**Title of the publication:**
Anti-inflammatory effects of ivy leaves dry extract: influence on transcriptional activity of NFκB.

**Method:**
In vitro cell culture experiments

**Background:**
Anti-inflammatory properties of EA 575 have been shown before, yet more details also concerning the mechanism of action were to be investigated. For this end, murine macrophages (J774.2) as well as various human cell lines (HEK 293, THP-1, A549) were genetically altered via transfection. These transfections caused the cells to express Nanoluciferase upon activation of the central inflammation activator and transcription factor NFκB. Under stimulation with inflammatory signals like TNFα or lipopolysaccharide (LPS), Nanoluciferase permits the measurement of NFκB activity and the influence of EA 575 thereon directly via light emission. This should permit to shed more light onto the mechanism of action of the anti-inflammatory effect.

**Results:**
- Stimulation with 25 ng/mL TNFα or 100 ng/mL LPS resulted in an increase of NFκB transcriptional activity in all cells that stably expressed Nanoluciferase
- In the presence of 40 µg/mL or more EA 575 a statistically significant reduction of NFκB transcriptional activity could be observed in stably transfected human HEK 293 cells (Figure 1 d)
- At a concentration of 240 µg/mL, EA 575 was also able to reduce NFκB transcriptional activity in statistically significant manner in human immune (THP-1) and lung epithelium (A549) cells
- The reduced translocation of NFκB into the nucleus could be demonstrated via fluorescence microscopy by implementation of Hoechst staining and immunohistochemistry (Figure 1 a–c)

**Summary**
- The mechanism of action underlying the anti-inflammatory effect of EA 575 (Prospan®) is based on the reduced migration of inflammation activator NFκB into the cell nucleus
- The anti-inflammatory effect could also be observed in human lung epithelium cells
- The anti-inflammatory properties of EA 575 was reinforced with a variety of experiments. In this way, patient benefit in the phytotherapy of acute and chronic inflammatory airway diseases was confirmed again
Figure 1:

a) Cell nucleus (blue) in J774.2 cells. Under basal conditions, NFKB (red) is mostly found in the cytosol.

b) After stimulation with LPS (100 ng/mL) NFKB translocates into the cell nucleus, where it initiates the transcription of pro-inflammatory genes.

c) In the presence of EA 575 (80 µg/mL) far less NFKB translocates into the cell nucleus even under stimulating conditions.

d) Concentration-dependent reduction of TNFα-induced NFKB transcriptional activity under EA 575 starting at a concentration of 40 µg/mL.


Recording to local prescribing information.