Metabolomic analysis of urine from interleukin-10-deficient mice supplemented with kiwifruit extracts

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Aims

To assess the *in vivo* anti-inflammatory activity of kiwifruit extracts in IL10$^{-/-}$ mice by analysing the urinary metabolite profile of inflammation.

To determine the metabolic effects of the kiwifruit extracts on IL10$^{-/-}$ mice by analysing the global urinary metabolite profile.

Introduction

Inflammatory bowel diseases (IBD) result in recurring intestinal inflammation attributed to a dysregulated immune response towards intestinal microbiota.

IBD animal models such as the interleukin-10-deficient (IL10$^{-/-}$) mouse are used to test potential functional foods that may modulate IBD disease activity.

A urinary metabolite profile of inflammation was previously identified in IL10$^{-/-}$ mice by GCMS metabolomic analysis (Lin *et al* 2010 J Prot Res 9: 1965). This profile consist of increased levels of xanthurenic acid, fucose, 5-aminovaleric acid, uracil and 11 unknown metabolites.

Kiwifruit is an example of a food with immunomodulatory activity. Fruit extracts of yellow or green-fleshed kiwifruits inhibited the production of cytokines by murine macrophages and IL10$^{-/-}$ intestinal cells *in vitro*.

Methods

IL10$^{-/-}$ and wildtype mice were fed with fruit extracts of yellow or green-fleshed kiwifruit for 6 weeks (Table 1).

Table 1. Details of dietary treatments.

Experiment	Name of dietary treatment	Diet constituents (% w/w) AIN76A	Diet constituents (% w/w) Kiwifruit extract**	Kiwifruit extract* Kiwifruit type	Kiwifruit extract* Extraction solvent
Yellow kiwifruit experiment	control	100	-	-	-
	AQ yellow	95	5	yellow-fleshed	water
	EA yellow	99.9	0.1	yellow-fleshed	ethyl acetate
Green kiwifruit experiment	control	100	-	-	-
	AQ green	95	5	green-fleshed	water
	EA green	99.9	0.1	green-fleshed	ethyl acetate

AQ, aqueous; EA, ethyl acetate

*Prepared by solvent extraction of lyophilized fruits

**Original quantity of kiwifruit used to prepare extracts were the same for all diets. Estimated amount of kiwifruit contributing to amount of extract in a 4 g daily intake of diet is 1.5 g for all the diets

Spot urine samples were collected at 3 time points approximately 2 weeks apart (Table 2).

Table 2. Urine samples analysed by GCMS.

	Mice	Dietary treatment	Number of mice	Number of urine samples analyzed 1st time point	2nd time point	3rd time point
Yellow kiwifruit experiment	IL10$^{-/-}$	control	15	12	13	13
		AQ yellow	15	13	13	8
		EA yellow	15	12	14	10
	wildtype	control	8	8	7	6
		AQ yellow	8	5	6	3
		EA yellow	8	7	7	7
Green kiwifruit experiment	IL10$^{-/-}$	control	10	9	8	4
		AQ green	10	9	7	4
		EA green	10	6	5	5
	wildtype	control	6	5	5	4
		AQ green	7	5	7	5
		EA green	7	4	7	6

Urine samples were subjected to urease treatment, deproteinisation and TMS derivatisation before GCMS analysis using a Shimadzu QP5050A with a HP-5 GC column (yellow kiwifruit experiment) or ZB-5MS GC column (green kiwifruit experiment).

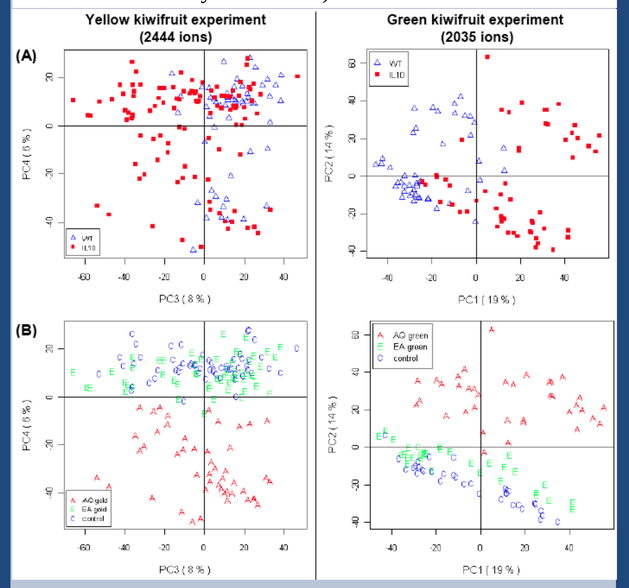
GCMS data files were pre-processed with XCMS, normalised for analytical batch and urine concentration differences (Probabilistic Quotient Normalisation).

T-tests ('multtest', R) and ANOVA (general linear model, Minitab) were used to identify peak areas that were significantly different between treatment groups.

Results & Discussion

The global urinary metabolite profile of IL10$^{-/-}$ and wildtype mice were altered by the kiwifruit extracts (Figure 1).

Figure 1. PCA score plots of all GCMS ions detected by XCMS, representing the global urinary metabolite profile. (Each datapoint is a urine sample. A: labeled as sample type. B: labeled as dietary treatments.)



However, the metabolite profile of inflammation was not altered by any of the kiwifruit extracts (Figure 2).

Histology analysis of intestinal tissues confirmed that inflammation was not reduced by the kiwifruit extracts.

Nine kiwifruit-derived metabolites detected in urine of mice fed with aqueous extracts of kiwifruits, of which one was identified as hippuric acid.

Five of these nine kiwifruit-derived metabolites have higher levels in urine of IL10$^{-/-}$ mice compared with wildtype (Figure 3).

The increased urinary excretion of these kiwifruit-derived metabolites by IL10$^{-/-}$ mice may be caused by a higher intestinal permeability or differences in intestinal microbiota.

Figure 2. Urinary levels of xanthurenic acid, one of the metabolite marker of inflammation, was not altered by the kiwifruit extracts. (Levels are relative to a reference sample)

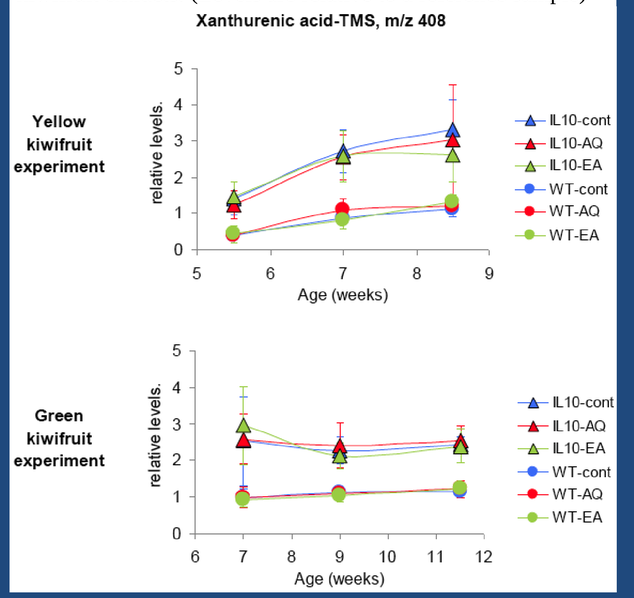
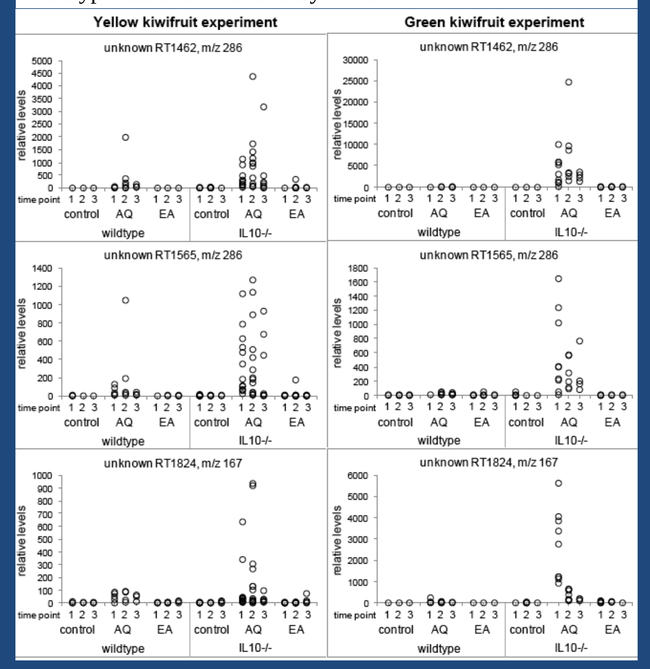


Figure 3. Examples of kiwifruit-derived metabolites with IL10$^{-/-}$-wildtype differences in urinary levels.



Conclusion

Kiwifruit extracts did not reduce intestinal inflammation in IL10$^{-/-}$ mice.

IL10$^{-/-}$ mice display differences in metabolism of kiwifruit extracts compared with wildtype mice.

Metabolism differences arising from impaired gut function may affect the efficacy of functional foods for IBD treatment.

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