Fruit polyphenolics may have potential in alleviating allergy-induced inflammation. One such mechanism is the induction of heat shock proteins (HSP) and there is evidence developing that such compounds may have immune modulating properties. IL-13 and IL-4 induce eotaxin-3 generation from lung epithelial cells [3] which activate eosinophils to further eosinophilic induced inflammation (Figure 1).

Studies suggest a correlation with healthy lung function and fruit consumption [2, 3]. Fruits are high in various polyphenolic compounds and there is evidence developing that such compounds may have immune modulating properties which are additional to the antioxidant properties. One such mechanism is the induction of heat shock proteins (HSP) by fruit polyphenolic compounds, which may lead to time protection. Fruit polyphenolics may have potential in alleviating allergy-induced inflammation.

**Introduction**

Eosinophilic induced inflammation enhances the airway hyper-responsiveness and obstruction observed in asthma [1]. Inflammation cytokines liberated from the T-lymphocyte subset (Th2) during the course of asthma are instrumental in exacerbating its symptoms by promoting further eosinophilic induced inflammation (Figure 1).

**Methodology**

A phenolic extract from blackcurrant (Ribes nigrum L cv Ben Ard) harvested from New Zealand sources was used in this study. Two lung epithelial cell-lines (alveoli [A549] and bronchial [BEAS2B] cells respectively) were preincubated with phenolic extract of blackcurrant and then analyzed initially for small heat shock protein expression (HSP) following by exploring their putative involvement in the pathology of Th2 cytokine-induced eosinophilic airway inflammation.

**Preparation of polyphenolic extracts**

- Fruits were harvested and snap frozen. Polyphenolics were isolated from the aqueous extract by absorption onto XAD-7 followed by elution with methanol.
- The methanol was evaporated to dryness leaving a red powder, which was dissolved in DMSO for use in study (stock concentration 100mg/ml).

**Analysis of small HSP expression**

- Cells (1x10⁶ cells) were grown in 75 Flasks until confluency and then incubated with blackcurrant extract (5ug/ml) for specified time (0-6hrs).
- Protein (10μg), extracted from the cells, was separated by SDS-electrophoresis and analyzed by Western blotting using monoclonal antibodies directed against specific small HSPs: HSP32/30, HSP27, HSP20, aB-crystallin.

**Evaluation of Th2-induced CCL26 expression**

- Lung epithelial cells (1x10⁶ cell/well) were grown to confluency in 12 well plates then preincubated with the blackcurrant phenolic extract (0.5-10μg/ml) or hemin chloride (0.05-10μg/ml) and then challenged with IL-4 & IL-13 (10ng/ml) each for 24hrs.
- Accumulation of CCL26 was determined over 24hrs using a specific CCL26 DuoSet ELISA (R&D systems).
- Preincubation (3-6hrs) with hemin chloride (known generator of HSP32/30) prior to stimulation of lung cells with Th2 cytokines (IL-4 & IL-13) for 24hrs mediated a concentration-dependent suppression of both IL-4- and IL-13-induced CCL26 expression on both lung cell lines (Figure 3). The blackcurrant phenolic extract had no effect on CCL26 expression or viability of both lung epithelial cell lines (data not shown).

**Results**

The Blackcurrant phenol extract (10μg/ml) of Th2 cytokines (IL-4 & IL-13) for 24hrs caused a prominent increase in HSP32/30-HSP expression (optimum after 6hrs) in both lung epithelial cell-lines. The blackcurrant phenolic extract had no effect on the expression of other small HSP; HSP27, HSP20 and aB-crystallin (Figure 2).

Simultaneous incubation of the blackcurrant phenolic extract (0-10μg/ml) with Th2 cytokines; IL-4 & IL-13 for 24hrs mediated a concentration-dependent suppression of both IL-4- and IL-13-induced CCL26 expression on both lung cell lines (Figure 3). The blackcurrant phenolic extract had no effect on CCL26 expression or viability of both lung epithelial cell lines (data not shown).

**Conclusions**

Our results suggest that the blackcurrant phenolic extract upregulates HSP32/30 in lung epithelial cells, which may serve to modulate Th2 cytokines (IL-4/IL-13) induced eosinophilic inflammation. Fruits or derived functional foods from New Zealand blackcurrants may be beneficial in alleviating airway inflammation.

**References**

Blackcurrant and Boysenberry fruit extracts enhance the ability of IFNγ to suppress IL-4/IL-13-induced CCL26 expression in lung epithelial cells

Introduction

Allergen-induced expression of Th2 cytokines (IL-4 and IL-13) in the lung promotes eosinophilic inflammation. Inflammation cytokines liberated from the T-lymphocyte subset (Th2) during the course of asthma are instrumental in exacerbating its symptoms by promoting further eosinophilic induced inflammation91, Figure 10.

Studies suggest a correlation with healthy lung function and fruit consumption. Polyphenols are abundant in fruits, vegetables, and nuts, and there is evidence evaluating the possibility that such components may have immune-modulatory actions which are additional to antioxidant properties. Recent findings suggest that some polyphenolic compounds may suppress the induction of eosinophilic chemotactants such as eotaxins (e.g. CCL26), which plays a role in asthma (poster presentation this meeting by Hurst SM). Furthermore, the Th1 cytokine IFNγ has also been shown to modulate both Th2 cytokine expression and eosinophilic inflammation.

Aim

The aim of this study was to explore the effect of phenolic extracts of blackcurrant and boysenberry fruits on Th2 cytokine IL-4 and IL-13-induced CCL26 expression in human lung epithelial cells and to explore the potential for disrupting the Th2 cytokine phenotype with the phenolic extracts.

Methodology

Phenolic extracts from blackcurrant and boysenberry were prepared from New Zealand sources. The phenolic extracts were pre-incubated with lung epithelial cells. The cells were then grown to confluency and stimulated with IL-4 (10 ng/ml) for 24 hrs. The cells were then washed and incubated with actinomycin D for 24 hrs. The induced mRNA was then extracted from the lung epithelial cells and analyzed by RT-PCR.

Assessment of CCL26 transcript

CCL26 transcript stability: Lung epithelial cells (5x10⁵ cells/well) grown to confluence and stimulated with IL-4 (10 ng/ml) for 4 hrs. The cells were then washed and incubated with actinomycin D (5ug/ml) +/- blackcurrant phenolic extract (5ug/ml) for 4 hrs.

CCL26 mRNA expression: Lung epithelial cells (5x10⁵ cells/well) grown to confluence and incubated with blackcurrant phenolic extract (5ug/ml) for specified times (1-4 days). The cells were then washed and incubated with IL-4 (10 ng/ml) for 4 hrs.

Total RNA was extracted and RT-PCR using specific primers directed against human CCL26 were performed. GAPDH was used as control for RNA loading.

Determinations of STAT-6 phosphorylation

Lung epithelial cells (5x10⁵ cells/well) were grown to confluency and were incubated with blackcurrant, boysenberry or IFNγ or specified combination for 2 hrs.

Cells were then washed and stimulated with IL-4 (5ng/ml) for 15 mins, placed immediately on ice and nuclear proteins extracted. Immunoprecipitation was carried out using anti-STAT6 and Western blot analysis was carried out using specific antibodies directed against either STAT6 (R&D systems) or phosphorylated STAT6 (Abcam Biotechnology).

Results

In all experiments, the blackcurrant phenolic extract showed no adverse affect on the bioavailability of IL-4 (data not shown) in experiments. Pre-incubation (6-24 hrs) of blackcurrant phenolic extract (5ug/ml) with the lung cells demonstrated an attenuation of IL-4- induced CCL26 expression, which was evident by 6hrs. Furthermore, pre-incubation of blackcurrant phenolic extract (5ug/ml) with the lung cells alone did not induced CCL26 mRNA expression (Figure 6).

Conclusions

These data suggest that blackcurrant and boysenberry phenolic extracts are capable of suppressing IL-4/IL-13-induced CCL26 expression, which involves the prevention of STAT6- phosphorylation and subsequent CCL26 transcription. IFNγ enhances the ability of blackcurrant phenolic extract to suppress IL-4-induced CCL26 expression, which in part, involves the prevention of STAT6 phosphorylation. These data give mechanistic insights into the potential value of fruits or derived-functional foods that might be beneficial in alleviating airway inflammation.

References