

BACKGROUND

Tea is the most popular beverage after water in the world. Especially in black and green tea are a lot of polyphenols like flavanoids. EGCG [(-)-Epigallocatechin Gallate] and (+)-Catechin are flavanoids which are known as anticancer and healthy drugs. The flavanoids are often tested on stable cell lines, but not often on primary cell culture.

GOALS

- To investigate the influence of numerous physiological concentrations of EGCG and Catechin;
- To test the effects of co-stimulation with ConA and polyphenols;
- To test the effect of ConA stimulation on WBC expression pattern;
- To investigate the time course of mRNA expression of various pro-inflammatory factors (**TNF- α** , **IL-1 β** , **IL-6**) as well as transcription factor **cFos** and **Histon H3**;

MATERIAL & METHODS

Primary white blood cells (WBC) were isolated from healthy dairy cow and cultivated in 3 ml RPMI medium with FCS and Gentamycin (1x10⁶ cells/ml).

We tested the following approaches:

- ConA stimulation **6 h before** EGCG or Catechin treatment.
- Investigated flavanoid concentrations: **0 (control), 0.1, 1, 10, 30 & 100 μ M**
- Investigated ConA concentrations: **0 (control), 0.01, 0.1 & 1 μ g ConA/ml**

WBC were harvested one day after stimulation and total RNA was extracted. Each combination was tested in five replicates.

Relative expression levels of cytokines TNF- α , IL-1 β , IL-6, transcription factor cFos, and Histon mRNAs were quantified with fully quantitative real-time RT-PCR (LightCycler), normalised by non-regulated **GAPDH** housekeeping gene expression.

Mathematical and statistical analyses

Data are presented as means and SEM. For statistical analysis, a two way ANOVA (Sigma Stat) was performed. Differences were considered significant if $p < 0.05$

RESULTS

Effect on total RNA expression

Total RNA concentration decreased with high polyphenol concentrations, in EGCG treated WBC more significant ($p < 0.001$) than in the Catechin treated WBC ($p < 0.05$).

Effects derived from flavanoids

Histon H3 and GAPDH mRNA levels were constantly expressed and mRNA expressions were not regulated by ConA or flavanoid treatments.

Expression of pro-inflammatory TNF α showed no regulation. High EGCG concentrations (>30 μ M) caused a down-regulation of the cFos mRNA expressions in a dose dependent manner. IL-6 and IL-1 β showed lower and stable expression levels under various EGCG treatments, independent on concentration, compared to Catechin treatments.

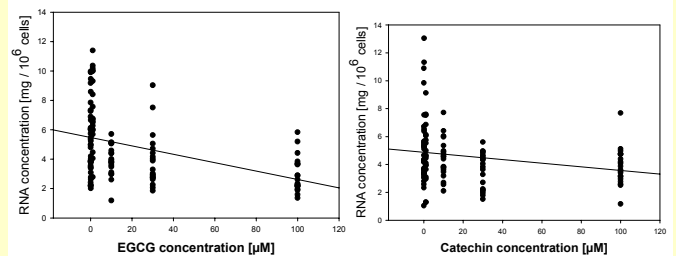
Effects derived from ConA

The effects of the super-antigen ConA were more pronounced in EGCG as in Catechin treatment group.

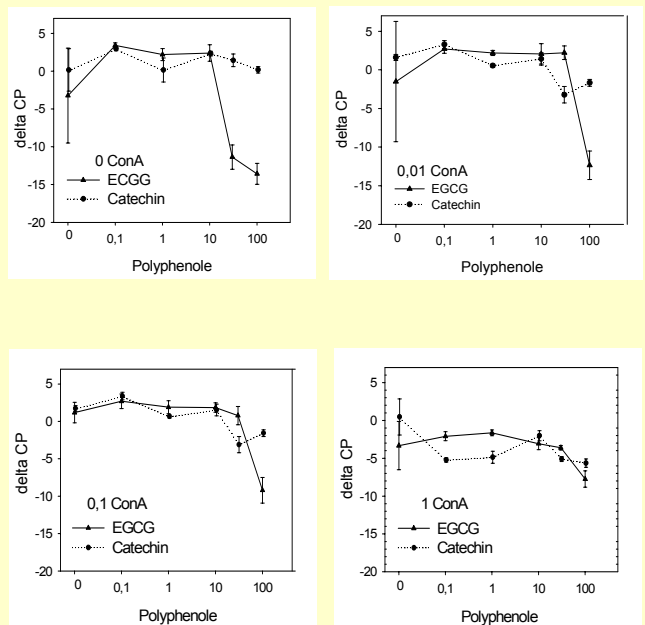
With ConA stimulation the cFos, IL1 β , TNF- α and IL-6 mRNA expression levels were highly up-regulated ($p < 0.01$).

Histon H3 was not influenced by ConA stimulation.

Influence of EGCG and Catechin on mRNA synthesis



cFos mRNA expression



CONCLUSION

We suggested that both flavanoids have preventive and silencing effect on WBC expression pattern for pro-inflammatory cytokines as well as transcription factor cFos.

In EGCG and Catechin treated WBC a slight down-regulation of pro-inflammatory genes is given, especially for IL-1 β and IL-6 expression. EGCG downregulates cFos.

ConA can stimulate primary WBC and enhance the transcription of pro-inflammatory cytokines, like TNF- α and interleukins in a dose dependent manner.

In conclusion, we suggested that the both flavanoids have mainly preventive effect in activation of immune relevant cytokines as well as transcription factors. Higher flavanoid concentration had more pronounced effects than lower, whereas EGCG shows a more potent suppression of gene expression than Catechin.