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The role of the phenethyl ester of caffeic acid (CAPE) in the inhibition of rat lung cyclooxygenase activity by propolis

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Abstract

In this study we investigated the effect of an ethanolic extract of propolis, with and without CAPE, and some of its components on cyclooxygenase (COX) activity. Propolis (0.00003–0.03%) significantly and concentration-dependently inhibited COX activity from lung homogenate of saline- or LPS-treated rats. Same results were obtained with CAPE (0.1–100 μM). COX activity from lung homogenate of saline- or LPS-treated rats was also inhibited by galangin (0.1–100 μM), although the inhibition induced by the lowest concentration was not significant. Caffeic, ferulic, cinnamic and chlorogenic acids and pinocembrin, (0.1–100 μM) did not affect COX activity. The inhibition curves showed that CAPE and propolis were equipotent inhibitors, whereas galangin was significantly ($P<0.001$) less potent than propolis and CAPE. In order to better investigate the role of CAPE, we tested the action of an ethanolic extract of propolis (0.00003–0.03%) without CAPE. This extract significantly and concentration-dependently inhibited COX activity from lung homogenate of saline- or LPS-treated rats, however, it resulted to be approximately 10 times less potent than the extract containing CAPE. The analysis of the inhibition curves of the extract with and without CAPE showed a significant ($P<0.001$) difference. These results suggest that both CAPE and galangin contribute to the overall activity of propolis, CAPE being more effective.

Keywords: Cyclooxygenase; CAPE; Lung; Prostaglandins; Propolis

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1. Introduction

Propolis (bee glue) is a resinous dark-coloured material which is collected by honeybees from the buds of living plants mixed with bee wax and salivary secretions. Crude extracts of propolis have very complicated compositions, resulting from variation in geographical and botanical origin [1].

Propolis is well known for its medical effects, including anti-inflammatory, antiviral, immunostimulatory and carcinostatic activities [2]. The wide spectrum of activity of propolis was mainly attributed to the large number of flavonoids [3]. In addition to flavonoids, propolis contains cinnamic derivatives such as caffeic, ferulic, cinnamic, chlorogenic acids and its esters [4]. Recently it has been reported that a component of propolis, caffeic acid phenethyl ester (CAPE), exerts a potent anti-inflammatory activity. The anti-inflammatory properties of CAPE have been attributed to suppression of prostaglandin (PG) and leukotriene synthesis [4].

PG, which plays a central role in the inflammatory process, is generated from arachidonic acid (AA) via the action of the enzyme cyclooxygenase (E.C. 1.14.991; COX). Two isoforms of COX have been identified: the constitutive isoform (COX-1), which seems to be almost ubiquitous and the inducible isoform (COX-2) which is expressed by several cell types in response to inflammatory and immunological stimuli [5]. Lung is a very rich source of PG mainly PGE_2 and 6-keto-$PGF_{1\alpha}$. COX-1 seems to be present in the whole lung homogenate of saline-treated rats, whereas a time- and dose-dependent up-regulation of COX-2 seems to occur after LPS challenge [6].

In order to investigate the role of CAPE and other components in the overall anti-inflammatory activity of propolis we investigated the effect of an ethanolic extract of propolis, with and without CAPE, and some of its components on COX activity from lung homogenate of saline- or LPS-treated rats, measured as PGE_2 and 6-keto-$PGF_{1\alpha}$ production from exogenously added AA.

2. Experimental

2.1. Drugs tested

The dried ethanol extract of propolis, with and without CAPE, was obtained from Carlo Sessa, Milan, Italy. Total flavonoid content of propolis with CAPE, expressed as galangin, was 9.04%; CAPE content was 10.44%. Total acidity, expressed as caffeic acid, was 13%. Total flavonoid content of propolis without CAPE, expressed as galangin, was 8.5%. CAPE, caffeic, ferulic, cinnamic, chlorogenic acids and galangin were obtained from Sigma-Aldrich, Milan, Italy. Pinocembrin was obtained from Trimital, Milan, Italy.

Stock solutions of test compounds were prepared in ethanol, an equivalent amount of ethanol was included in control samples.

2.2. Materials

Arachidonic acid was obtained from SPIBIO, Paris, France. Bacterial lipopolysaccharide (LPS) and all other reagents and compounds used were obtained from Sigma-Aldrich, Milan, Italy.

2.3. Animals

Male Wistar rats (200–250 g; Mario Negri Sud, Chieti, Italy) were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with the EEC regulations O.J. of E.C.L 358/1 12/18/1986.

2.4. Assessment of COX activity

Animals were treated i.p. with 6 mg/kg of LPS or saline (0.9% NaCl; 2 ml/kg). Six hours after LPS or saline injection rats were anaesthetised with CO_2, lungs were removed and homogenised at 4 °C in protease inhibitor buffer, in a ratio 5:1 (v/w). The protein concentration in the homogenates was measured by the Bradford assay [7], with bovine serum albumin (BSA) used as standard. Homogenates were pre-incubated for 5 min in the absence (controls) or presence of test compounds and subsequently incubated at 37 °C for 30 min in the presence of excess arachidonic acid (1 mM). Test compounds were the following: propolis, with and without CAPE, (0.00003–0.03%), CAPE, galangin, pinocembrin, caffeic, ferulic, cinnamic and chlorogenic acids (0.1–100 μM). The concentrations of CAPE and galangin (0.1–100 μM) were the same as in the extracts. The samples were boiled and centrifuged at 10 000 × *g* for 30 min. The amount of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the supernatants was measured by radioimmunoassay [8]. Inhibition of prostanoid generation by test compounds is calculated as percentage of activity in the presence of drugs vs. the activity in the control samples.

2.5. Statistics

Results are expressed as the mean for four experiments performed in triplicate. Data fit was obtained using the sigmoidal dose–response equation (variable slope) (GraphPad software). The IC_{50} and the 95% confidence intervals were calculated by GraphPad Instat program (GraphPad software). Inhibition curves were analysed and compared with two-way analysis of variance (ANOVA). A *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Inhibition of COX activity from lung homogenate of saline-treated rats

The ethanolic extract of propolis (0.00003–0.03%) significantly and concentration-dependently inhibited, at all the concentrations tested, both PGE_2 and 6-keto-

$PGF_{1\alpha}$ with an IC_{50} of 0.00062% (0.00024–0.00158%) and 0.00094% (0.00058–0.00153%), respectively (Fig. 1).

Same results were obtained with CAPE (0.1–100 μM) which significantly and concentration-dependently inhibited, at all the concentrations tested, prostanoid generation (Fig. 1). The IC_{50} values for PGE_2 and 6-keto-$PGF_{1\alpha}$ were 3.3 μM (1.6–6.8 μM) and 3.9 μM (1.6–9.8 μM), respectively.

The production of PGE_2 and 6-keto-$PGF_{1\alpha}$ was also significantly inhibited by galangin (0.1–100 μM) but only from the concentration of 1 μM (Fig. 1). The IC_{50} values were 22 μM (10–47 μM) for PGE_2 and 25 μM (13–49 μM) for 6-keto-$PGF_{1\alpha}$.

The other compounds tested—pinocembrin, caffeic, chlorogenic, ferulic and cinnamic acids—did not inhibit, at all, the concentrations tested (0.1–100 μM), prostanoid generation (data not shown).

In order to better investigate a better investigation, the role of CAPE in the inhibition of PG biosynthesis by propolis we tested the action of an ethanolic extract of propolis without CAPE. This extract (0.00003–0.03%) was approximately 10 times less potent than the extract containing CAPE since the inhibition of PGE_2 and 6-keto-$PGF_{1\alpha}$ production was significant only from the concentration of 0.0003% (Fig. 1). The IC_{50} values were 0.0053% (0.0020–0.0140%) for PGE_2 and 0.0072% (0.0017–0.0306%) for 6-keto-$PGF_{1\alpha}$.

The comparison of the inhibition curves showed a significant difference ($P < 0.001$) between propolis with and without CAPE, whereas the inhibition curves of propolis and CAPE were almost superimposable. When we compared the inhibition curves of propolis and galangin we found a significant ($P < 0.001$) difference. On the contrary, the inhibition curve of propolis without CAPE was not statistically different from the inhibition curve of galangin.

3.2. Inhibition of COX activity from lung homogenate of LPS-treated rats

The ethanolic extract of propolis (0.00003–0.03%) significantly and concentration-dependently inhibited, at all the concentrations tested, both PGE_2 and 6-keto-$PGF_{1\alpha}$ production with IC_{50} values of 0.00029% (0.00016–0.00053%) and 0.0012% (0.0009–0.0017%), respectively (Fig. 2).

Same results were obtained with CAPE (0.1–100 μM) which significantly and concentration-dependently inhibited, at all the concentrations tested, prostanoid generation (Fig. 2). The IC_{50} values for PGE_2 and 6-keto-$PGF_{1\alpha}$ were 0.88 μM (0.21–3.64 μM) and 4.7 μM (1.1–19.8 μM), respectively.

Galangin (0.1–100 μM) was also effective, but the inhibition was significant only from the concentration of 1 μM (Fig. 2). The IC_{50} values were 29 μM (13–66 μM) for PGE_2 and 45 μM (26–77 μM) for 6-keto-$PGF_{1\alpha}$.

The other compounds tested—pinocembrin, caffeic, chlorogenic, ferulic and cinnamic acids—did not inhibit, at all the concentrations tested (0.1–100 μM), prostanoid generation (data not shown).

Next we tested the action of an ethanolic extract of propolis without CAPE. This extract (0.00003–0.03%) significantly and concentration-dependently inhibited

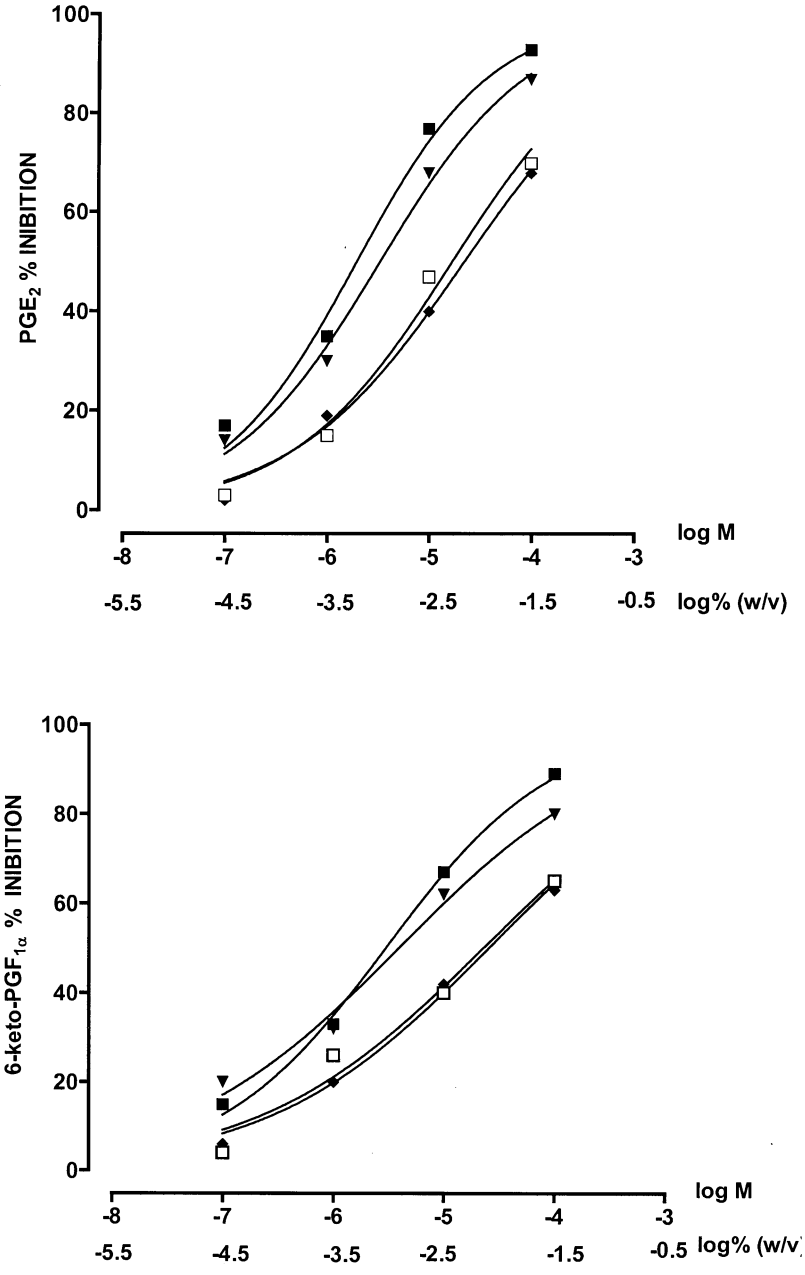

Fig. 1. Inhibitory effect of an ethanolic extract of propolis with (■) and without (□) CAPE, CAPE (▼) and galangin (◆) on PGE_2 and 6-keto-$PGF_{1\alpha}$ production by lung homogenates, from saline-treated rats, exposed to arachidonic acid for 30 min. Lung homogenates were incubated for 5 min with test compounds before the addition of arachidonic acid. The concentrations of propolis extracts are expressed as log% (w/v), whereas the concentrations of CAPE and galangin are expressed as log M. Each point represents the mean of four experiments performed in triplicate.

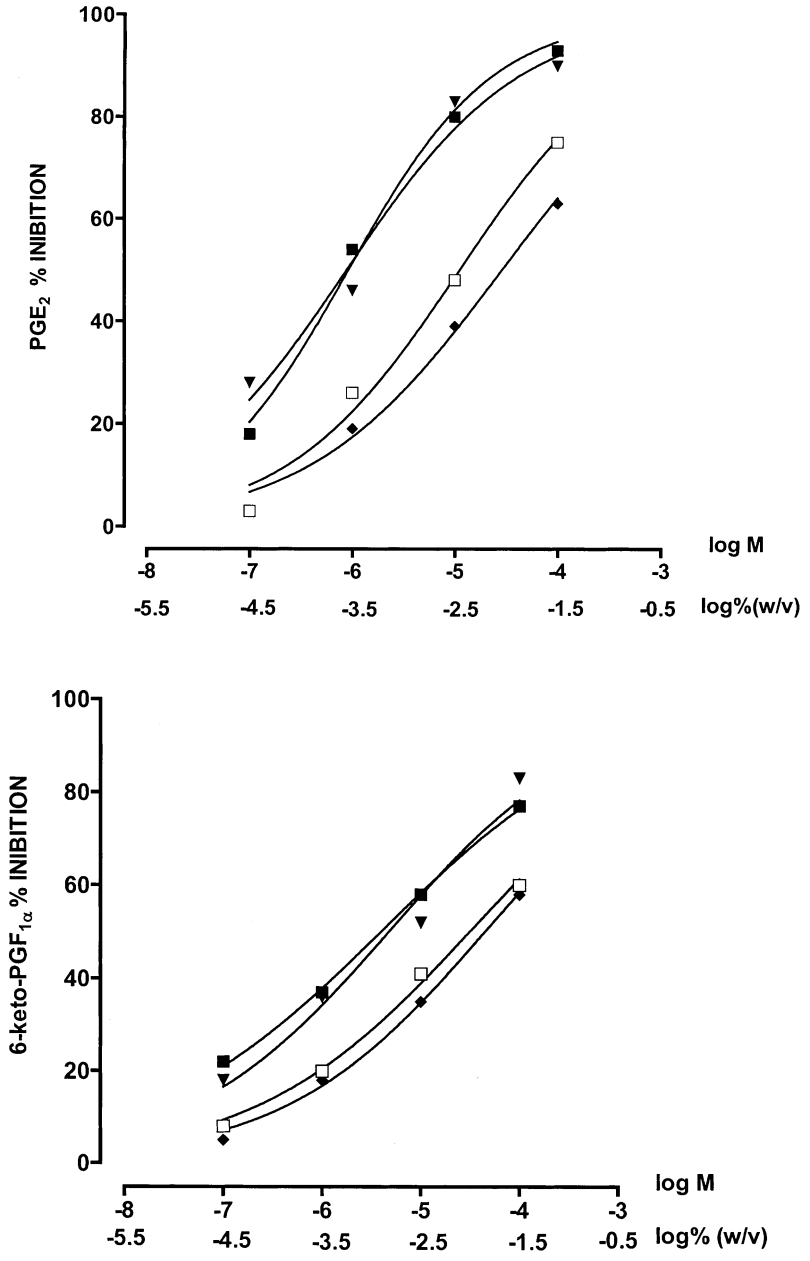

Fig. 2. Inhibitory effect of an ethanolic extract of propolis with (■) and without (□) CAPE, CAPE (▼) and galangin (♦) on PGE_2 and 6-keto-$PGF_{1\alpha}$ production by lung homogenates, from LPS-treated rats, exposed to arachidonic acid for 30 min. Lung homogenates were incubated for 5 min with test compounds before the addition of arachidonic acid. The concentrations of propolis extracts are expressed as log% (w/v), whereas the concentrations of CAPE and galangin are expressed as log M. Each point represents the mean of four experiments performed in triplicate.

PGE_2 and 6-keto-$PGF_{1\alpha}$ production but only from the concentration of 0.0003% (Fig. 2). The IC_{50} values were 0.0035% (0.0013–0.0099%) for PGE_2 and 0.0099% (0.0053–0.0185%) for 6-keto-$PGF_{1\alpha}$.

The comparison of the inhibition curves showed a significant difference ($P < 0.001$) between propolis containing CAPE and propolis without CAPE. the inhibition curves of propolis and CAPE were almost superimposable, on the contrary the inhibition curves of propolis and galangin were significantly ($P < 0.001$) different, whereas the inhibition curve of propolis without CAPE was not statistically different from the inhibition curve of galangin.

4. Discussion

The anti-inflammatory activity of propolis is well known although the exact mechanism of action and the compounds mainly responsible of this action are still under debate. Research has been mainly focused on AA metabolism, since PG play a central role in the inflammatory process [9]. Recently it has been reported that CAPE, the caffeic acid phenethyl ester, which is present in the ethanolic extract of propolis, inhibits AA metabolism in mouse peritoneal macrophages stimulated with LPS or A23187 ionophore [4]. Moreover, CAPE seems to inhibit the activation by tumour necrosis factor and other anti-inflammatory agents, such as phorbol ester, ceramide, hydrogen peroxide and okadaic acid, of the transcription factor NF-kB [10] which regulates the expression of many genes involved in inflammation [11].

In this study we have studied the effect of an ethanolic extract of propolis, with and without CAPE, and some of its components on COX activity from lung homogenate of saline- and LPS-treated rats, measured as PG production from exogenously added AA. Our results show that propolis concentration-dependently inhibits COX activity from lung homogenate of saline- or LPS-treated rats. Among the compounds tested only CAPE and galangin were active. However, the comparison of the inhibition curves and the analysis of the IC_{50} indicate that, in our experimental model, CAPE is more potent than galangin. The central role of CAPE is further supported by the evaluation of the effect of the extract of propolis without CAPE. In fact this extract was a less potent (approx. 10 times) inhibitor of COX activity when compared to the extract containing CAPE. The inhibitory activity of the extract without CAPE seems to be due to the presence of galangin.

These results suggest that although both CAPE and galangin contribute to the overall inhibitory activity of propolis, CAPE gives a greater contribution.

In our experimental model we could not detect, any selectivity of test compounds towards the two isoforms of COX. Further studies using a more selective experimental model are needed in order to better clarify this aspect.

References

[1] E.L. Ghisalberti, Bee World 60 (1997) 59.
[2] J.W. Dobrovolski, S.B. Vohora, K. Sharma, S.A. Shah, S.A.H. Nagvi, P.C. Dandiya, J Ethnopharmacol 35 (1991) 77.

[3] B. Vennat, A. ArvouetGrand, D. Gross, A. Pourrat, J Pharm Belg 50 (1995) 438.
[4] O.K. Mirzoeva, P.C. Calder, Prostaglandins, Leukotrienes and Essential Fatty Acids 55 (1996) 441.
[5] W.L. Smith, D.L. Dewitt, Semin Nephrol 15 (1995) 179.
[6] L. Ermert, M. Ermert, M. Merkle, et al., Am J Pathol 156 (2000) 1275.
[7] M.M. Bradford, Anal Biochem 72 (1976) 248.
[8] L. Sautebin, A. Ianaro, L. Rombolà, A. Ialenti, A. Sala, M. Di Rosa, Inflamm Res 48 (1999) 503.
[9] J.R. Vane, Nature New Biol 231 (1971) 232.
[10] K. Natarajan, S. Singh, T.R. Burke Jr., D. Grunberger, B.B. Aggarwal, Proc Nat Acad Sci 93 (1996) 9090.
[11] F.G. Wulczyn, D. Krappmann, C. Scheidereit, J Mol Med 74 (1996) 749.