UNIVERSITI PUTRA MALAYSIA

IN VITRO ANTICANCER PROPERTIES OF LINAMARIN CONTROLLED RELEASE FROM BIODEGRADABLE POLY-LACTIC CO-GLYCOLIC ACID NANOPARTICLE

WEDAD ASHOUR AL FOURJANI.

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By

WEDAD ASHOUR AL FOURJANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATIONS

To my husband and my son Abdo

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Master of Science.

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WEDAD ASHOUR ALFOURJANI

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Chairman: Norhafizah Abdullah, PhD

Faculty : Engineering

There are many interests in finding new chemotherapeutic agents for cancer. The current work involved screening of linamarin as the therapeutic agent on different cancer cells, as no such study has been performed previously. Improved bioavailability and delivery of the linamarin to the targeted tumour cells can be engineered by proper selection of its carrier. There are many advantages of choosing biodegradable nanoparticles as a drug carrier. These include an improved bioavailability and efficacy of the drug. It also offers a controlled release mechanism in which the activity of the drug can be prolonged at the affected sites. Besides, the biodegradability character of the carrier means these particles are easily dissolved in the system without exerting any side effects to the body. The

present study investigated fabrication of linamarin encapsulation into biodegradable nanoparticles to kill cancer cells.

The present study was initiated with an investigation of the toxic effect of linamarin on cancer cells and their cell cycles. The *in vitro* study on the effect of linamarin was performed on two tumour cell lines, HeLa (cervical tumour cell line) and CAOV3 (ovarian tumour cell line). The cytotoxicity of linamarin was determined by the MTT assay. Both cell lines showed significant cell death when exposed to linamarin with the IC50 values well within the efficacious limit (IC50 of 30 mg/ml and 58 mg/ml for HeLa and CAOV3 cell lines, respectively, when exposed to pure linamarin). This result indicated that linamarin has the potential as a for drug candidate for cancer treatment. The subsequent cell cycle analysis performed by flow cytometry to determine the arrested point of linamarin within the cell cycle. Results showed significant effect of linamarin on the G1 phase of the cell cycle. In other words, a significant number of cells were being arrested in the G1 phase. However, no significant effect was observed on the S and G2-M stage of the cell cycle stage after treatment with the linamarin for 24 hours.

The second part of the study was on fabrication of biodegradable linamarin loaded nanoparticles. Poly (lactic-co-glycolic acid) (PLGA) was chosen as the polymeric material of the nanoparticles. The water-in-oil-in-water emulsification process was the method of choice for the encapsulation of linamarin inside polymeric particles. The linamarin nanoparticles based on two different mole fraction of PLGA copolymer (50/50 and 85/50 of lactic acid/glycolic acid, respectively) were successfully fabricated using water-in- oil-in-water double emulsion extraction/evaporation technique. The SEM

analysis on the morphologies of the nanoparticles showed the particles are spherical in shape with porous surface structure and well within nano-scale in size.

A preliminary investigation on *in vitro* drug (linamarin) release was also carried out. The *in vitro* drug (linamarin) release was characterised by an initial burst and incomplete dissolution of the drug. When decreasing the polymer/drug ratio, the release appeared more controlled and prolonged up to 8hr. It can be concluded that nanoparticles prepared by water-in-oil-in-water emulsification followed by solvent evaporation is a good potential for a controlled released-drug carriers for linamarin.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SIFAT-SIFAT ANTIKANSER LINAMARIN SECARA IN-VITRO DAN PELEPASAN TERKAWALNYA DARIPADA NANOZARAH ASID PLGA BOLEH BIOROSOT

Oleh

WEDAD ASHOUR AL FOURJANI

November 2005

Pengerusi: Norhafizah Abdullah, PhD

Fakulti : Kejuruteraan

Terdapat banyak minat dalam penemuan agen kemoterapi yang baru untuk penyakit kanser. Kajian ini melibatkan penyaringan linamarin sebagai agen terapi untuk pelbagai sel-sel kanser memandangkan tiada kajian yang sama dijalankan terdahulu. Peningkatan bioavailabiliti dan penghantaran linamarin ke sel-sel tumor yang ingin ditujui boleh dijuruterakan dengan pemilihan pembawa yang sesuai. Terdapat banyak kebaikan dalam memilih nanopartikel yang boleh dibiodegradasikan sebagai pembawa ubat. Ini termasuk peningkatan bioavailabiliti dan keberkesanan ubat. Ia juga membolehkan pengawalan terhadap mekanisma pelepasan di mana aktiviti ubat tersebut boleh diperpanjangkan di kawasan yang terjangkit. Disamping itu, sifat pembawa yang boleh dibiodegradasikan juga bermakna partikel-partikel tersebut mudah larut dalam sistem tanpa membawa sebarang kesan sampingan kepada badan. Kajian ini bertujuan mengaji fabrikasi linamarin yang dikapsulkan dalam nanopartikel yang boleh dibiodegradasikan untuk tujuan pembunuhan sel-sel kanser.

Kajian ini dimulakan dengan menyiasat kesan ketoksikan linamarin terhadap sel-sel kanser serta kitaran selnya. Pengajian kesan linamarin di luar tubuh badan dilakukan ke atas dua jujukan sel tumor, iaitu HeLa (jujukan sel tumor servik) dan CAOV3 (jujukan sel tumor ovari). Sitotoksiksiti linamarin ditentukan dengan asei MTT. Kedua-dua jenis jujukan sel menunjukkan kematian sel yang nyata apabila didedahkan kepada linamarin pada nilai IC_{50} yang berada di dalam julat keberkesanan. (Nilai IC50 untuk HeLa adalah 30 mg/ml dan 58 mg/ml untuk sel CAOV3 apabila kedua-dua sel ini didedahkan kepada linamarin yang tulen. Keputusan ini menunjukkan bahawa linamarin mempunyai potensi sebagai calon ubat dalam rawatan kanser. Kitaran sel yang kemudiannya dianalisasikan dengan flow sitometri menunjukkan kesan linamarin yang nyata pada fasa G1 kitaran sel. Ini bermakna terdapatnya nombor sel yang nyata yang telah disekat pada fasa G1. Walaubagaimanapun, tiada kesan yang nyata yang diperhatikan pada fasa S dan G2-M kitaran sel selepas dirawatkan dengan linamarin selama 24 jam.

Bahagian kedua kajian ini adalah mengenai fabrikasi nanopartikel dengan muatan linamarin yang boleh dibiodegradasikan. Poli (laktik – ko – asid glikolik) (PLGA) dipilih sebagai bahan polimerik nanopartikel. Air-dalam-minyak-dalam air adalah proses pengemulsian yang dipilih untuk mengkapsulkan linamarin ke dalam partikel polimerik. Nanopartikel linamarin yang berasaskan dua pecahan mol kopolimer PGLA yang berlainan (50/50 dan 85/15 masing-masing untuk pecahan mol asid laktik kepada asid glikolik) telah berjaya dihasilkan dengan teknik pengekstrakan/ penyejatan dua kali ganda pengemulsian air-dalam-minyak-dalam-air. Analisis morfologi nanopartikel

dengan SEM menunjukkan bahawa partikel-partikel yang dihasilkan adalah dalam bentuk sfera dengan struktur permukaan yang berliang dan saiz yang berada dalam skala nano. Kajian pada peringkat awal tentang pelepasan ubat (linamarin) di luar tubuh badan juga dijalankan. Pelepasan ubat (linamarin) di luar tubuh badan bercirikan peletusan pada permulaan dan keterlarutan ubat yang tidak lengkap. Apabila nisbah polimer kepada ubat dikurangkan, pelepasan ubat didapati lebih terkawal dan berlanjutan sehingga 8 jam. Kesimpulannya, nanopartikel yang disediakan dengan pengemulsian air-dalam-minyak-dalam-air dan diikuti dengan penyejatan pelarut merupakan satu potensi yang baik untuk pelepasan pembawa ubat linamarin yang terkawal.

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LIST OF ABBREVIATIONS

ACA	alkyl cyanocrylate
ANOVA	analysis of variance
CN^-	cyanide ion
CO_2	carbon dioxide
DCM	dichloromethane
DMAB	didodecyl dimethyl ammonium bromides
DMSO	dimethylsulphoxide
DNA	dideoxyribonucleic acid
DSC	differential scanning calorimetry
FDA	Food and Drug Administration
GAS	gas anti solvent
HCN	hydrogen cyanide
HPLC	high performance liquid chromatography
IV	intravenous
MPS	mononuclear and phagocytic system
MTT	3-4, 5-dimethylthizol-2-yl)-2-5-diphenyl tetrazolium bromide solution
MW	molecular weight
OD	optical density
PACA	Poly alkyl cyanorylate
PBS	phosphate buffer saline

PDLLA	poly D, L lactic acid
PGA	poly glycolide
PI	propidium iodide
PLA	poly lactide
PLGA	poly lactic glycolic acid
PLLA	poly L-lactic acid
PVA	polyvinyl alcohol
RESS	rapid expansion of supercritical
RPMI media	Roswell Park Memorial Institutes media
RNA	ribonucleic acid
SAS	supercritical anti solvent
SEM	scanning electron microscope
W1/O	water-in-oil
W1/O/W2	water-in-oil- in-water

CHAPTER 1

INTRODUCTION

1.1 Introduction

The drug delivery system is a system which delivers or carries the drug to the infected sites. The system is characterised by its ability to incorporate drugs without damaging them, long *in vivo* stability, its tuneable release kinetics and targeting to specific organs and tissues. This tuneable release kinetics is a characteristic for a controlled drug delivery mechanism. The controlled drug delivery offers many advantages over conventional dosage forms, including improved efficacy, reduced toxicity, improved patient compliance, and cost effective therapeutic treatment. In particular, the controlled release mechanism is strongly required for unconventional drugs, such as proteins and oligopeptides.

In recent years, there has been significant effort to develop nanotechnology for drug delivery since it offers a suitable means for delivering small molecular weight drugs, as well as macromolecules such as protein, peptide or genes. Most of the works focus on formulation of therapeutic agents in biocompatible nano-composites such as nanoparticles, nanocapsules, micellar system, and conjugates. These systems are often polymeric based matrix and submicron in size.

These nanotechnology systems can be used to provide targeted delivery of drugs, to improve the oral bioavailability and to sustain drug effect in cancer tissues. They can

also be used to solubilize drugs for intravascular delivery and to improve the stability of therapeutic agents against enzymatic degradation. Much work in the past found that nanoparticulates drug carrier made of polymer appear to be more stable when in contact with biological fluids than other colloidal drug carriers (Kreuter *et al.*, 1988; Zambaux *et al.*, 1998). They also have been proposed as drug delivery systems for different routes of administration and for different types of active ingredients such as anticancer agents (Feng *et al.*,2003and Fonseca *et al.*, 2002), anti-inflammatory compounds (Chacon *et al.*,1999), oligonucleotides (Lambert *et al.*, 2001; Ulbrich *et al.*, 2004) and peptides (Lemoine and Preat, 1998).

Polymers can be used as a base matrix for nanoparticles. Polymeric nanoparticles generally vary in size from 10 to1000 nm. The fabrication of polymeric nanoparticles is via dissolvement, entrapment, encapsulation or attachment of the drug to a polymer matrix. The polymers used to make the nanoparticles for administration into the human body are significantly limited to a few types of polymers due to their biocompatibility and biodegradation although various polymers can be employed to make nanoparticles.

There has been intensive research in the development of nanoparticles of biodegradable polymers as an effective drug delivery system for medical practice, especially for chemotherapy and gene delivery. Progress in nanoparticles technology, material science of biodegradable polymers and cellular and molecular physiology and pathology have contributed to the advancements in chemotherapy and gene therapy of cancer and other

disease with polymeric nanoparticles been considered as promising carriers for the therapeutic agent.

Nanoparticulate delivery systems, based on poly (lactic-co-glycolic acid) (PLGA) polymers have been studied extensively for many years (Song.C.X, 1997). PLGA (lactic-co-glycolic acid) and its homo- or copolymers are the most widely used biodegradable polymers for fabricating nanoparticles. PLGA polymers have the advantage of being well characterized and have been commercially used as a microparticulate drug delivery systems. They are biocompatible, biodegradable and bio-resorbable.

1.2. Problem Statement

Chemotherapy is a complicated procedure in which many factors are involved in determining its success or failure. It carries a high risk due to drug toxicity and usually the more effective drugs tend to be more toxic. Problems related to drug side effects still exist even for successful chemotherapy, with patients not only have to tolerate the severe side effects but also sacrifice their quality of life. The effectiveness of chemotherapy depends on many factors, including the drug (s) used, the condition of the patient, the dosage and its form and schedule and others.

Most anticancer drugs are highly hydrophobic, and hence are not soluble in water and most pharmaceutical solvents. Adjuvants have to be used for the clinical administration of many anticancer drugs and this may cause serious side effects, some of which are life threatening. Development of effective carriers with little side effects for anticancer