Research Grant – Successful Submission

Rachel Pritchard – PhD Research Student – Awarded £2000

Development of a aerosolisable nanocomposite microcarriers for the pulmonary delivery of novel chemotherapeutic combinations to reverse drug resistance in lung cancer cells.

1. Aim of the Project

To encapsulate natural flavonoids with chemotherapeutic agents in respirable nanocomposite microparticles for deposition within the lungs. To evaluate particle deposition, toxicity and ability to overcome drug resistance in lung cancer cells.

Current cancer treatments can fail if cells stop responding to a drug. We will combine plant derived substances with cancer drugs and package them into particles for inhalation into the lung to overcome drug resistance and better treat lung cancer.

In the treatment of lung cancer, chemotherapeutic drug resistance is currently a major problem. Research into the effect of natural products on cancer cells has shown that some flavonoids have anticancer properties. Moreover, we have demonstrated that drug resistance to the anticancer drug epirubicin can be overcome using specific grapefruit derived flavonoids in combination with epirubicin resulting in the drug resistant cancer cells becoming susceptible to epirubicin again. However, flavonoids have poor oral bioavailability and undergo extensive first pass metabolism so cannot be delivered orally. We have previously shown that biodegradable polymeric nanoparticles contained within a dry powder microcarrier can be used for the pulmonary delivery of macromolecules. Further optimisation of this technology would enable the formulation and delivery of drug combinations directly to drug resistant cancer cells in the lung via dry powder inhalation.

2. Programme of Work

To meet the aims, this research project is divided into three studies, each related to the optimal outcomes of the study before.

Study 1: To evaluate the potential of a variety of natural flavonoids to overcome lung cancer drug resistance.

Workplan:

- Determine the effect of typical cancer drugs (doxorubicin, vinblastine and cisplatin) and various natural flavonoids (apigenin, luteolin, genistein,narigenin) on drug susceptible human lung carcinoma cells. The A549 cell line will be cultured and the IC50 of each drug and flavonoid independently measured using relevant cell biology assays (MTT assay).

- Development of drug resistant sub-cultures of A549 cells to test the toxicity, efficacy and uptake of flavonoid/drug combinations and the ability to reversing drug resistance. A549 cells will be exposed to increasing amounts of drug until drug resistance is observed. These drug resistant cells will then be exposed to solutions of flavonoid and drug/flavonoid combinations to determine if reversal of drug resistance occurs.

- Investigate the mechanism of flavonoid reversal of drug resistance thought to be via inhibition of P-gp efflux pumps that are over expressed in drug resistance via western blot and nuclear fluorescence spectroscopy. Also evaluate if any flavonoids have any cytotoxic effects of cancer cells using MTT assay.

Study 2: To formulation and evaluate polymeric nanoparticles containing optimal flavonoid and drug combinations for reversal of drug resistance.

- Nanoparticles of varying charge, size and drug loading will be prepared by altering formulation parameters and the type and concentrations of the polymers and surfactants used. Particles will be prepared using emulsion solvent evaporation methods and analysed using a Malvern zetasizer (size and charge) and hplc (drug loading and release)

- Optimal nanoparticles containing flavonoid and drug will be selected in terms of size, charge, toxicity and the efficacy in treating drug susceptible and drug resistant A549 cells.

Study 3: To evaluate flavonoid:drug containing nanoparticles formulated as nanocomposite microparticles (NCMP) for inhalation.

- Design of experiment (Taguchi method) will be applied to optimise the formulation parameters for microcarriers containing unloaded nanoparticles. Spray drying conditions and the ratio and combination of excipients (sugars, amino acids, chitosan) will be optimised to form aerosolisable NCMP.

- NCMP will be evaluated for morphology (SEM), aersolisability (tap density), lung deposition (next generation impactor) and nanoparticle stability (size, charge and drug loading as per study 2).

- Potent flavonoid/drug loaded nanoparticle formulations will be formulated into optimal NCMP and evaluated in vitro with drug susceptible and drug resistant A549 cells using the methods described in study 1.

3. Potential Applications

Current treatments for lung cancer don't always provide a cure, often due to the development of drug resistance. There are also numerous side effects to current treatment. However, the potential of using chemotherapeutic drugs alongside novel compounds that overcome drug resistance could be optimised for cancer treatment. This would deliver treatment directly to the site of the cancer providing a higher bioavalability with reduced side effect profile. If successful, then this novel approach could ultimately be applied into the clinical setting to treat lung cancer patients.

There has been little progress in lung cancer treatment since the 1970's, patient outcomes are poor and drug resistance adds to this problem. New delivery routes to replace paternal treatment are also gaining attention. Hence, the outcome of this research will be relevant to both the Pharma industry and clinicians working with lung cancer patients with whom we will establish collaborations.

The duration of this project will be 3 years.