Abstracts for the 228th Otago Medical School Research Society Summer Student Speaker Meeting sponsored by the Otago Medical Research Foundation

Wednesday 13 May, 2015

Fruit consumption is not positively associated with serum urate or risk of prevalent gout

R Wrigley1, T Flynn1, L Stamp2, N Dalbeth3, TR Merriman1

1Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin. 2Department of Medicine, University of Otago, Christchurch. 3Department of Medicine, Faculty of Medical and Health Sciences, University of Auckland.

Gout, the second most common form of arthritis in New Zealand, is caused by reaction of the innate immune system to monosodium urate crystals that form in synovial fluid when serum urate (SU) levels are elevated. There are many dietary risk factors for high SU and gout; dietary changes are an important part of managing gout. This project aimed to determine if fruit consumption affects SU and gout risk, as previous evidence is limited and conflicting.

Linear regression was used to investigate association of fruit with SU, and logistic regression to investigate association of fruit consumption with prevalent gout. Data from the Atherosclerosis Risk in Communities study, Cardiovascular Health Study, and stage three of the Framingham Heart Study Generation 3, which collected information on individuals of European ancestry were used for the SU analysis. Data collected from NZ subjects of East Polynesian (NZ Māori and Cook Island Māori), West Polynesian, mixed East and West Polynesian, or European ancestry were used for the gout analysis.

There was significant inverse association of fruit consumption with SU with standard adjustment (BMI, whole-genome principal component analysis vectors 1 and 2, calorie intake, and menopausal status) (mean, 95% CI, ß= –0.328, -0.461 to -0.194 µmol.L⁻¹/weekly serving of fruit; P=1x10⁻⁴, n=12,674), but not when also adjusted for other dietary factors (ß=–0.121, -0.282 to 0.040 µmol.L⁻¹/weekly serving of fruit; P=0.14, n=12,572). Similarly, inverse associations of apples, bananas, citrus fruit, or peaches with SU were not significant when adjusted for other dietary factors. There was no evidence for association of fruit consumption with gout in Polynesians (n=1,549) or Europeans (n=834).

These results do not support reduction of fruit intake in management of gout. These results do not rule out the possibility that fruit consumption is a marker for another urate-lowering behaviour.

Supported by a Department of Biochemistry Summer Research Studentship

The use of styrene maleic acid nanomicelles encapsulating the cannabinoid synthetic analogue WIN55, 212-2 for the treatment of breast and prostate cancer

S Xian, N Parayath, H Nehoff, N Giles, K Greish

Department of Pharmacology and Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin.

Synthetic cannabinoid WIN55,212-2 (WIN) has shown promise as an anti-cancer agent with minimal toxicity. However, cannabinoids cross the blood brain barrier (BBB) to cause psychoactive side-effects. Their poor solubility in blood also makes clinical application difficult.

In the present study, styrene-maleic acid (SMA)-WIN nanomicelles were synthesised to test whether the encapsulation of WIN would solubilise the drug, increase drug efficacy and reduce side-effects. SMA-WIN55,212-2 micelles were characterised and their in vitro cytotoxicity compared to free WIN in triple-negative breast (MDA-MB-231), hormone-receptor positive breast (MCF-7) and castrate-resistant prostate cancer (PC3) cell lines, each treated with concentrations ranging from 0-10µM for 72 hours.

WIN was encapsulated in amphipathic copolymer SMA to form a water-soluble spherical nanostructure. The average diameter was 132.7 nm, a size exceeding the threshold to cross the BBB, kidney and endothelial junctions of normal blood vessels, but still able to move through defective tumour vasculature. As lymphatic drainage of tumour tissue is often also impaired, the drug can selectively accumulate in the tumour tissue – exploiting what is known as the enhanced permeability and retention (EPR) effect, allowing increased drug delivery and reduced adverse effects.

Equal potency against all cell lines was found for both micellar and free WIN. The concentration of drug required to produce 50% of maximum
cell death (IC₅₀) was found to be 3.8 ± 0.1, 4.8 ± 0.0 and 6.1 ± 0.5 μM for free WIN, compared to 4.1 ± 0.2 μM, 5.9 ± 0.2 μM and 6.3± 0.4 μM for MDA-MB-231, MCF-7 and PC3 cell lines respectively, treated with equal drug concentrations of SMA-WIN. A 2-tailed T-test comparing variations between data points at each concentration of free versus micellar WIN found P-values >0.05 across all cell lines, rendering them not statistically significant.

These results show that SMA-WIN nanomicelles can be synthesised which possess evident cytotoxicity against breast and prostate cancer cells, and characteristics which may improve in vivo bio-distribution and drug efficacy and decrease adverse effects. SMA-WIN may show promise as a novel anti-cancer treatment.

Supported by an Otago School of Medical Sciences Summer Research Studentship

The repair protein MGMT and scavenger receptor CD163 are independent prognostic factors for metastatic brain tumours

S Kirs1, A Taha2, J Royds3, T Slatter4, N Hung5

1Department of Surgical Sciences. 2Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin.

For those with metastatic brain tumours the survival time is highly variable ranging from 2–30 months. Molecular markers may aid to better predict prognosis leading to personalised treatment. The repair protein 0-6- methylguanine-DNA methyltransferase (MGMT), paired box transcription factor 8 (PAX8), and scavenger receptor CD163 are implicated in the prognosis of primary tumours. This pilot study aimed to find if these molecular markers were present in brain metastases and whether these markers should be studied in a larger cohort to better estimate patient prognosis.

A total of 23 brain metastases procured from surgery between 2008 and 2014 were chosen. Tumour sections from paraffin embedded tissue were stained for each marker using immunohistochemistry. A double-stain was performed for MGMT and CD163 in which MGMT positive cells were detected using DAB and CD163 detected using Warp Red chromogens. A single stain was performed for PAX8 using DAB. Positive cells were identified by light microscopy. Molecular marker results were correlated with patient survival.

MGMT status was a positive prognostic factor for brain metastases. MGMT positive metastases had a mean survival time of 13.4 months (95% CI 9.6- 17.2, n=15) whereas MGMT negative tumours had a mean survival of 8.5 months (95% CI 4.2-12.8, n=8); P=0.03 (logrank test). CD163 status was also a positive prognostic factor with a mean survival of 39.0 months (95% CI 19.2 - 58.8, n=10) compared to 8.5 months (95% CI 4.0 -12.9, n=13), P=0.02 for CD163 positive and negative metastases respectively. Sixty-seven percent of brain tumour metastases were PAX8 positive; however, no association with patient survival was found.

The CD163 and MGMT markers were associated with patient survival and may provide an improved estimation of prognosis for individual patients. Patients with MGMT negative tumours may benefit from temozolomide as they are sensitive to this type of chemotherapy.

Supported by a Summer Research Studentship from Otago Medical Research Foundation and PriceWaterhouseCoopers Foundation

Inability of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry to differentiate between different Staphylococcus aureus strains

S Grainger1, A van der Linden1, J Ussher1

1Department of Pathology, 2Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, Dunedin.

Rapid and accurate discrimination between methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible S. aureus (MSSA) is essential for effective treatment and prevention of transmission. This project investigated the ability of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF MS) to discriminate between MRSA and MSSA and between the seven major MRSA strains found in New Zealand.

The mass spectra for 35 pre-characterised MRSA isolates, representing a collection of the seven major MRSA strains in New Zealand, and 42 clinical specimens were acquired using a mass spectrometer. The spectra were then processed and analysed to create an unsupervised hierarchical dendrogram.

The dendrograms showed that MALDI-ToF MS is not sensitive enough to discriminate S. aureus beyond species level identification. No distinctive clusters could be associated with MRSA, MSSA or the seven MRSA strains. The formic acid extraction preparation method resulted in mass spectra of higher quality than the direct preparation method, however it had minimal effect on the dendrograms with no obvious clustering of MRSA and MSSA.

Rapid detection of methicillin resistance using MALDI-ToF MS would be extremely useful, however this project has shown that MALDI-ToF MS lacks the resolution to discriminate beyond the species level for S. aureus. Therefore we cannot recommend the use of MALDI-ToF MS for identification of MRSA isolates, nor for discriminating the seven major MRSA strains found in New Zealand.

Supported by an OMRF Summer Research Studentship

Circulating miR-34a as biomarker for diabetes mellitus

S Gandhi1, J Fomison-Nurse1, V Cameron2, R Katare3

1Department of Physiology, Otago School of Medical Sciences,
Diabetic heart disease (DHD) is associated with increased cardiomyocyte apoptosis and senescence, although the underlying mechanisms are unknown. MicroRNAs (miRs) have been shown to have pathological effects in the development of heart disease and diabetes. miRs are short, non-coding, single-stranded RNA molecules that regulate gene expression. Among several miRs, miR-34a is predominantly expressed in the diseased heart and importantly, demonstrated to have implications in cardiomyocyte apoptosis and senescence. The aim of this research is to determine the role of miR-34a in the onset of DHD.

Plasma samples were collected from Type II diabetic and age-matched non-diabetic volunteers without any history of heart disease at varying stages of diabetes, <5 years, 5–10 years, and >10 years. Total RNA was extracted from plasma, reverse transcribed to cDNA with miR34a specific probe and amplified by real time PCR analysis to determine the level of miR-34a. As miRs are bound to high-density lipoprotein (HDL) in circulation, we also measured the level of HDL to normalise the level of circulating miR-34a.

RT-PCR analysis showed significant increase in the level of miR-34a in all the diabetic groups (<5 years 1.8 ± 0.4, P<0.05; 5–10 years 2.8 ± 0.5, P<0.01; >10 years 2.6 ± 0.7, P<0.05, unpaired t-test vs non-diabetic). ELISA showed marked decrease in the level of HDL in people with diabetes (P<0.001 vs non-diabetic, n=25). Importantly, normalisation of miR-34a expression to the HDL level eliminated the significance in <5 years (<5 years 1.1 ± 0.2, P>0.05; 5–10 years 1.7 ± 0.3, P>0.05; >10 years 1.8 ± 0.5, P>0.05 vs. non-diabetic).

Results suggested expression of circulating miR-34a may increase before the development of clinical manifestations, indicating that measurement of circulating miR-34a could be a potential diagnostic biomarker for heart disease in people with diabetes. Importantly, the results warrant the need to consider the level of HDL while determining the expression of circulating miRs.
produced by hypothalamic neurons and has recently been shown to induce stress responses. To demonstrate a role for endogenous RFRP-3 in stress and affective disorders, we developed a mouse model that specifically ablates RFRP-3 expressing neurons by crossing a new mouse line in which the \textit{Rfrp} gene also produces Cre recombinase with a line that enables Cre-dependent expression of the diphtheria toxin receptor (DTR). Diphtheria toxin (0.5, 1.0 or 1.5 mg/kg) was injected subcutaneously into 7-week-old \textit{Rfrp}-DTR mice. Pronounced ablation of RFRP-3 neurons occurred at all doses compared to Cre-negative controls 3 weeks post-treatment (1.5 ± 0.33 vs 9.25 ± 1.7 neurons/brain section respectively; \(P<0.05\); unpaired\( t\)-test). Because the highest dose caused adverse health effects, we selected the 0.5 mg/kg dose for the next experiment. In this pilot study (3 Cre-expressing and 3 Cre-negative control mice) we assessed anxiety behaviour (elevated plus maze [EPM] and light/dark box tests), obsessive-compulsive behaviour (marble burying test) and glucocorticoid hormone responsiveness (acute restraint test) 3 weeks post-diphtheria toxin treatment.

In the EPM, RFRP-3 deficient mice spent more time in the aversive open arms compared to controls (8.02 ± 2.47 vs 1.46 ± 0.75 seconds respectively; \(P<0.05\)). Non-significant trends towards improved performance were recorded in the light/dark box (46.81 ± 15.6 vs 10.47 ± 3.48 seconds in lighted area respectively; \(P=0.41\)) and marble burying tests (2.5 ± 0.5 vs 4.5 ± 2.5 marbles buried respectively; \(P=0.19\)).

We have established an effective and safe technique for RFRP-3 neuronal ablation using diphtheria toxin. Preliminary behavioural data indicate that endogenous RFRP-3 promotes anxiety responses. The technique will be used in a larger study to further characterise roles of RFRP-3 in affective disorders.

Supported by a Otago Medical Research Foundation Summer Research Studentship