

Potential Honours Projects for 2010

Project 1: To develop and evaluate a diagnostic test for early-stage gastric cancer (GC) using a metallothionein-null mouse model of *Helicobacter felis*-induced gastric cancer.

GC is the second leading cause of cancer death worldwide claiming more than 6,000 lives per year in Australia alone. The gold-standard method for accurate GC diagnosis involves an invasive and expensive endoscopic examination that identifies primarily intermediate to late-stage disease. Despite advances in surgical treatment and chemotherapy, at this point the prognosis is often poor with an expected five-year survival rate of <30%. Mortality rates could be dramatically reduced if disease was detected earlier. The existing serum biomarkers for GC, carcinoembryonic antigen and carbohydrate antigen 19-9, are neither sufficiently sensitive nor specific for routine screening applications and are primarily used to monitor the progression of disease following cancer treatment. Thus, an effective assay for detecting early, pre-clinical disease is urgently required.

Although the aetiology of GC is acknowledged to be multi-factorial by far the leading causative factor is infection with *Helicobacter pylori*. This has led to the classification of *H. pylori* as a Group 1 definite carcinogen by the World Health Organization. In Australia, approximately 25-35% of the general population is infected. *H. pylori* infection rates amongst Indigenous Australians, however, are disproportionately high ranging from 60% in urban communities and up to 91% in remote areas.

The proposed Honours project will utilise a metallothionein (MT)-null *H. felis*-induced gastritis, gastric ulcer and subsequently gastric cancer mouse model established by **Dr Cuong Tran**. This model is based on MT-null mice on a C57BL/6 background that are deficient in both MT-I and MT-II. MT is involved in metal detoxification and has been shown to protect against inflammatory disease by sequestering reactive oxygen species. MT-null mice are highly susceptible to *H. pylori* colonisation and the subsequent development of gastritis with the disease being more pronounced when infected with the alternative *Helicobacter* strain *H. felis*. The project will investigate serum markers of different stages of gastric inflammation and early-stage gastric cancer using state-of-the-art proteomic techniques established by **Drs Penno and Hoffman** at the Adelaide Proteomics Centre (University of Adelaide).

Key references

1. Gøbel R, Symonds EL, Butler RN, Tran CD. Association between *Helicobacter pylori* infection in mothers and birth weight. *Dig Dis Sci* 2007;52(11):3049-3053.
2. Tran CD, Campbell MAF, Kolev Y, Chamberlain S, Huynh HQ, Butler RN. Short-term zinc supplementation attenuates *Helicobacter felis*-induced gastritis in the mouse. *J Infect* 2005; 50(5):417-24.
3. Tran CD, Huynh H, van den Berg M, van der Pas M, Campbell MA, Philcox JC, Coyle P, Rofe AM, Butler RN. *Helicobacter*-induced gastritis in mice not expressing metallothionein-I and II. *Helicobacter*. 2003;8(5):533-41.
4. Aslani MR, Pascoe I, Kowalski M, Michalewicz A, Retallick MAS, Colegate SM. In vitro detection of hepatocytotoxic metabolites from *Drechslera biseptata*: A contributing factor to Acute Bovine Liver Disease? *Aust. J. Exp. Agric.* 2006;46(5):599-604.

Potential Honours Projects for 2010

Project 2: The efficacy of zinc and probiotic supplementation on the alleviation of methotrexate-induced gut damage in mice.

Zinc is an essential trace element and is required for normal growth and development in both animals and humans. Zinc is required for the biological function of more than 300 enzymes. In particular, zinc is essential and directly involved in catalysis by the enzymes. Zinc plays structural and functional roles in several proteins involved in DNA replication and reverse transcription. Zinc ion bioavailability is essential for immune function. Zinc is mainly absorbed by the small intestine and it is pivotal that the gut is matured by weaning to allow maximum absorption of zinc required for the growing animal to develop normally.

Probiotics are described as non-pathogenic living micro-organisms that when ingested exert positive health benefits to the host beyond any inherent general nutritional value. Several rat models have demonstrated the protective effects of probiotics for chemotherapy-induced mucositis. *Lactobacillus bulgaricus* significantly reduced the incidence of diarrhoea and bacterial translocation. In a model of methotrexate-induced mucositis, prophylactic administration of *Streptococcus thermophilus* TH-4 was able to partially attenuate the severity of small intestinal damage.

The proposed Honours project will utilize a mouse model of methotrexate-induced small bowel damage which has been established by **Dr Cuong Tran** and **A/Prof Gordon Howarth** (rat model). It has been shown independently by **Dr Tran** and **A/Prof Howarth** that zinc alone and probiotic alone has partial protection from methotrexate-induced gut damage. However, the combination of zinc and probiotic has not been investigated. **Thus, the project will investigate the efficacy of the combine therapy of zinc and probiotic on the alleviation of methotrexate-induced gut damage in mice.**

Key references

1. Tran CD, Howarth GS, Coyle P, Philcox JC, Rofe AM, Butler RN. Dietary supplementation with zinc and a growth factor extract derived from bovine cheese whey improves methotrexate-damaged rat intestine. *Am J Clin Nutr.* 2003;77(5):1296-303.
2. Tran CD, Ball JM, Sundar S, Coyle P, Howarth GS. The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model. *Dig Dis Sci* 2007;52(9):2113-21.
3. Tooley KL, Howarth GS, Lynn KA, Lawrence A, Butler RN. Oral Ingestion of *Streptococcus thermophilus* Diminishes Severity of Small Intestinal Mucositis in Methotrexate Treated Rats. *Cancer Biol Ther* 2006; 5(10):1275-81.

Potential Honours Projects for 2010

Project 3: Zinc supplementation as an adjuvant therapy for small intestinal recovery in children with Coeliac Disease.

Coeliac Disease is a common, but frequently unrecognized, disease. Screening studies have shown that Coeliac Disease is significantly under-diagnosed, with a prevalence of approximately 1 to 1.5% in Caucasians. Assuming a conservative prevalence of 1%, this corresponds to over 200,000 Coeliac Disease cases in Australia. Approximately 85% of these cases are unrecognized and thus also untreated. The detection rate of Coeliac Disease greatly underestimates its prevalence due to a lack of awareness of the many manifestations of the condition and the requirement for at least one small intestinal biopsy for diagnosis. The diagnosis of Coeliac Disease requires histologic examination of intestinal mucosa obtained by duodenal biopsy that shows the characteristic findings of intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. These changes of intestinal damage should respond to a gluten-free diet, particularly in children who generally show better improvement of intestinal histology than adults; **however histological recovery measured using intestinal biopsy remains variable.**

Numerous studies have documented the impact of nutrient malabsorption caused from Coeliac Disease in both children and adults. Reports have shown that zinc deficiency is associated with the severity of villous atrophy and children. A characteristic feature of zinc deficiency is histological abnormalities in the gut that are similar to those observed in the gut of young children with Coeliac Disease. The inflamed and flattened small intestinal mucosa may compromise zinc absorption. These data may indicate the importance of adequate supplementation of zinc in children with Coeliac Disease. Furthermore, zinc supplementation may be useful as an adjuvant therapy to the gluten-free diet. Studies have not specifically investigated the efficacy of nutrient supplementation in treatment of Coeliac Disease. In addition, improving nutrient malabsorption and co-morbidity conditions related to nutritional deficiencies may safely hasten zinc as a therapy. **Thus, the primary aim of this proposal is to determine the efficacy of zinc as an adjuvant therapy in the histologic recovery of children with Coeliac Disease compared to those receiving a gluten-free diet and zinc placebo.**

The proposed Honours project will involve recruiting children with Coeliac Disease at the Women's and Children's Hospital. The subjects will then be randomized to receive either 0 or 20 mg of zinc in conjunction with their gluten-free diet. Zinc supplements will be administered twice weekly before breakfast for 6 weeks. The gut function breath test will be conducted at 0, 2, 4 and 6 weeks to monitor intestinal inflammation/integrity and symptoms questionnaires will be conducted weekly. Plasma zinc will be assessed before and after treatment. The primary outcomes are the change in the gut function breath test and plasma zinc.

Key references

1. Tran CD, Miller LV, Krebs NF, et al. Zinc absorption as a function of the dose of Zn sulfate in aqueous solution. *Am J Clin Nutr.* 2004;80:1570-3.
2. Ritchie BK, Brewster DR, Davidson GP, Tran CD, McNeil Y, Hawkes JS, Butler RN. The ¹³C-Sucrose Breath Test: a novel non-invasive biomarker of environmental gut health. *Pediatr.* 2009 (in press).