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Inhibition of granulocyte elastase activity by caffeic acid derivatives

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In former studies it was shown that bornyl derivatives may contribute to the anti-inflammatory activity of some drugs, such as *Verbesina turbacensis* [1] *Notopterygium incisum* [2] or *Coreopsis mutica* [3]. It was reported that (−)-bornyl caffeate, (−)-bornyl ferulate and (−)-bornyl *p*-coumarate significantly inhibit the TPA-induced mouse ear edema. IC_{50} values were similar to that obtained for indomethacin [3]. This anti-inflammatory effect can be partly explained by their inhibitory activity towards 5-lipoxygenase and cyclooxygenase-1 [1, 2]. Additionally, it was proved that these compounds were able to reduce the production of reactive oxygen species in human granulocytes, which may have further beneficial effects in inflammatory processes [1].

Against this background we investigated whether these three bornyl derivatives isolated from *Verbesina turbacensis* may also influence the activity of neutrophile elastase. Elastase and cathepsin G are the two main proteinases in neutrophils and released during inflammatory processes. They are localized in the azurophil granules, together with proteinase 3 and further acidic cathepsins [4]. The neutrophile elastase contributes to the destruction of basement membranes during inflammation and is directed to the degradation of type IV procollagen [5]. One typical feature of active inflammation is an elevated plasma level of neutrophile elastase [6].

In an *in vitro* assay we used isolated elastase from human leukocytes and determined the inhibitory effect of the bornyl derivatives in comparison to caffeic, ferulic and *p*-coumaric acid. All bornyl derivatives inhibited the enzyme activity, down to a concentration of approximately 50 μmol/l. Ferulic acid and *p*-coumaric acid did not show a significant inhibitory potential, whereas caffeic acid exhibited a strong inhibition (IC_{50} value of 16 μg/ml = 93 μmol(l). (−)-Bornyl caffeate was the most active compound in the with an IC_{50} value of 0.5 μg/ml (1.6 μmol/l) (Table). The stronger inhibition of the neutrophile elastase activity by the bornyl esters compared to the pure acids may be due to the enhancement of lipophilicity by esterification with borneol. Thus, the esters can easier interact with the active site of the enzyme localized not on the surface of the protein but in regions which require a high lipophilicity for interaction.

Table: Effect of selected cinnamate derivatives on the enzymatic activity of human leukocyte elastase

Substance	IC_{50} (μmol/l)
Caffeic acid	93
Bornyl caffeate	1.6
Bornyl ferulate	78
Bornyl coumarate	69
Ferulic acid	>1 mM
p-Coumaric acid	>1 mM

Our study shows again, that in particular (−)-bornyl caffeate seems to be a powerful anti-inflammatory agent with a broad spectrum of action within the inflammatory process. It would be interesting to study whether other caffeic acid esters frequently occuring in a variety of medicinal plants used for treatment of inflammatory disorders also exert an inhibitory potential against the proinflammatory neutrophile elastase from human leukocytes.

Experimental

1. Substances

(−)-Bornyl caffeate, (−)-bornyl ferulate and (−)-bornyl *p*-coumarate were isolated from *Verbesina turbacensis* as described earlier [1]. Caffeic acid, ferulic acid and *p*-coumaric acid were purchased from ICN Biomedicals (Germany).

Neutrophile elastase from human leukocytes (E.C. 4.4.21.37) was supplied by Fluka (Germany), the enzyme substrate MeO-Suc-Ala-Ala-Pro-Val-pNA was purchased from Bachem (Switzerland). All other chemicals were from Sigma (Germany).

2. Enzyme assay

The determination of neutrophile elastase activity was performed according to Stein [7] with human leukocyte elastase. Briefly, 250 μl substrate solution (700 μM MeO-Suc-Ala-Ala-Pro-Val-pNA in Tris-HCl-buffer, pH 7.5) were mixed with 100 μl test solution (test substances solubilized in Tris-HCl-buffer, pH 7.5) and vortexed. After the addition of 250 μl enzyme solution (approximately 0.5 mU) the samples were incubated for 1 h at 37 °C. The reaction was stopped by addition of 500 μl soybean trypsin inhibitor solution (2 mg/ml Tris-HCl-buffer, pH 7.5) and places in an ice bath. After vortexing the absorbance was read at 405 nm and the enzyme activity was calculated from a standard curve obtained with *p*-nitraniline.

All assays were performed at least three times, IC_{50}-values were determined by linear regression of the dose-inhibition-curve.

References

1 Lobitz, G. O.; Heilmann, J.; Zschocke, S.; Bauer, R.; Merfort, I.: Pharm. Pharmacol. Lett. **3**, 115 (1998)
2 Zschocke, S.; Lehner, M.; Bauer, R.: Planta Med. **63**, 203 (1997)
3 Maldonado, E.; Ramirez Apan, T.; Pérez-Castorena, A. L.: Planta Med. **64**, 660 (1998)
4 Baggiolini, M.; Schnyder, J.; Bretz, U.; Dewald, B.; Ruch, W.: Ciba Found. Symp. **75**, 105 (1979)
5 Pipoly, D. J.; Crouch, E. C.: Biochemistry **26**, 5748 (1987)
6 Fischbach, W.; Becker, W.; Mossner, J.; Ohlemuller, H.; Koch, W.; Borner, W.: Digestion **37**, 88 (1987)
7 Stein, R. L.: J. Am. Chem. Soc. **105**, 5111 (1983)

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