Pharmacological Profiling of the Thymus vulgaris Preparation BNO 1018

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Material and Methods

Extract

For the preparation of BNO 1018, the comminuted herb of *Thymus vulgaris* (Plantamed Lot 500181) was percolated with a mixture of water/96% ethanol 3/7 (v/v). After filtration the resulting extract had a residual dry mass of 16,2 mg/g resp. 14,47 mg/ml and an ethanol concentration of 66,9 % (v/v) (GC). The concentrations of thymol and carvacrol were found to be 0,072 % and 0,005 %, respectively, as determined by capillary gas chromatography.

Radical Scavenging Activity

The scavening effect on superoxid radicals was investigated by measuring the quenching effect of the chemiluminescence of luminol.
The assay was performed in a 96 microtiter plate (Dynatec). In a total volume of 250µl each well contained 50mmol/l (pH 7,8) Sörensen buffer, 200µmol/l luminol (Sigma), 1U/ml horseradish peroxidase (Sigma) and 50µl dilution of plant extract.
The plate was placed in the reader (Luminometer / Berthold) and the reaction was started by adding 50µl H2O2 (25µmol/l) (Sigma) to give the final volume of 250µl. The measurement was stopped when the peak of luminescence reached the base line.
The amount of radicals was determined by integrating the area under the curve and comparing the AUC of the test compounds with the control (buffer only) which was set to100 percent.

PGE2 Assay

This assay was used to investigate the influence of BNO 1018 on the PGH synthase. It was performed with the monocyte-macrophage cell-line MM6 which expresses both isoforms of the enzyme.

The assay was performed in a microtiter plate with 100µl RPMI medium containing 10mg/ml lipopolysaccharid (LPS) (Sigma), 20µM arachidonic acid (Cayman Chemical) and the dilution of the plant extract to be tested. Indomethacin (Sigma) was used as positive control at a concentration of 5µM.
1,5 x 105 cells in 100µl of the MM6 cell-suspension were added to 100 µl test solution.
The cells were incubated for 7 hours at 37°C and 100µl of the supernatant were taken for further assays.
To determine the amount of PGE2 synthesized, a one in five dilution of the supernatant was measured by the standard procedure of a commercial ELISA-kit (Cayman Chemical).

Antimicrobial Assay

Antimicrobial activity was quantified by an agar diffusion assay, applying 100µl of the extract into a whole of the agar layer in the petri dish with the bacterial strain to be tested. The diameter of the inhibtion zone was corrected by the respective control of ethanol concentration

Antiviral Assay

For the testing of antiviral activity the plaque forming capacity of the Influenza A, strain Chile (H1N1) and the Respiratory Syncytial virus was used. 5 - 7 days after infection the number of plaques was checked after fixation and Giemsa-staining of the feeder cells.

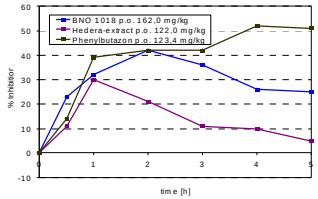

Fig. 1: Effect of p.o. thyme(BNO 1018) and ivy extract and Phenylbutazon in Carrageenin-induced paw edema in rats

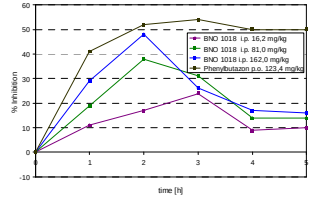

Fig. 2: Dose dependant effect of thyme extract BNO 1018 (i.p.) and Phenylbutazon in Carrageenean-induced paw edema in rats

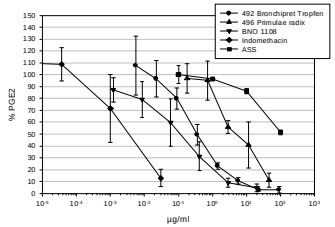

Fig. 3: Influence of plant extracts and synthetic compounds on the release of PGE2 from MM6 cells

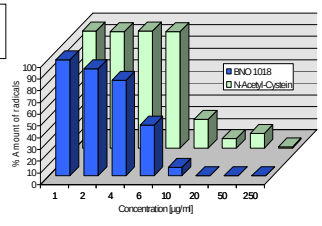

Fig. 4: Comparison of the antioxidative effect of BNO 1108 and N-Acetyl-Cystein on superoxide radicals.

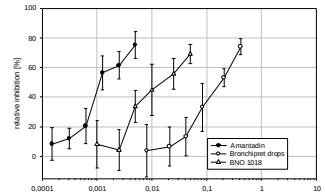

Fig. 5: Inhibition of the plaque formation of Influena A virus/Chile/183 (H1N1) propagated on MDCK cells by thyme extract and Bronchipret drops.

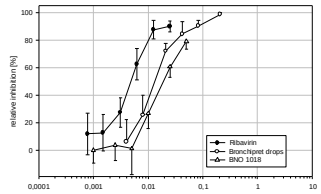

Fig. 6: Inhibition of the plaque formation of Respiratory Syncytial Virus propagated on HEp-2 cells by thyme extract and Bronchipret drops

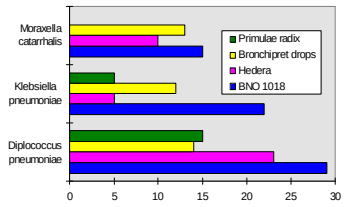

Fig. 7: Antibacterial activity of plant extracts in the agar diffusion assay. Diameter of inhibition zone in mm

Results

BNO1018 revealed an antiviral activity in vitro towards Respiratory Syncytial virus and Influenza A virus which are most frequently the eliciting agents of infection. The sensitivity of RSV, which is predominantly causing infections in young children, was very high and about one tenth of the strength of the control ribavirin.
BNO1018 also showed antimicrobial activity in the agar diffusion assay towards the pneumotropic bacteria Moraxella catarrhalis, Klebsiella pneumoniae and Diplococcus pneumoniae.
The plant extract had a strong antiinflammatory effect in the edema model of the carrageenean injected rat paw when it was administered orally. In the early and mid phase of the acute inflammation the effect of 162 mg/kg bw. was comparable to 123 mg/kg bw. phenylbutazon. A dose dependent effect could be demonstated for i.p. application.
The formation of the inflammatory mediator PGE2 was suppressed in monocyte-macrophage like cell-line MM6 with an IC50 of about 0,1µg/ml (indomethacin: 0,004; ASS: 100µg/ml).

BNO 1018 can scavenge superoxid-radicals with an effectiveness comparable to N-acetyl-cystein.

Discussion

The thyme preparation BNO 1018 was screened for pharmacological effects which may be clinically beneficial to the treatment of the infection and inflammation of the airways. The findings of antiviral and antimicrobial activity, the antiinflammatory effect in the rat and in MM6 cells on the formation of PGE2 and the radical quenching effect may positively contribute to the treatment of causal agents and the relieve of symptoms related to the disease.

References

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