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The effect of an aqueous extract of *Tanacetum parthenium* L. on arachidonic acid metabolism by rat peritoneal leucocytes

FRANCESCO CAPASSO, *Department of Experimental Pharmacology, University of Naples, via L. Rodinò 22, 80138 Naples, Italy*

The effect of feverfew (*Tanacetum parthenium* L., Schultz Bip.) as a whole plant on an aqueous extract equivalent to 20 mg dried plant per ml, has been examined on both cyclo-oxygenase and lipoxygenase activity in rat leucocytes in-vitro. At 10–25 μg ml^{-1} feverfew had no effect on the formation of arachidonate metabolites while at highest concentrations (50–200 μg ml^{-1}) it inhibited both cyclo-oxygenase and lipoxygenase metabolic products.

Feverfew (*Tanacetum parthenium* L., Compositae) is a yellowish-green aromatic perennial with ridged stems, pinnate or bipinnate leaves, and the flower heads as a corymb. It grows abundantly against walls, on waste ground and along hedges. Traditionally the leaves or infusions of the herb have been used as a febrifuge and to relieve menstrual and rheumatic pain and migraine (Berry 1984). A recent clinical report by Johnson et al (1985) demonstrated the efficacy of feverfew as a prophylactic treatment of migraine in several patients. Since these complaints may involve arachidonic acid metabolism we have investigated extract of feverfew on both cyclo-oxygenase and lipoxygenase activity of rat peritoneal leucocytes.

Methods

A whole feverfew plant in flower was cut off at soil level, chopped into short pieces and air dried. Pieces were homogenized in Krebs solution, filtered and a stock solution made equivalent to 20 mg dried plant in 1 ml. The resultant extract was centrifuged at 2500 *g* for 30 min and the clear supernatant tested.

Rat peritoneal leucocytes were obtained from male Wistar rats (200–250 g) by the method of Capasso (1981). Aliquots of the leucocyte suspension (1 ml) were pre-incubated (37 °C, 15 min) alone or containing different concentrations of feverfew extract (10–200 μg ml^{-1}). This was followed by further incubation at 37 °C for 5 min with calcium ionophore A23187(2-[(3β,9α,11β-trimethyl)-8-(2-pyrrolecarboxymethyl)-1,7-dioxaspiro[6,6]undecyl-2β-methyl]-5-methylamino-benzoxazole-4-carboxylic acid) (0·5 μg) and [1-^{14}C]-arachidonic acid (0·1 μCi, 1·7 nM) in a final volume of 1 ml. Enzymic activity was terminated with methanol–formic acid (2 ml: 20 μl), after which the eicosanoids were extracted with diethyl ether (4 ml × 2) and evaporated to dryness. For the lipid extraction of the labelled arachidonic acid products, the samples were chromatographed on silica gel thin layer plates (organic phase of ethyl acetate–hexane–acetic acid–water; 56 : 24 : 12 : 60), and autoradiographs prepared (Kodak, NS -2T; exposure 10 days) according to the method of Capasso et al (1985).

Drugs. [1-^{14}C]Arachidonic acid (Radiochemical Centre, Amersham), indomethacin (Merck Sharp and Dohme), BW755c (Wellcome). Other chemicals were reagent grade. All drugs were made up in Krebs solution.

Statistics. The results are shown as percent control and s.e., analysed statistically by Student's *t*-test for paired data.

Results

Rat peritoneal leucocytes stimulated by the calcium ionophore A23187 metabolized [^{14}C]arachidonic acid via both the cyclo-oxygenase and lipoxygenase pathways. PGE_2, PGF_{2a}, PGD_2, TXB_2, 6-keto PGF_{1a}, LTB_4, and 5-HETE were characterized by comparison with authentic standards.

In control experiments conversion into cyclo-oxygenase and lipoxygenase products was, respectively, 18·9 ± 2·0% and 7·0 ± 0·9% (n = 8). The order of amounts of cyclo-oxygenase products present was PGE_2 > PGF_{2a} > TXB_2 > PGD_2 > 6-keto PGF_{1a}. Their formation was inhibited by feverfew extract (50–200 μg ml^{-1} 25–57%, $P < 0{\cdot}05$–$0{\cdot}005$) in a concentration-related manner. Lower concentrations (10–25 μg ml^{-1}) had no significant effect. Indomethacin (1 μg ml^{-1}, 59%, $P < 0{\cdot}01$–$0{\cdot}001$) or BW755c (1 μg ml^{-1}, 60%, $P < 0{\cdot}01$–$0{\cdot}005$ strongly inhibited the formation of the cyclo-oxygenase products. The predominant lipoxygenase products were 5-HETE > LTB_4. Their formation was inhibited by the feverfew extract in a concentration-dependent manner (50–200 μg ml^{-1}, 28–62%, $P < 0{\cdot}05$–$0{\cdot}1$). Both of these compounds were inhibited by BW755c (1 μg ml^{-1}, 64%, $P < 0{\cdot}005$–$0{\cdot}001$), but not by indomethacin (1 μg ml^{-1}), confirming that they were not cyclo-oxygenase products.

Discussion

Rat peritoneal leucocytes stimulated with calcium ionophore A23187 converted [^{14}C]arachidonic acid to PGE_2, PGF_{2a}, PGD_2, TXB_2, LTB_4 and 5-HETE, and into some other products that were not characterized. The formation of these products was inhibited by an

Table 1. Effect of feverfew extract on [^{14}C]arachidonic acid metabolism by rat peritoneal leucocytes.

Drugs μg ml^{-1}	n-	PGE_2	$PGF_{2\alpha}$	TXB_2	PGD_2	6-keto	LTB_4	5-HETE
Feverfew extract								
10	4	100·3	105·0	99·9	103·2	100·0	98·7	101·4
25	5	90·0	91·3	91·4	98·0	93·1	91·1	88·4
50	8	70·4a	78·4	70·1a	80·3	73·4	70·7a	73·4a
100	9	57·1b	67·3a	60·3b	70·1a	67·3b	58·3b	61·7b
200	6	34·3d	43·9c	40·7c	50·3b	47·8b	36·5c	39·7b
Indomethacin								
1	5	33·9c	49·1c	38·7d	44·9c	40·3c	200·70	297·1
BW755c								
1	5	37·0d	39·0d	40·3d	40·7d	44·8c	31·8e	39·0d

Results are expressed as % control and analysed by Student's *t*-test.
a $P < 0{\cdot}05$, b $P < 0{\cdot}02$, c $P < 0{\cdot}01$, d $P < 0{\cdot}005$, e $P < 0{\cdot}001$.

aqueous (Krebs solution) extract of feverfew (concentrations equivalent to 50–200 μg dried plant). In most respects the profiles of activity of BW755c, a dual cyclo-oxygenase/lipoxygenase inhibitor, and feverfew were similar while indomethacin differed from feverfew extract in that it did not affect the formation of LTB_4 and 5-HETE by rat peritoneal leucocytes. Indomethacin markedly increased the formation of the lipoxygenase products, possibly by stimulating lipoxygenase activity (Siegel et al 1981) and/or by removing the inhibitory effect of prostaglandins formed via cyclo-oxygenase (Malmsten 1984).

Feverfew extract inhibits PG synthesis (Collier et al 1980) and platelet aggregation (Makheja & Bailey 1982) by a mechanism involving cellular phospholipase inhibition. Subsequently Heptinstall et al (1985) found that feverfew extracts could inhibit the secretion of granules by platelets and polymorphonuclear leucocytes but that a mechanism other than phospholipase inactivation was involved. The present data show that extract of feverfew contains a material that is water-soluble and that inhibits arachidonate products of the cyclo-oxygenase and lipoxygenase metabolic pathways, in contrast with non-steroidal anti-inflammatory drugs such as indomethacin which inhibit only the cyclo-oxygenase pathway.

In acute inflammation, prostanoids interact with substances such as histamine and bradykinin to augment pain (Ferreira et al 1978) and vascular permeability (Williams et al 1983). In recent years it has also become clear that other metabolites of prostaglandin precursors produced by the action of lipoxygenases, such as the leukotrienes and non peptide-fatty acids, may be important in inflammation (Samuelsson 1983). Therefore the effects of feverfew on arachidonate metabolism may account for its effectiveness as a herbal remedy in arthritis, pain and migraine (Johnson et al 1985). Numerous compounds including sesquiterpene lactones (parthenolide, santamarine, canin, artecanin), pinene derivatives, germacranolides, guaianolides and spiroketal enol ether polyines have been demonstrated in extracts of feverfew (Romo et al 1970; Evans & Schmidt 1980; Stefanovic et al 1980; Bohlmann & Zdero 1982; Berry 1984) but it is not known to what extent these contribute to the inhibited eicosanoid synthesis.

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