UNIVERSITI PUTRA MALAYSIA

ENHANCEMENT OF *IN VITRO* EFFECTS OF POLYMERIC NANOPARTICLE ENCAPSULATED TAMOXIFEN COMPARED TO TAMOXIFEN IN MCF-7 BREAST CANCER CELL LINE

Tung En En

FPSK(m) 2012 30

ENHANCEMENT OF *IN VITRO* EFFECTS OF POLYMERIC NANOPARTICLE ENCAPSULATED TAMOXIFEN COMPARED TO TAMOXIFEN IN MCF-7 BREAST CANCER CELL LINE

TUNG EN EN

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2012

ENHANCEMENT OF *IN VITRO* EFFECTS OF POLYMERIC NANOPARTICLE ENCAPSULATED TAMOXIFEN COMPARED TO TAMOXIFEN IN MCF-7 BREAST CANCER CELL LINE

By

Tung En En

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

May 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Masters of Science

ENHANCEMENT OF *IN VITRO* EFFECTS OF POLYMERIC NANOPARTICLE ENCAPSULATED TAMOXIFEN COMPARED TO TAMOXIFEN IN MCF-7 BREAST CANCER CELL LINE

By

TUNG EN EN

May 2012

Chair: Abdah Md Akim, PhD

Faculty: Medicine and Health Sciences

Tamoxifen (TMX) is one of the common hormone therapies for breast cancer treatments. It acts as an anti-estrogen on breast cancer tissues by inducing apoptosis which is regulated by a variety of cellular signalling pathways such as tumour suppressor protein p53 and caspase-9. However, it is associated with side effects at high doses. This suggests the use of nanoparticles (NP) to deliver a lower dose of TMX with an enhance efficiency. Thus, the objective of this research was to assess and compare the *in vitro* effects of synthesized polymeric NP-encapsulated TMX to TMX toward MCF-7 breast cancer cell line. NP composed mainly of N-isopropylacrylamide (NIPAAm) were synthesized and loaded with TMX. Photon Cross Correlation Spectroscopy Nanophox (PCCS-Nanophox), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR) were used to determine the size, morphology and infrared spectrum of the NP, respectively. The drug release pattern of the NP was investigated through dialysis. To study the cytotoxicity properties, MTT assay was performed on

breast cancer cell line, MCF-7. The apoptotic effects were determined qualitatively through acradine orange and propidium iodide (AO/PI) stains with fluorescent microscopy, and quantitatively through FITC Annexin V and PI staining with flow cytometry. The expression levels of tumour suppressor protein p53 and caspase-9 were determined through ELISA. Finally, the data collected were analyzed by One Way Analysis of Variance (ANOVA), Tukey's test. In this study, the polymeric NP were successfully prepared by gamma irradiation, forming spherical NP at a size of 49.89 ± 0.55 nm in diameter. TEM images showed that the particles were spherical in shape with a distinct core-shell structure. It was demonstrated that the void polymeric NP were non-toxic, and were able to release the drug in a sustained manner with 50.99 ± 1.21 % entrapment efficiency, underscoring the potential of these NP as a carrier for drugs. The proliferation of MCF-7 cells was significantly inhibited by the TMX-NP with a lower 50% inhibitory concentration (IC_{50}) value of 24.63 ± 1.56 µM at 48 hr. It also possessed a greater apoptotic effect, resulting in a percentage of 68.53 ± 3.81% at 32.0 µM. Furthermore, higher levels of p53 (23.22 ± 2.79 U/ml) and caspase-9 (85.35 ± 11.11ng/ml) were detected in a dose-dependent manner. In conclusion, the therapeutic effects of the synthesized TMX NP were enhanced when compared to TMX. Its potential is not limited to anti-cancer drugs, but may also be applied in other drugs and diseases.

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

PENINGKATAN KESAN-KESAN *IN VITRO* NANOPARTIKEL POLIMER TAMOXIFEN DIBANDINGKAN DENGAN TAMOXIFEN DALAM SEL KANSER PAYUDARA MCF-7

Oleh

TUNG EN EN

Mei 2012

Pengerusi: Abdah Md Akim, PhD

Fakulti: Perubatan dan Sains Kesihatan

Tamoxifen (TMX) merupakan salah satu terapi hormon yang biasa digunakan dalam rawatan kanser payudara. Ia bertindak sebagai anti-estrogen pada tisu kanser payudara melalui apoptosis, di mana apoptosis dikawal oleh pelbagai laluan selular isyarat seperti protein p53 penekan tumor dan caspase-9. Walau bagaimanapun, ia boleh mengakibatkan kesan-kesan sampingan pada dos yang tinggi. Ini mencadangkan penggunaan nanopartikel (NP) menyampaikan TMX pada dos yang lebih rendah dengan meninggikan kecekapannya. Justeru, objektif kajian ini adalah untuk membandingkan kesan *in vitro* NP polimer yang mampu memuatkan TMX dengan TMX asal ke atas sel kanser payudara, MCF-7. NP yang terdiri daripada N-isopropylacrylamide (NIPAAm) telah disintesiskan dan dimuatkan dengan TMX. *Photon Cross Correlation Spectroscopy Nanophox* (PCCS-Nanophox), *Transmission Electron Microscopy* (TEM), dan *Fourier Transform Infrared Spectroscopy* (FTIR) digunakan untuk menentukan saiz, morfologi dan spektrum inframerah NP tersebut, masing-masing. Dialisis dilakukan

untuk menentukan ciri-ciri pelepasan TMX dari NP. MTT dijalankan ke atas sel MCF-7 untuk mengkaji ciri-ciri sitotoksiti. Kesan kualitatif apoptosis ditentukan dengan pewarnaan acradin oren dan popidium iodida melalui mikroskop floresen, manakala kesan kuantitatif ditentukan dengan FITC Annexin V dan propidium iodida melalui flositometer. Tahap ekspresi p53 dan caspase-9 ditentukan melalui ELISA. Akhir sekali, data yang diperoleh dianalisis dengan menggunakan Analisis Varian Satu Hala (ANOVA), uji Tukey. Dalam kajian ini, NP polimer telah berjaya disediakan melalui sinaran gama, dengan bentuk sfera yang bergaris pusat 49.89 ± 0.55m. Imej TEM menunjukkan bahawa partikel-partikel tersebut berstruktur sfera dan mempunyai lapisan luar dan dalam. NP tersebut adalah tidak bertoksik dan TMX yang dimuatkan dilepaskan secara perlahan-lahan dengan kecekapan pengkapsulan 50.99 ± 1.21 %. Ciri-ciri ini menjadikan NP tersebut sebagai penhantar ubat yang efektif. Proliferasi sel MCF-7 dihalang oleh TMX-NP dengan nilai perencatan 50% (IC_{50}) yang lebih rendah, iaitu 24.63 ± 1.56 µM pada 48 hr. Ia juga mempunyai kesan apoptosis yang lebih besar dengan 68.53 ± 3.81% pada 32.0 µM. Selanjutnya, tahap p53 (23.22 ± 2.79 U/ml) and caspase-9 (85.35 ± 11.11ng/ml) juga adalah lebih tinggi dalam sel-sel yang dirawat dengan TMX-NP. Kesimpulannya, kesan terapeutik TMX-NP yang disintesiskan adalah lebih tinggi apabila dibandingkan dengan TMX. Potensi NP ini tidak terhad kepada ubat antikanser sahaja, tetapi boleh diaplikasikan juga dalam ubat-ubatan dan penyakit-penyakit lain.

ACKNOWLEDGEMENTS

First and most importantly, I would like to thank God for being the best company throughout my Masters Degree. Throughout the process of research and writing, He has guided me through experiences of tough times and good times. All glory to God in my study completion. Secondly, my deepest gratitude goes to my research supervisor, Dr. Abdah MD Akim, who is the main inspiration of this study. Thank you for your supervision, patience and constant support which helped me to overcome hardships throughout the whole process. I would like to thank my co-supervisors Dr. Khairul Zaman Hj. Mohd Dahlan and Assoc. Prof. Dr. Chong Pei Pei who have also played a very important role in giving advice and guidance for this study. Furthermore, I would like to specially thank Mr. Mohd Yusof Hamzah for giving me unconditional help in PCCS-Nanophox, Ms. Marsitah Abdul Jalil in flow cytometry, and Mr. Kairul in TEM. I am also grateful for Universiti Putra Malaysia and Malaysian Nuclear Agency for providing all the facilities for this study. Finally, I am also thankful to my lab mates, friends and family who have never failed to give various forms of support and encouragement during this period of time. Your love and concern for me made all these possible. Thank you from the very bottom of my heart.

I certify that a Thesis Examination Committee has met on the 25th May 2012 to conduct the final examination of Tung En En on her thesis entitled "**Enhancement of *in vitro* effects of polymeric nanoparticle encapsulated tamoxifen compared to tamoxifen in MCF-7 breast cancer cell line**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

Roslida Abd Hamid, PhD
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Sabrina Sukardi, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Zuraini Ahmad, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Fadzelly Abu Bakar, PhD
Institute for Tropical Biology and Conservation
Universiti Malaysia Sabah
Malaysia
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science**.** The members of the Supervisory Committee were as follows:

Abdah Md Akim, PhD
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Khairul Zaman Hj. Mohd Dahlan, PhD
Malaysian Nuclear Agency
(Member)

Chong Pei Pei, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

TUNG EN EN

Date: 25 May 2012

TABLE OF CONTENTS