INTESTINAL ZINC AND METALLOTHIONEIN: ROLE IN CHEMOTHERAPY-INDUCED MUCOSITIS

BY

CUONG DUY TRAN

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The Centre for Paediatric and Adolescent Gastroenterology,

Women's and Children's Hospital, Adelaide, South Australia

and

Department of Physiology, The University of Adelaide, Adelaide, South Australia

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ABSTRACT

Intestinal zinc and metallothionein: role in chemotherapy-induced mucositis
Cuong Duy Tran; Women's and Children's Hospital and The University of Adelaide
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The aim of this thesis is to examine the regional localisation of zinc (Zn) and its intracellular binding protein, metallothionein, in determining susceptibility to damage and potential for repair in the gut. Zn and metallothionein concentrations in seven contiguous regions of the gastrointestinal tract were measured and their intracellular distribution demonstrated by specific antibody and fluorescence techniques. The effects of Zn supplementation with or without bovine whey growth factor extract (WGFE) on the severity of, and recovery from, methotrexate-induced intestinal mucositis were investigated.

Zn and metallothionein were co-localised in the base of crypt cells, mainly stem and Paneth cells most visibly in the ileum, using Zinquin fluorescence and immunohistochemistry, respectively (Chapter 2). This work was extended to examine the distribution gut Zn and metallothionein under different Zn feeding regimens (10, 100, 400 mg Zn/kg) in the rat (Chapter 3) and mouse (Chapter 4). Irrespective of dietary Zn content, Zn concentration was 20% higher in the ileum than other gut regions. Zn was 94% membrane-bound and 6% cytosolic. Metallothionein increased in all gut regions at dietary Zn at or above 100 mg/kg and was 40% higher in the ileum. The role of metallothionein in these findings was investigated using metallothionein-knockout mice ($MT^{-/-}$) (Chapter 4). Zn concentrations were higher in the distal gut at increasing Zn intakes in normal, but to a lesser extent in $MT^{-/-}$ mice. Without metallothionein, there was little modification of regional gut Zn concentration in response to excess dietary Zn and poorer regulation of Zn. Glucagon administration stimulated gut as well as liver metallothionein, implicating it as a major component of the metallothionein response to fasting.

Intracellular intestinal Zn and metallothionein in response to methotrexate-induced intestinal damage was characterised in Chapter 5. Methotrexate (2.5 mg/kg) was administrated subcutaneously for 3 d to induce gut mucositis. Intestinal damage was minimal in the ileum, moderate in the duodenum and most severe in the jejunum. Increase in gut metallothionein was greatest in the ileum, less in the jejunum and least in the duodenum. Plasma Zn decreased markedly (33%) after methotrexate at d 6, and then gradually recovered to control levels. Hepatic metallothionein progressively increased, reaching a maximum (5-fold) six days after the first methotrexate injection and rapidly decreased to control levels by d 7.

The effects of Zn and WGFE supplementation on methotrexate-induced gut damage were investigated in Chapter 6. Zn+WGFE supplementation resulted in an improvement on methotrexate-induced intestinal damage during the recovery phase. Zn, WGFE and Zn+WGFE supplementation to rats reduced intestinal permeability during the early phase and recovery phase of methotrexate-induced damage suggesting improved tight junction integrity during the early and recovery phase of bowel damage. A potential benefit of Zn and WGFE in the treatment of intestinal mucositis is thus indicated.