# Stimulation of isolated white blood cells with two types of catechin [ Epigallocatechin Gallate & (+)-Catechin ] and ConA

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## BACKGROUND

EGCG [(-)-Epigallocatechin Gallate] and (+)-Catechin are flavanoids which are known as anticancer and healthy drugs. Apple, red wine and green tea are rich in such polyphenols. The flavanoids are often tested on stable cell lines, but not often on primary cell culture.

## GOALS

- Investigation of immune-stimulatory effect of the superantigen and T-cell mitogen ConA on primary WBC;
- Test the influence of numerous physiological concentrations of EGCG and Catechin;
- Test the effect of ConA stimulation on WBC expression pattern;
- Investigate the course of mRNA expression of various pro-inflamatory factors (**TNF-α, IL-1ß, IL-6 mRNAs**) as well as transcription factor **cFos mRNA** and **Histon mRNA**;

## MATERIAL & METHODS

Primary white blood cell (WBC) culture (1x10$^6$ cells/ml), isolated from healty dairy cow, were cultivated using RPMI medium with FCS and Gentamycin.

**We tested three approaches:**

- ConA stimulation **6 h before** EGCG/Catechin treatment;
- ConA stimulation **in parallel** with EGCG/Catechin treatment;
- ConA stimulation **6 h after** EGCG/Catechin treatment;

Investigated flavanoid concentrations: **0 (control), 0.1, 1, 10, 30 & 100 µM;**

Investigated ConA concentrations: **0 (control) & 0.1 µg ConA/ml;**

WBC were harvested one day after stimulation and total RNA was extracted.

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

### 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

### Effects derived from flavanoids

When ConA was given before flavanoids, cFos was high significantly down-regulated und Histon high significantly up-regulated in the EGCG group. With Catechin treatment TNF-α, IL-6 and Histon was highly up-regulated.

When the immune stimuli and flavanoid treatment were in parallel, TNF-α was down-regulated with EGCG and cFos was up-regulated with Catechin.

If the ConA stimuli was given after the flavanoid treatment cFos and TNF-α are down-regulated with EGCG. Catechin has various effects on cFos, TNF-α IL-6 and IL-1β. Expressions of all four factors were down-regulated.

### Effects derived from ConA

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

When ConA was given before EGCG, cFos, TNF-α and IL-6 were highly up-regulated. If the ConA stimuli was given after EGCG cFos, IL-6 and IL-1β were down-regulated.

In the Catechin treatment, only cFos and Histon were influenced.

## Influence of EGCG and Catechin on the mRNA synthesis

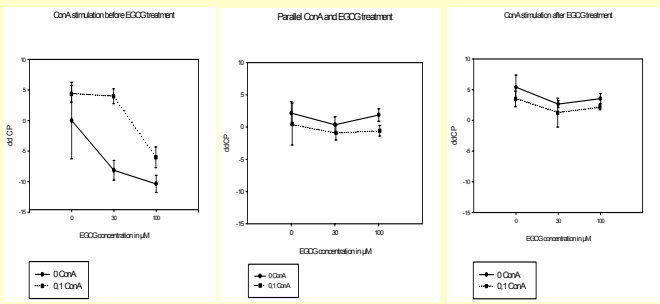
| EGCG | ConA stimulation before EGCG treatment | Parallel ConA and EGCG treatment | ConA stimulation after EGCG treatment | Catechin | ConA stimulation before Catechin treatment | Parallel ConA and Catechin treatment | ConA stimulation after Catechin treatment |
|---|---|---|---|---|---|---|---|
| cFos | ↓↓↓ P<0.001 | n.s. | ↓↓ P=0.008 | cFos | n.s. | ↑↑↑ P<0.001 | ↓↓↓ P<0.001 |
| TNFalpha | n.s. | ↓ P=0.017 | ↓ P=0.030 | TNFalpha | ↑↑↑ P<0.001 | n.s | ↓ P=0.032 |
| IL-6 | ↓ P=0.043 | n.s. | n.s. | IL-6 | ↑↑↑ P<0.001 | n.s | ↓↓ P=0.002 |
| IL-1beta | n.s. | n.s. | n.s. | IL-1beta | n.s | n.s | ↓↓↓ P<0.001 |
| Histon | ↑↑↑ P<0.001 | n.s. | n.s. | Histon | ↑ P=0.025 | n.s | n.s |

## Influence of ConA on the mRNA synthesis

| EGCG | ConA stimulation before EGCG treatment | Parallel ConA and EGCG treatment | ConA stimulation after EGCG treatment |
|---|---|---|---|
| cFos | ↑↑↑ P<0.001 | ↓↓ P=0.005 | ↓↓ P=0.008 |
| TNFalpha | ↑↑ P=0.004 | n.s. | n.s. |
| IL-6 | ↑↑↑ P<0.001 | n.s. | ↓ P=0.020 |
| IL-1beta | n.s. | n.s. | ↓↓ P=0.007 |
| Histon | n.s. | n.s. | n.s. |

| Catechin | ConA stimulation before Catechin treatment | Parallel ConA and Catechin treatment | ConA stimulation after Catechin treatment |
|---|---|---|---|
| cFos | n.s. | ↑ P=0.027 | n.s. |
| TNF-alpha | n.s. | n.s. | n.s. |
| IL-6 | n.s. | n.s. | n.s. |
| IL-1beta | n.s. | n.s. | n.s. |
| Histon | n.s. | ↓ P=0.038 | n.s. |

## cFos mRNA expression




## CONCLUSION

We suggested that ConA can stimulate primary WBC and enhance the transcription of primary unspecific pro-inflammatory cytokines, like TNF-α and interleukins.

In EGCG and Catechin treated WBC a significant down-regulation of such pro-inflammatory genes is given (TNF-α, IL-1β, IL-6 ).

Both flavanoids have preventive and silencing effect on WBC expression pattern of pro-inflammatory cytokines as well as transcription factor cFos.

Higher flavanoid concentration had more pronounced effects than lower, whereas EGCG showed a more potent suppression of gene expression than Catechin.