Inhibition of Lipoxygenase by Phenolic Compounds

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ABSTRACT — Eugenol dose-dependently inhibited 5-HETE (5-hydroxy-5,8,10,14-eicosatetraenoic acid) and 15-HETE formation by human polymorphonuclear leucocytes. p-Chlorophenol, guaiacol and phenol also inhibited the lipoxygenases. Formation of HETEs by rat dental pulp was inhibited by eugenol and p-chlorophenol. The concentrations of the phenolics required to inhibit lipoxygenases were in the similar range with those used for inhibiting cyclooxygenase. These results showed that phenolic compounds inhibited lipoxygenases and thus suggest that these compounds may be dual inhibitors of lipoxygenase and cyclooxygenase.

Phenolic dental medicaments such as phenol, guaiacol and eugenol have been used for relief of pain and to suppress inflammation in pulpitis, post-pulpal extraction alveolitis, dentine hyperalgesia and other conditions. However, relatively little attention has been paid to the mode of pharmacological action of phenolic dental medicaments, and their therapeutic effects are believed to be due to bactericidal and non-specific counter-irritant actions.

It has been reported that phenolic compounds inhibited the cyclooxygenase activity of various tissues including dental pulp (1-4). Some of the phenolic compounds have a potent anti-inflammatory action (5). The evidence may suggest that the phenolic dental medicaments produce the anti-inflammatory effects by inhibiting the production of prostaglandins (PGs).

Arachidonic acid metabolites via the lipoxygenase pathway appear to participate in the generation of inflammatory reactions. Lessard et al. showed an increase in LTC₄ level by inflammatory treatments in canine tooth pulp (6). Okiji et al. demonstrated that arachidonic acid was converted to 12-HETE (12-hydroxy-5,8,10,14-eicosatetraenoic acid) by incubation with rat dental pulp homogenate and the conversion increased in the inflamed pulp (7). The evidence suggests that the lipoxygenase system is present in dental pulp tissue and may be involved in the development of pulp inflammation. Thus, there is a possibility that phenolic dental medicaments produce anti-inflammatory effects by influencing the lipoxygenase pathway. The present study examined whether phenolic compounds affect lipoxygenase activities using human polymorphonuclear leucocytes (PMN) and rat dental pulp.

The pulp tissue was isolated from the incisors of male Wistar rats and rinsed in ice-cold phosphate-buffered saline (pH 7.4). The pulp was sonicated at 20 kHz for 15 sec in 2 vol. of 0.1 M potassium phosphate buffer (pH 7.4) and used as the enzyme preparation as previously reported (7). PMN were isolated from peripheral blood collected from healthy volunteers as described previously (8). PMN suspensions was sonicated, centrifuged at 105,000 x g for 60 min, and the resultant su-
pernatant was used as the enzyme preparation.

Lipoxygenase activity was assayed as described previously (8). Proteins of enzyme samples (3–4 mg protein of dental pulp or 0.1–0.3 mg protein of PMN) were preincubated for 2 min in the presence of 10 μM indomethacin with or without phenolic compounds in the total volume of 1 ml (pH 7.4) and then incubated for an additional 2 min with [1-14C]-arachidonic acid (11 kBq, 30 μM) at 37°C. Phenolic compounds were dissolved in ethanol and added in a final concentration of less than 0.3%. The reaction was stopped by adding ice-cold diethyl ether. The extraction with ether was repeated twice, and the organic phase was collected. The extracts were chromatographed on silica gel plates in a solvent system of ethyl acetate – iso-octane – acetic acid – water, 11:5:2:10, V/V. The radioactivity of the reaction products was localized by autoradiography. The silica gel zones corresponding to authentic 5-HETE, 12-HETE and 15-HETE were scraped off, and the radioactivity was determined in a liquid scintillation spectrometer.

The reaction products of PMN homogenate incubated with [14C]-arachidonic acid appeared on TLC with Rf values corresponding to that of authentic 5-HETE and 15-HETE in agreement with the presence of both 5-lipoxygenase (predominant activity) and 15-lipoxygenase in the cytosol of human PMN (9). On the other hand, the most abundant product formed by rat dental pulp was 12-HETE (or 15-HETE). 12-HETE and 15-HETE were not distinguishable on TLC. Okiji et al. further analyzed the lipoxygenase products of rat dental pulp by reverse-phase HPLC and showed that 12-HETE is a major product with little formation of 15-HETE (7). The lipoxygenase activity of rat dental pulp calculated from 12-(15-)HETE (expressed as HETEs) formation was 7.1 pmoles/mg protein/min, which was much lower than 5-lipoxygenase activity of PMN (340 pmoles/mg protein/min).

Eugenol dose-dependently inhibited the formation of 5-HETE and 15-HETE in PMN (Fig. 1 and Table 1A). Other phenolic compounds such as p-chlorophenol, guaiacol and phenol also inhibited the lipoxygenases of PMN (Table 1A). Eugenol was found to be the most potent inhibitor among the tested compounds. Eugenol and p-chlorophenol inhibited the formation of HETEs in rat dental pulp (Table 1B). The inhibitory effects of eugenol were less potent and those of p-chlorophenol were more potent than those in PMN. The results demonstrated that these phenolic compounds...
compounds are lipoxygenase inhibitors.

We have previously shown that phenolic compounds stimulated PG synthesis by bovine dental pulp microsomes and that by rabbit kidney medullary microsomes at concentrations of 0.1–1.0 mM and inhibited the synthesis at higher concentrations (4). No stimulatory effect of phenols on lipoxygenase was observed. The concentrations of phenolic compounds required to inhibit lipoxygenases are in the similar range or less than the range for inhibition of cyclooxygenase. For example, the IC₅₀ values of eugenol and p-chlorophenol for inhibiting cyclooxygenase in microsomes of rabbit renal medulla were 0.2 and 3.0 mM, respectively (4). The order of potency of phenolic compounds for inhibiting lipoxygenase is roughly in agreement with that for cyclooxygenase inhibition. These results show that the phenolic compounds, especially eugenol, are potent dual inhibitors of cyclooxygenase and lipoxygenase.

Phenol and its derivative compounds are primarily protein denaturants. They damage the cell membrane and are thus bactericidal. However, the concentration of these compounds required to produce such nonspecific effects were much higher than those for inhibiting PG biosynthesis, and thus it has been suggested that the mechanism for cyclooxygenase inhibition differs from the antibacterial mechanism (4). Lands suggested that a small amount of hydroperoxide is required to initiate and maintain the free-radical cyclooxygenase reaction, although large amounts of the peroxide inactivate the enzyme (10). Therefore, the quantity of hydroperoxides present within the cells determines whether cyclooxygenase is activated or inactivated. Radical trapping antioxidant agents such as phenol could decrease the radical intermediates to lower levels which do not permit the reaction to continue. It is proposed that the lipoxygenase reactions requires hydroperoxide activation to initiate and continue the reaction, as in the case of the free radical chain reaction in the cyclooxygenase system (11). Thus the termination of the chain reaction with phenolic antioxidants could inhibit both cyclooxygenase and lipoxygenase. It has been also shown that the peroxide level modifies the anticyclooxygenase potency of such compounds of which the mechanism of inhibition is due to antioxidative properties. For example, the inhibitory effects of the phenolic antioxidative type of inhibitors such as phenylbutazone and MK 447, but not other types of inhibitors such as ibuprofen and indomethacin, increased when peroxides were decreased by glutathione peroxidase (10). Thus the clinical effectiveness of such inhibitors in vivo would be influenced by the pathophysiological state. It has been proposed that the general hyperalgesic conditions in which the cellular peroxide may not be greatly elevated would be responsive to the treatment with such agents as analgesics (10). The peroxide level contained in the assay system could also affect the inhibitory potency of these agents in vitro. The enzyme preparation from PMN, due to its cellular function, may contain a higher level of peroxides than the preparation from dental pulp, and this might explain why the inhibitory effect of p-chlorophenol on the lipoxygenase of PMN was less potent than the effect on pulp lipoxygenase. However, the inhibitory effect of eugenol on the pulp lipoxygenase was less potent than on PMN. If the mechanisms for lipoxygenase inhibition by eugenol involve the direct interaction of eugenol with the enzyme protein in addition to its antioxidative ability, the anti-lipoxygenase action would be hindered if large amounts of non-enzyme proteins contaminated the pulp enzyme preparation. However, the exact mechanism of action of eugenol is not known at present.

Phenolic antioxidants such as nordihydroguaiaretic acid (NDGA) and 3-amino-1-[m-trifluoromethylphenyl]-2-pyrazoline (BW-755C) and naturally occurring phenolics, caffic acid and curcuminoids, are inhibitors of 5-lipoxygenase (12). Baicalein and esculetin are inhibitors of 12-lipoxygenase (13). Some of the phenolics have the ability to inhibit cyclooxygenase. Corticosteroids, by inhibiting arachidonic acid mobilization from phospholipids, block the formation of the arachidonic acid
derived mediators of inflammation through both the cyclooxygenase and lipoxygenase pathways and thus are potent anti-inflammatory agents. Drugs that simultaneously inhibit cyclooxygenase and lipoxygenase would be expected to possess similar anti-inflammatory capacities. On this basis, dual inhibitors of both pathways are currently undergoing development. It is noteworthy that phenolic compounds are dual inhibitors of cyclooxygenase and lipoxygenase. Topically applied phenolic compounds such as eugenol and guaiacol actually inhibited the formation of edema induced by croton oil in the mouse ear edema assay (14). Lipoxygenase pathways, in addition to cyclooxygenase pathways, may be involved in the pathogenesis of pulpal and periapical diseases. Phenolic dental preparations are applied locally as pulpal, root canal, post-pulpal extraction and periapical antiseptic dressings. These phenolic preparations contain high concentrations (2–5 M) of phenolic compounds which can penetrate into tissues. Phenol irreversibly blocks the nerve conduction in desheathed cutaneous nerve with high concentrations of 50–100 mM (15). Thus the phenolic compounds may produce diverse effects depending on their concentration in the tissue such as non-specific denaturation of tissue and specific inhibition of prostaglandin and leukotriene biosynthesis, resulting in the analgesic and anti-inflammatory effects of these compounds in endodontic therapy.

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REFERENCES

1 Lindgren, J. A., Claesson, H.-E. and Hammerström, S.: Inhibition of prostaglandin synthesis in mouse 3T3 fibroblasts and human platelets by substituted phenol. Prostaglandins 13, 1093–1102 (1977)
2 Dewhirst, F. E.: Structure-activity relationships for inhibition of prostaglandin cyclooxygenase by phenolic compounds. Prostaglandins 20, 209–222 (1980)
3 Hirafuji, M.: Inhibition of prostaglandin I2 biosynthesis in rat dental pulp by phenolic dental medicaments. Japan. J. Pharmac. 36, 544–546 (1984)
4 Anamura, S., Dohi, T., Shirakawa, M., Okamoto, H. and Tsujimoto, A.: Effects of phenolic dental medicaments on prostaglandin synthesis by microsomes of bovine tooth pulp and rabbit kidney medulla. Arch. Oral Biol. 33, 555–560 (1988)
5 Kuchl, F. A., Jr., Humes, J. L., Egan, R. W., Ham, E. A., Beveridge, G. C. and Van Arman, C. G.: Role of prostaglandin endoperoxide PGH2 in inflammatory processes. Nature 265, 170–173 (1977)
6 Lessard, G. M., Torabinejad, M. and Swope, D.: Arachidonic acid metabolism in canine tooth pulps and the effects of nonsteroidal anti-inflammatory drugs. J. Endodont. 12, 146–149 (1986)
7 Okiji, T., Morita, I., Kobayashi, C., Sunada, I. and Murota, S.: Arachidonic-acid metabolism in normal and experimentally-inflamed rat dental pulp. Arch. Oral Biol. 32, 723–727 (1987)
8 Tamai, K., Dohi, T., Ogawa, T., Okamoto, H. and Tsujimoto, A.: Some properties of gingival 12-lipoxygenase activity in human and dog. Arch. Oral Biol. 35, 575–581 (1990)
9 Soberman, R. J. and Austen, K. F.: Kinetics of the arachidonic acid 5- and 15-lipoxygenases of the human polymorphonuclear leukocyte. Adv. Prostaglandin Thromboxane Leukotriene Res. 15, 169–171 (1985)
10 Lands, W. E. M.: Actions of anti-inflammatory drugs. Trends Pharmacol. Sci. 2, 78–80 (1981)
11 Lands, W. E. M.: Biological consequences of fatty acid oxygenase reaction mechanisms. Prostaglandins Leukotrienes Med. 13, 35–46 (1984)
12 Koshihara, Y., Neichi, T., Murota, S., Lao, A., Fujimoto, Y. and Tatsuno, T.: Selective inhibition of 5-lipoxygenase by natural compounds isolated from Chinese plant, Artemisia rubripes Nakai. FEBS Lett. 158, 41–44 (1983)
13 Sekiya, K. and Okuda, H.: Selective inhibition of platelet lipoxygenase by baicalein. Biochem. Biophys. Res. Commun. 150, 1090–1095 (1982)
14 Dohi, T., Terada, H., Anamura, S., Okamoto, H. and Tsujimoto, A.: The anti-inflammatory effects of phenolic dental medicaments as determined by mouse ear edema assay. Japan. J. Pharmacol. 49, 535–539 (1989)
15 Dodt, H. U., Strichartz, G. R. and Zimmermann, M.: Phenol solutions differentially block conduction in cutaneous nerve fibers of the cat. Neurosci. Lett. 42, 323–327 (1983)