Anti-inflammatory Effect of Propolis through Inhibition of Nitric Oxide Production on Carrageenin-Induced Mouse Paw Edema

Koichi Tan-no,* Takeharu Nakajima, a Takehiro Shojo, a Osamu Nakagawa, a Fukuie Niijima, a Masaaki Ishikawa, b Yasuo Endo, c Takumi Sato, d Susumu Satoh, d and Takeshi Tadano*  

a Department of Pharmacology, Tohoku Pharmaceutical University; b Department of Pharmacology and Toxicology, Tohoku Pharmaceutical University; c 4–4–1 Komatsushima, Aoba-ku, Sendai 981–8558, Japan; d Department of Molecular Regulation, Graduate School of Dentistry, Tohoku University; Sendai 980–8575, Japan; and e Department of Pharmacology and Pharmacotherapy, Nihon Pharmaceutical University; Kitaadachi-gun 362–0806, Japan. Received August 17, 2005; accepted October 25, 2005; published online October 27, 2005

The anti-inflammatory effect of propolis was compared with that of diclofenac, a non-steroidal anti-inflammatory drug, and 1-\(N^\text{G}\)-nitro arginine methyl ester (\(\text{L-NAME}\)), a nitric oxide synthase inhibitor, using carrageenin-induced mouse paw edema. When administered 10 min prior to carrageenin injection, propolis (1:1000, 1:100, p.o.) diclofenac (12.5, 50 mg/kg, p.o.) and \(\text{L-NAME}\) (10, 100 mg/kg, s.c.) showed a significant anti-inflammatory effect. The anti-inflammatory effects of propolis and \(\text{L-NAME}\) were significantly inhibited by \(\text{L-arginine}\) but not by \(\text{d-arginine}\). In contrast, the anti-inflammatory effect produced by diclofenac was not inhibited by either \(\text{n-arginine}\) or \(\text{l-arginine}\). These results indicate that the anti-inflammatory effect of propolis on mouse paw edema acts via the inhibition of nitric oxide production, similar to that of \(\text{L-NAME}\) but not diclofenac.

Key words propolis; carrageenin; paw edema; \(\text{L-NG}\)-nitro arginine methyl ester; diclofenac; mouse

Propolis, a natural product derived from plant resins and collected by honey bees, has been used in folk medicine throughout the world; it possesses several biological properties such as anti-inflammatory, anticancer, antioxidant, antibacterial and antifungal activities (for review, see refs. 1, 2). Regarding the anti-inflammatory effect of propolis, Borrelli et al. 3) reported that caffeic acid phenethyl ester (CAPE) but not galangin, primary components of propolis, inhibits carrageenin hind paw edema, carrageenin pleurisy and adjuvant arthritis in rats, suggesting that the anti-inflammatory activity of propolis is due to CAPE. Moreover, in vitro experiments, CAPE inhibits the production of nitric oxide (NO) 4) and the activation of nuclear transcription factor NF-\(\kappa\)B, 5) which are associated with inflammation. Therefore, these reports lead us to propose that the anti-inflammatory effect of propolis is due to the CAPE-induced inhibition of NO production and NF-\(\kappa\)B activation. However, the anti-inflammatory effect of propolis in vivo is not fully understood.

In the present study, we examined the anti-inflammatory effect of an ethanol extract of Brazilian propolis and its mechanism from the viewpoint of the NO system using carrageenin-induced mouse paw edema.

MATERIALS AND METHODS

Animals Male ddY-strain mice (Japan SLC, Hama-matsu, Japan), weighing 23–28 g were used in all experiments. Animals were given standard food (Clea, Osaka, Japan) and tap water ad libitum in an air-conditioned room at 23±1 °C and 55±5% relative humidity with a standard 12 h light–dark cycle (lights on 8 a.m. to 8 p.m.).

Drugs and Chemicals The following drugs and chemicals were purchased and used: \(\lambda\)-carrageenin, \(\text{L-NG}\)-nitro arginine methyl ester hydrochloride (\(\text{L-NAME}\)), \(\text{l-arginine}\), \(\text{d-arginine}\) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and diclofenac sodium (Sigma Chemical Co., St. Louis, MO, U.S.A.). The ethanol extract of Brazilian propolis (CB Propolis®) and its vehicle (containing 45% ethanol) were provided from ChatBlanc Inc. (Tokyo, Japan) and diluted in distilled water. The maximum concentration of ethanol injected into animal was 0.45%. Diclofenac was dissolved in distilled water, and \(\text{L-NAME}\), \(\text{l-arginine}\) and \(\text{d-arginine}\) were dissolved in physiological saline.

Carrageenin-Induced Mouse Paw Edema Edema was induced on the right hind paw by intraplantar injection of carrageenin (2 w/v % in physiological saline, 30 \(\mu\)l). Paw volume was measured before and after carrageenin injection up to 6 h, using a plethysmometer MK-550 (Muromachi Kikai Co., Tokyo, Japan). For each animal, the percent of edema, which is expressed as the percentage of the increase in paw volume before carrageenin injection, and the area under the curve (AUC) was calculated to evaluate the anti-inflammatory effect.

Analysis of Data Data are presented as the means and S.E.M. Significant differences between groups were determined by Fisher’s PLSD post hoc test for multiple comparisons after analysis of variance (ANOVA). In all statistical comparisons, \(p<0.05\) was used as the criterion for statistical significance.

RESULTS

Anti-inflammatory Effects of Propolis, Diclofenac and \(\text{L-NAME}\) on Mouse Paw Edema Caused by Carrageenin Intraplantar injection of 30 \(\mu\)l of 2% carrageenin caused edema after 30 min in the control mice treated orally with vehicle (containing 0.45% ethanol) 10 min before the injection of carrageenin. The paw edema increased along with the time course and was observed at 6 h after the injection (Fig. 1a). Whereas propolis, at a dilution of 1:100 and 1:1000, significantly inhibited the paw edema at 2–4 h and at 2 h after carrageenin injection, respectively. When the anti-inflammatory effect of propolis was evaluated as AUC during the period from 30 min to 6 h after carrageenin injection, propolis...
(1:1000, 1:100) showed a significant anti-inflammatory effect (Fig. 1b). Propolis (1:1000, 1:100) administered 3 h after carrageenin injection also showed a significant anti-inflammatory effect during the period from 4 to 6 h after carrageenin injection, as evaluated by AUC (data not shown).

The anti-inflammatory effects of diclofenac, a non-steroidal anti-inflammatory drug, and l-NAME, a nitric oxide synthase (NOS) inhibitor, on carrageenin-induced mouse paw edema were examined in order to compare with the anti-inflammatory effects of propolis. The time course of paw edema is shown in Figs. 2a and 3a when diclofenac (3.1—50 mg/kg, p.o.) or L-NAME (1—100 mg/kg, s.c.) was administered 10 min prior to carrageenin injection. In the evaluation of AUC during the period from 30 min to 6 h after carrageenin injection, diclofenac (12.5, 50 mg/kg) and L-NAME (10, 100 mg/kg) showed a significant anti-inflammatory effect (Figs. 2b, 3b).

Effects of l-Arginine and D-Arginine on the Anti-inflammatory Actions of Propolis, Diclofenac and L-NAME

In order to determine the participation of NO system in the anti-inflammatory actions of propolis, diclofenac and l-NAME, the effects of l-arginine and d-arginine on the anti-inflammatory actions of these compounds were examined. l-Arginine or d-arginine was intraperitoneally administered 2 h before the peak time of the anti-inflammatory effects. Namely, each arginine was administered to propolis, diclofenac and L-NAME mice at 1 h, 3 h and 3 h after carrageenin injection; the AUC during the period from 1 h after arginine injection to 6 h after carrageenin injection was calculated and evaluated. The anti-inflammatory effects of propolis (1:100) and l-NAME (100 mg/kg) were significantly inhibited by l-arginine (300 mg/kg), but not by d-arginine (300 mg/kg) (Figs. 4, 5). Whereas, the anti-inflammatory effect produced by diclofenac (50 mg/kg) was not inhibited by either d-arginine or l-arginine (Fig. 6). As shown in Figs. 4—6, a single administration of both arginines (300 mg/kg) did not significantly affect the paw edema.

DISCUSSION

The intraplantar injection of carrageenin in rats leads to paw edema, the first phase of which results from the concomitant release of histamine, serotonin and kinins and second phase is correlated with the elevated production of prostaglandins, oxygen-derived free radicals, and the production of inducible cyclooxygenase (COX-2) and the local neutrophil infiltration and activation.8—10 Although less explored than rat paw edema, mouse paw edema elicited by car-
Rageenin has been shown to be a useful model for the study of inflammation. Essentially the same mediators are involved as in the rat model and the models yield similar profiles for various anti-inflammatory drugs. In the present study, propolis (1:1000, 1:100) administered 10 min prior to carrageenin injection showed a significant anti-inflammatory effect, with nearly the same efficacy as diclofenac and L-NAME though the duration was shorter than that of diclofenac and L-NAME. Borrelli et al. reported that ethanol extract of propolis containing CAPE at doses of 300 and 600 mg/kg but not 100 mg/kg significantly inhibits carrageenin-induced rat paw edema. Because lyophilized ethanol extract of propolis used in the present study is equivalent to 18.5 mg propolis per 100 μl, 1:100 dilutions of propolis correspond to 1.85 and 18.5 mg/kg, respectively. Therefore, propolis used in the present study was 5.4—54 times more potent than that reported by Borrelli et al. though there was a difference between mouse and rat.

NO is produced from L-arginine by NOS, for which three isoforms have been reported. Two of these syntheses, namely endothelial and neuronal NOS (e-NOS and n-NOS, respectively), are calcium-dependent and constitutively expressed.
enzymes. The third type, inducible NOS (i-NOS), is calcium-independent and can be induced in several cell types, including macrophages and vascular smooth muscle cells, following activation by bacterial endotoxin and/or inflammatory cytokines (for review, see ref. 17). It is also known that inhibition of i-NOS activity18 and expression19 produces an anti-inflammatory effect on rat paw edema. Moreover, we have previously reported that i.p.20 or intraplantar21 injection of l-NAME inhibits the nociceptive behavior during only the second phase in the mouse formalin test, which is induced due to the formation of edema via the release of inflammatory mediators. These reports provide that NO produced by i-NOS is involved in the inflammatory response. In the present study using carrageenin-induced mouse paw edema, l-NAME is involved in the inflammatory mediators. These reports provide that NO produced by i-NOS is involved in the inflammatory response. In the present study using carrageenin-induced mouse paw edema, l-NAME (10, 100 mg/kg) showed a significant anti-inflammatory effect similar to propolis. Moreover, the anti-inflammatory effects of propolis and l-NAME were significantly inhibited by l-arginine, a substrate of NOS, but not by d-arginine; also l-arginine did not inhibit the diclofenac-induced anti-inflammation mediated through cyclooxygenase inhibition. The results are in agreement with a previous report stating that l-arginine completely reverses the inhibition of endotoxin-induced rat paw edema produced by l-NAME.18 On the other hand, it has been indicated that activation of NF-κB is involved in i-NOS expression because there is an NF-κB consensus site in the upstream promoter region of the i-NOS gene.22 Song et al.23,24 reported that the ethanol extract of propolis and CAPE inhibit not only the directly catalytic activity of i-NOS but also the i-NOS gene expression at the transcriptional level through the suppression of NF-κB activation. Though l-arginine almost completely inhibited l-NAME-induced anti-inflammation, it did not completely inhibit that of propolis. The difference in the results may be explained by the fact that propolis inhibits both i-NOS expression and the catalytic activity of i-NOS whereas l-NAME inhibits only the direct catalytic activity of i-NOS. Regardless of the detailed mechanism, it is true that propolis produces an anti-inflammatory effect through the inhibition of NO production.

In conclusion, propolis produces an anti-inflammatory effect on carrageenin-induced mouse paw edema. The mechanism underlying propolis-induced anti-inflammation may be mediated through the inhibition of NO production.

Acknowledgements The authors express their sincere thanks to Y. Ohtomo and J. Morimura for help in performing the blind experiments. We also thank ChatBlanc Inc. for providing the ethanol extract of Brazilian propolis (CB Propolis®).

REFERENCES