Dietary titanium dioxide particles and intestinal health

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Abstract

The purpose of this dissertation was to investigate the relationship between food-grade titanium dioxide particles and intestinal health, in particular the development of Crohn's disease after uptake of titanium dioxide particles in intestinal lymphoid tissues.

Crohn's disease is a common form of inflammatory bowel disease. It is characterised by chronic inflammation of the gastrointestinal tract and affects approximately 1 in 1,000 people. The aetiology of Crohn's disease is unclear, but both genetic and environmental factors are involved in the development of the disease.

The gene that is most commonly associated with Crohn's disease is the nucleotide-binding oligomerisation domain (*NOD*) 2 gene. The diet is one of the most likely environmental factors that have been proposed to play a role in Crohn's disease. It has been hypothesised that uptake of titanium dioxide particles, which are used as a whitening agent in processed foods, toothpaste, and pharmaceuticals, by macrophages in intestinal lymphoid tissues negatively affects intestinal health and contributes to the development of Crohn's disease.

To investigate this hypothesis, immune cell-stimulating properties of titanium dioxide were first assessed *in vitro* with macrophages derived from wild-type mice and mice with a Crohn's disease-like *Nod2* gene variant. These mouse models were also used to determine particle uptake in intestinal lymphoid tissues *in vivo* after exposure to titanium dioxide with the diet and effects of this dietary exposure on intestinal health and urine metabolites.

The results from the *in vitro* studies showed that titanium dioxide induced the release of the pro-inflammatory cytokine interleukin-1β. For the first time, it has been shown that accumulation of particles in intestinal lymphoid tissues was a consequence of titanium dioxide intake with the diet. However, this had no negative effects on growth performance and intestinal health of both wild-type mice and mice with a Crohn's disease-like *Nod2* gene variant. Nevertheless, differences in urine metabolite profiles between wild-type mice exposed to titanium dioxide and unexposed wild-type mice indicated that consumption of a titanium dioxide-containing diet affected the metabolism.

This dissertation forms the foundation for future studies with animal models about the relationship between titanium dioxide and intestinal health.

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All animal experiments that were carried out during the course of this project were in compliance with the New Zealand Animal Welfare Act 1999 and were approved by the Grasslands Ethics Committee (Palmerston North, NZ).

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List of abbreviations

$[M+H]^+$	Positive molecular ion
$[M-H]^-$	Negative molecular ion
1,007fs	Frameshift mutation at amino acid position 1,007
A5	Annexin-V
AIN	American Institute of Nutrition
ANOVA	Analysis of variance
APC	Antigen presenting cell
ASC	Apoptosis-associated speck-like protein containing a CARD
ATG16L1	Autophagy-related 16-like 1 gene
B cell	Bursa-derived cell
BMDC	Bone marrow-derived DC
BMDM	Bone marrow-derived macrophage
CARD	Caspase recruitment domain
CCL	CC chemokine ligand
CD	Crohn's disease
CD[number]	Cluster of differentiation [number]
CX_3CR	CX_3C chemokine receptor
DAPI	4',6-Diamidino-2-phenylindole
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DSS	Dextran sodium sulphate
EDS	Energy-dispersive X-ray spectroscopy
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FAE	Follicle-associated epithelium
FBS	Foetal bovine serum
FDR	False discovery rate
FSC	Forward scatter
GALT	Gut-associated lymphoid tissue
GC-MS	Gas chromatography mass spectrometry
H&E	Haematoxylin and eosin
HSD	Honest significant difference

IBD	Inflammatory bowel disease
IFN	Interferon
IFR	Interfollicular region
Ig	Immunoglobulin
IL	Interleukin
Il10$^{-/-}$	*Il10* gene-deficient
IL12B	IL-12 p40 subunit-encoding gene
IL-1Ra	IL-1 receptor antagonist
ILF	Isolated lymphoid follicle
IκB	Inhibitor of NF-κB
LC-MS	Liquid chromatography mass spectrometry
LP	Lamina propria
LPMC	LP mononuclear cell
LPS	Lipopolysaccharide
M cell	Microfold cell
m/z	Mass-to-charge
MANOVA	Multivariate ANOVA
MCP	Monocyte chemotactic protein
MDP	Muramyl dipeptide
MFI	Median fluorescence intensity
MIP	Macrophage inflammatory protein
MLN	Mesenteric lymph node
mRNA	Messenger ribonucleic acid
NF	Nuclear factor
NLR	NOD-like receptor
NLRP	NOD, leucine-rich repeats domain, and pyrin domain
NMR	Nuclear magnetic resonance spectroscopy
NOD	Nucleotide-binding oligomerisation domain
NOD2	*NOD2* gene (human)
NOD2	NOD2 protein (human)
Nod2	*Nod2* gene (mouse)
Nod2	Nod2 protein (mouse)
Nod2$^{-/-}$	*Nod2* gene-deficient
Nod2$^{m/m}$	*Nod2* gene mutation

NTA	Nanoparticle tracking analysis
p	Probability
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PGN	Peptidoglycan
PI	Propidium iodide
PLS-DA	Partial least squares discriminant analysis
PMT	Photomultiplier tube
PP	Peyer's patch
PRR	Pattern recognition receptor
RIP	Receptor-interacting protein
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
SD	Standard deviation
SED	Subepithelial dome
SEM	Scanning electron microscopy
SNP	Single nucleotide polymorphism
SSC	Side scatter
T cell	Thymus-derived cell
TCM	Tissue culture medium
TEM	Transmission electron microscopy
TGF	Transforming growth factor
Th	T helper
TiO_2	Titanium dioxide
TLR	Toll-like receptor
TNBS	2,4,6-Trinitrobenzenesulfonic acid
TNF	Tumour necrosis factor
UC	Ulcerative colitis
WST	Water-soluble tetrazolium salt
WT	Wild-type