Research on the effect of oligomer procyanidins on the biological behavior of glioblastoma cells

PhD Student: Xiao Yu (xyu4@mail.usf.edu)

Department of Physics Advisor: Dr. Myung K. Kim (mkkim@usf.edu)

Name of Company: Pharmacology lab, Shenyang Pharmaceutical University, China

Address: 103 Wenhua Road, Shenhe, Shenyang, Liaoning, China, 110016


Industrial Site Advisor: Dr Jingyu Yang (yangjingyu2007@gmail.com)

Duration: 2012-7-26~2012-8-16

Brief Synopsis:

1. Study and reproduce the effect of oligomer procyanidins (F2) (degree of polymerization 2–15), a natural fraction isolated from grape seeds on the biological behavior of glioblastoma cells.

2. This helps the further study of F2 potently inhibited the chemotaxis and invasion of U-87 cell induced by formyl-methionyl-leucyl-phenylalanine (fMLF), an agonist of FPR.

3. Learned about the basic cells culture procedures and imaging system operation.

4. Developed more on biological, pharmacological theories and practical experiment skills.

5. Learned about the value of teamwork.
Industrial Practicum Report

I performed my industrial practicum this summer under the guidance of Prof. Yang in lab of pharmacology, Shenyang Pharmaceutical University, China. Shenyang Pharmaceutical University is a multidisciplinary, multilevel and multiform pharmaceutical institute of higher learning. It is also an important component unit of The Engineering Technique Center of the National Patent Medicines and the Shenyang National New Drug Safety Evaluation Center. The field of research covers the theory and practice of new dosage forms of pharmaceutical preparations-polyphase liposomes and solid preparations, chemical components and active components of traditional Chinese medicines and natural drugs, etc. The University has gained hundreds of international, national, provincial and ministry prizes of development of science and technology. It has also gained more than 50 certificates of new drugs. Some research results have brought outstanding economical and social benefit. Professor Jingyu Yang has been engaged in pharmacy education and scientific research for many years, and has acquired a great achievement in such aspects as neural pharmacology, traditional Chinese medicine pharmacology, etc. She has over 100 scientific papers at domestic and abroad academic publications and participated in compiling and publishing several books. She has certain influence in domestic pharmacy field.

Shenyang Pharmaceutical University, China

I have been working on digital holography microscopy on biological cells in my current PhD study. Since I have the experience of cells culture and optical imaging, Dr Yang assigned me to
join in the research with a senior student to reproduce the effect of oligomer procyanidins (F2) (degree of polymerization 2–15), a natural fraction isolated from grape seeds on the biological behavior of glioblastoma cells [1]. This helps the further study of F2 potently inhibited the chemotaxis and invasion of U87 cell induced by formyl-methionyl-leucyl-phenylalanine (fMLF), an agonist of FPR. I give a description of the work I have participated as followed.

1. Introduction of topic background
Gliomas are the most common and lethal tumor type in the brain. Malignant gliomas, due to their aggressiveness and invasiveness, has been increasingly recognized that single-target therapy for cancers is insufficient and agents that may control the invasive behavior of tumor cells combined with conventional therapy should be of greater benefit [2].

Grapes, one of the most widely consumed fruits in the world, have multiple health benefits; especially they constitute a reservoir of potential anticancer agents. Grapes seed are rich in proanthocyanidins and grape seed proanthocyanidins extract (GSPE) contains monomeric, dimeric, trimeric, tetrameric and other oligomeric proanthocyanidin bioflavonoids. Oligomer proanthocyanidins are generally recognized as the most biologically active fraction in the grape seed extract [3]. Dr Yang’s group have successfully isolated the oligomer proanthocyanidins (F2) from grape seeds and found that F2 enhanced the •OH scavenging ability of rat brain and protected mouse brain against ethanol-induced oxidative DNA damage [4].

2. Cells culture
This is one of the major parts I have participated in. The facilities of cells culture in their group are almost the same as what I am using in USF. Work environment is shown below. Human astrocytoma cell line U-87 and U-251, human hepatoma cells Hep3B (American Type Culture Collection, ATCC) were grown in DMEM with 10% fetal bovine serum (FBS), 100μg/ml streptomycin and 100 IU/ml penicillin in a humidified atmosphere of 95% air and 5% CO2. Human promyelocytic leukemia cells HL-60 and human A549 cells (ATCC) were cultured in RPMI 1640 with 10% FBS, 100g/ml streptomycin and 100 IU/ml penicillin in a humidified atmosphere.
of 95% air and 5% CO₂. All other reagents were purchased from Sigma (St. Louis, MO, USA) [10].

Grape seeds were finely ground using an ultra-centrifugal mill ZM 100 (Retsch GmbH & Co. KG, Haan, Germany). The grape seed powder was immediately extracted using first methanol–water (80:20) followed by acetonewater (75:25) to obtain a crude phenolic extract. After removing organic solvents, the crude phenolic extract was chromatographed by column on Lichroprep RP-18 (200mm×25mm i.d.; 25–40μm particle size) to isolate oligomeric procyanidin fractions, named as F2. F2 were evaporated at less than 30 °C to dryness and dissolved in water prior to lyophilization. For studies of its effects on U-87 cells, F2 was dissolved in pure water and frozen at ~20 °C.

3. Imaging and experimental results

As shown in figures below, U-87 cells treated with F2 at the concentrations of 0, 30 and 100μg/ml for 48 h displayed marked morphological changes. The adherent cells became round shaped, with extensive cytoplasmic vacuolation.
Fig. 1 Conventional microscopy of cultured U-87 glioblastoma cells. Cells were treated with F2 at (a) 0μg/ml, (b) 30μg/ml, and (c) 100μg/ml for 48 h.

For the further study of F2 potently inhibited the chemotaxis and invasion of U-87 cell induced by formyl-methionyl-leucyl-phenylalanine (fMLF), an agonist of FPR, the effect of F2 on the cell fluorescence microscopy observation changes in U-87 cells were recorded in Fig. 2. Cells were treated with different concentrations of F2 for 48 h. The images were recorded by ImageXpress Micro system, a high resolution cell imaging microscope, Fig. 3. However, these images are not ideal to reach a convincing conclusion. More work on the F2 treated U-87 cells needs to be done to determine through which receptor F2 inhibited the invasion of U-87 cells.

Fig. 2 Fluorescence microscopy of cultured U-87 glioblastoma cells. Cells were treated with F2 at (a) 0μg/ml, (b) 30μg/ml, (c) 60μg/ml, and (d) 100μg/ml for 48 h.
4. Conclusion

During this period of industrial practicum, I utilized my skills of cells culture and optical imaging, and more importantly I developed more on biological, chemical, pharmacological theories and practical experimental skills. Collaborated with the senior student, we successfully reproduced the basic process of the experiment on the effect of oligomer procyanidins (F2) on the biological behavior of glioblastoma cells. And this helps and improves the further study of F2 potently inhibited the chemotaxis and invasion of U87 cell induced by FPR. Dr Yang was satisfied with my work and we also discussed about combining their pharmacological research with our digital holography microscopy field. Our novel and robust imaging technique may reveal more information of the behavior of natural compound treated biological cells. Hopefully our two labs may work together and produce more scientific findings in the future. Dr Yang also provided me with some information and opportunities of the academic job market in China and this gives me more ideas about my future job-seeking which is the main purpose of this industrial practicum.

Reference

