxy polyethylene glycol (PEG_{750}) conjugated dipalmitoyl phosphatidylethanolamine
PEG_{5000}-DPPE	=	Methoxy polyethylene glycol(PEG_{5000}) conjugated dipalmitoyl phosphatidylethanolamine
PEG_{750}-DSPE	=	Methoxy polyethylene glycol (PEG_{750}) conjugated distearoyl phosphatidylethanolamine
PEG	=	Polyethylene glycol
PEG-PE	=	Polyethylene glycol conjugated phosphatidylethanolamine
RAREs	=	Retinoic acid response elements
RARs	=	Nuclear retinoic acid receptor proteins
rpm	=	Round per minute

ABBREVIATIONS (CONT.)

RXRs	=	Nuclear retinoids receptor proteins
SC	=	Stratum corneum
SD	=	Standard deviation
SE	=	Standard error
SLS	=	Sodium lauryl sulfate
TEM	=	Transmission electron microscopy
TMB	=	3,3,5,5-Tetramethyl benzidine
v/v	=	Volume by volume
w/v	=	Weight by volume

LIST OF CONTENTS

LIST OF CONTENTS (CONT.)

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (CONT.)

Figure **Page**

LIST OF FIGURES (CONT.)

Figure **Page**

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

Figure **Page**