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A Comparison of the Effects of an Extract of Feverfew and Parthenolide, a Component of Feverfew, on Human Platelet Activity In-vitro

W. A. GROENEWEGEN* AND S. HEPTINSTALL

Department of Medicine, University Hospital, Nottingham NG7 2UH, UK

Abstract—Extracts of the herb feverfew inhibit human blood platelet aggregation and secretion induced by a number of agents in-vitro and this may relate to the beneficial effects of feverfew in migraine. We previously identified several compounds with antisecretory activity in human blood platelets using adrenaline as the stimulant. In the present study, we have compared the inhibitory activity of one of these compounds, parthenolide, with that of crude feverfew extract. The effects of both on [^{14}C]5-HT secretion from platelets and on platelet aggregation induced by a number of different stimulants were determined. The activating agents studied included the phorbol ester PMA, ADP, arachidonic acid, collagen, the thromboxane mimetic U46619, the calcium ionophore A23187, the diacylglycerol analogue OAG and adrenaline. The results show that there are marked similarities between the effects of feverfew extract and of parthenolide on both [^{14}C]5-HT secretion and platelet aggregation, which is consistent with the effects of feverfew extract on platelets being brought about by parthenolide or similar compounds in the extract. Only in one case, when A23187 was used as the stimulatory agent, was there any discrepancy, which may have been due to materials in the extract other than parthenolide. Both feverfew extract and parthenolide were more effective as inhibitors of the [^{14}C]5-HT secretion and aggregation induced by some agents and not others, and were most effective as inhibitors of the [^{14}C]5-HT secretion (but not the aggregation) induced by PMA. This suggests that the effects of feverfew/parthenolide on the protein kinase C pathway warrants further study.

In the last decade there has been an increased interest in the ancient herb feverfew (*Tanacetum parthenium*) for use in conditions such as migraine and arthritis (Editorial Lancet 1985). Some evidence is now available about possible benefits of leaves taken orally to prevent migraine. In two studies, patients taking the herb were found to have reduced frequency and severity of migraine attacks (Johnson et al 1985; Murphy et al 1988). In addition, extracts of feverfew leaves inhibit secretion of intracellular storage granule contents in both platelets and neutrophils induced by a variety of stimulating agents in-vitro (Heptinstall et al 1985, 1987). Similarly, Hayes & Foreman (1987) found that feverfew extract inhibited histamine release from mast cell.

In a previous communication (Groenewegen et al 1986) we reported the identification of the components of feverfew extract responsible for the inhibition of [^{14}C]5-hydroxytryptamine ([^{14}C]5-HT) secretion from human blood platelets stimulated with adrenaline. These compounds are sesquiterpene lactones with an α-methylenebutyrolactone unit as a common part of their structure. Here we report a further study with one of these compounds, parthenolide. We carried out direct comparisons between the effects of crude feverfew extract and parthenolide on inhibition of [^{14}C]5-HT secretion and platelet aggregation induced by a range of platelet stimulating agents.

Materials and Methods

Materials

Feverfew leaves for extraction were obtained from feverfew plants (*Tanacetum parthenium* Schultz Bip.) grown in the Department of Botany, University of Nottingham. Parthenolide was a gift from Dr P. Hylands, King's College, London. Blood was obtained from healthy volunteers who denied taking aspirin during 14 days before blood sampling. [^{14}C]5-HT (spec. act. 57 mCi mmol^{-1}; 50 μCi mL^{-1}) was from Amersham International. Phosphate-buffered saline (PBS), pH 7·4 was prepared using buffer tablets from Oxoid Ltd. Acetylsalicylic acid, adrenaline, PMA, adenosine diphosphate (ADP), sodium arachidonate (AA) and A23187 were all from Sigma Chemical Co. Acetylsalicylic acid, adrenaline and ADP were each dissolved in 150 mM NaCl. PMA was stored as a stock solution in DMSO (500 μg mL^{-1}) and samples were diluted in saline immediately before use, to give a working solution of 12·5 μg mL^{-1}. AA was stored as a stock solution (30 mM) in n-hexane. The working solution (25 mM) was prepared by evaporating the hexane of a sample of the stock solution and redissolving the residue in aqueous Na_2CO_3 (0·1 M). A23187 was stored as a stock solution (10 mM) in ethanol. The working solution (1·25 mM) was obtained by diluting a sample of the stock solution in distilled water. Collagen was obtained from Hormon-Chemie, Munich and was used as directed. U46619 ((15S)-hydroxy-11α,9α-(epoxymethano) prosta-5Z,13E-dienoic acid) was from the Upjohn Company and samples from the stock solution (10 mM) in ethanol were diluted in saline. 1-Oleoyl 2-acetyl-sn-glycerol (OAG) was a gift from Dr H. Hodson, Wellcome Labs, Beckenham, Kent. To obtain a working solution (7·5 mg mL^{-1}), OAG (7·5 mg) was dissolved in DMSO (15 μL) and diluted in saline (985 μL). The solution was sonicated immediately before use.

Methods

Extraction of feverfew. Leaves were air-dried and powdered

Correspondence and present address*: W. A. Groenewegen, Smith Kline Beecham, The Frythe, Welwyn, Herts AL6 9AR, UK.

using a pestle and mortar. The powdered leaves were stirred vigorously with chloroform (feverfew, 50 mg mL^{-1} chloroform) for 30 min. The powder was removed by filtration and the chloroform evaporated. The remaining residue was resuspended in an equal volume of PBS such that the final solution was derived from feverfew 50 mg mL^{-1} PBS. Any insoluble material was removed by filtration.

Preparation of a stock solution of parthenolide. To ensure complete dissolution of parthenolide in an aqueous medium, it was necessary to use a vehicle solvent. To parthenolide crystals (2·5 mg), ethanol (200 μL) was added and the crystals were allowed to dissolve. The ethanol was then diluted out by dropwise addition of PBS such that the stock ethanol concentration was 2% and the concentration of parthenolide 1 mM.

[^{14}C]5-HT secretion from platelets. These measurements were performed in samples of platelet-rich plasma (PRP). A sample of [^{14}C]5-HT (6 μL/10 mL blood) was added to citrated human blood to label the intracellular storage granules of the platelets (Heptinstall & Fox 1983). The blood was centrifuged (160 *g*, 10 min) to obtain PRP which was diluted to 300×10^9 platelets L^{-1} with platelet-poor plasma, obtained by centrifugation (200 *g*, 10 min) of the blood after removal of the PRP. PRP (460 μL amounts) was stirred (1000 rev min^{-1}) at 37°C for 2 min in the presence of 100 μL of either feverfew extract or parthenolide. If smaller volumes of feverfew extract were used the volume was always made up to 100 μL with PBS; dilutions of parthenolide were made in PBS containing ethanol (2%) so that the ethanol concentration in the final sample of PRP would always be the same (0·33%). PRP in the presence of 100 μL of the diluting media was used as control. After the 2 min incubation, the agonist under investigation was added to give a final sample volume of 600 μL. The PRP-sample was stirred for a further 6 min after which 50 μL of the acetylsalicylic acid solution (14 mM) was added and the sample transferred to ice. The [^{14}C]5-HT that had been secreted from the platelets was measured in duplicate amounts (50 μL) of the supernatant of the platelet samples and expressed as a percentage of the total amount of [^{14}C]5-HT taken up by the platelets.

Platelet aggregation in PRP. This was monitored in a six-channel light absorbance aggregometer (Adams et al 1975). The procedure was the same as that for [^{14}C]5-HT secretion. The aggregation process was recorded for 6 min after addition of the platelet stimulating agent.

Results and Discussion

This study was carried out to obtain more information on the link between the biological activities of crude feverfew extract and one of its active components, parthenolide. We had previously identified active components using an assay for antisecretory activity against adrenaline (Groenewegen et al 1986). Here, we have studied the effects of both feverfew extract and parthenolide on both [^{14}C]5-HT secretion and aggregation induced by a range of stimulating agents.

First, we carried out a number of experiments in which the effects of crude feverfew extract and parthenolide on stimulant-induced [^{14}C]5-HT secretion from platelets were compared. For each experiment the platelets had been prepared from the same blood sample enabling a direct comparison of the data. The effects of different volumes of feverfew extract and a range of concentrations of parthenolide were studied on [^{14}C]5-HT secretion induced by adrenaline, the phorbol ester PMA, ADP, AA, collagen, the calcium ionophore A23187, the thromboxane mimetic U46619 or the diacylglycerol analogue OAG. Both parthenolide and feverfew extract inhibited [^{14}C]5-HT secretion in a dose-dependent way for each stimulant with one exception, and the degree of inhibition varied with the stimulating agent studied. The exception was when the effects of crude feverfew extract on [^{14}C]5-HT secretion induced by the calcium ionophore A23187 (10 μM) were studied. A marked inhibition was found in the presence of small volumes (10 μL or less) of feverfew extract. However, maximum, but not complete, inhibition was found in the presence of 10 μL of feverfew extract (73 ± 5% inhibition, mean ± s.e.m. for n = 3), whereafter the inhibition levelled off and larger volumes of feverfew extract did not inhibit [^{14}C]5-HT secretion further. This phenomenon was not found when the effects of parthenolide on A23187-induced [^{14}C]5-HT secretion were studied. Here, parthenolide inhibited [^{14}C]5-HT secretion in a dose-dependent way and the IC50 value (see below) was $33{\cdot}3 \pm 15$ μM (mean ± s.e.m., n = 3). It is possible that some additional components in the extract resulted in potentiation of [^{14}C]5-HT secretion at the larger volumes of feverfew extract used.

In all the cases where it was possible, IC50 values (the volume of feverfew extract or concentration of parthenolide which caused 50% inhibition) were determined. A plot of IC50 values obtained for parthenolide versus those obtained for feverfew revealed a very good correlation (Fig. 1). Thus these data indicate clearly that parthenolide is at least one of the active components of feverfew extract. Furthermore, over the range of agonist concentrations studied both parthenolide and feverfew varied in the extent of the inhibition. For example PMA and OAG-induced responses were very strongly inhibited (low IC50 values), whereas the collagen response was less potently inhibited (high IC50 values).

We carried out a further series of experiments in which the effects of feverfew extract and parthenolide on a range of concentrations of PMA, U46619 and collagen-induced [^{14}C]5-HT secretion were studied. Fig. 2 shows the results obtained for the effects of feverfew extract and parthenolide on PMA and collagen-induced [^{14}C]5-HT secretion. Both feverfew extract and parthenolide gave similar inhibition patterns with either PMA or collagen-induced [^{14}C]5-HT secretion. However, it is clear that there are marked differences between the type of effect of both inhibitors on [^{14}C]5-HT secretion induced by the two different stimulants. In the case of collagen-induced [^{14}C]5-HT secretion the inhibition was overcome by increasing the concentration of collagen and may thus be called surmountable inhibition. On the other hand, when [^{14}C]5-HT secretion was induced by PMA, an increase in the concentration of this stimulant did not alleviate the inhibition by either feverfew extract or parthenolide and may thus be called insurmountable inhibition. The effects of feverfew extract and parthenolide on [^{14}C]5-HT secretion induced by U46619 also gave similar patterns of

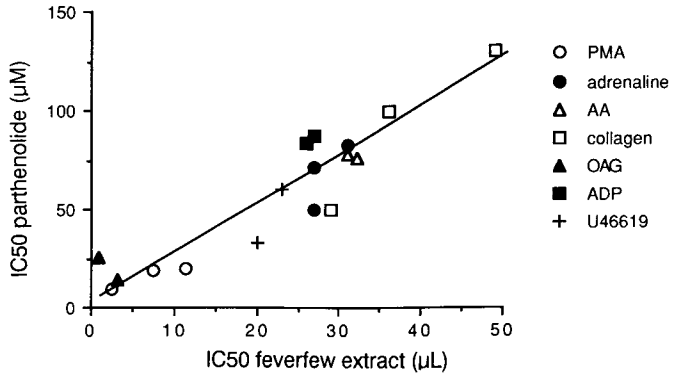

FIG. 1. IC50 values for the effects of feverfew extract (μL) and parthenolide (μM) on [^{14}C]5-HT secretion induced by adrenaline (100 μM, n = 3), PMA (500 ng mL^{-1}, n = 3), ADP (10 μM, n = 2), AA (1 mM, n = 2), collagen (6 μg mL^{-1}, n = 3), U46619 (3 μM, n = 2) or OAG (500 μg mL^{-1}, n = 2); r = 0·96.

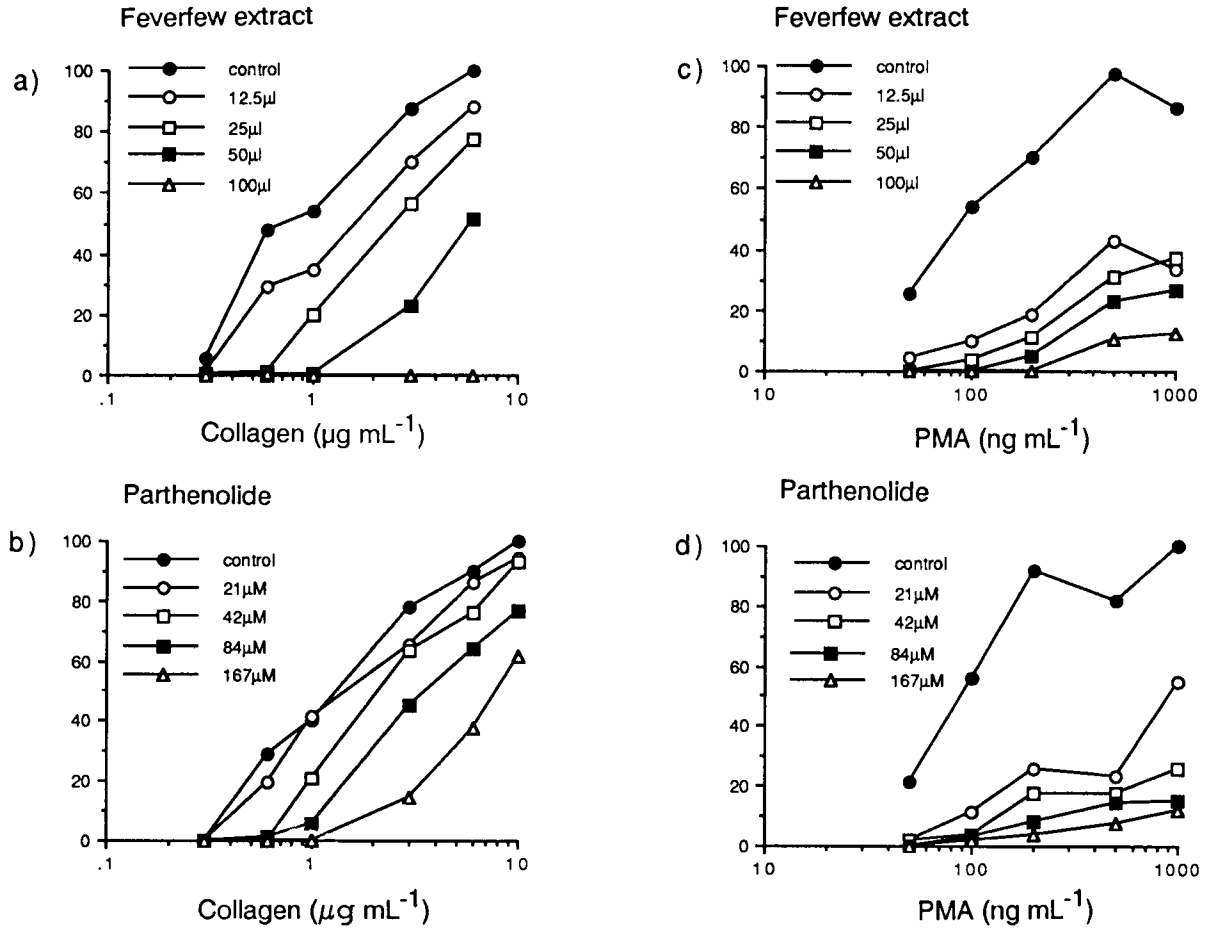

FIG. 2. The effects of feverfew extract (μL) and parthenolide (μM) on [^{14}C]5-HT secretion induced by either collagen (a and b) or PMA (c and d). The results are expressed as a percentage of the maximum amount of [^{14}C]5-HT secretion found in the controls. The means of three experiments are shown. For the sake of clarity, error bars have been omitted.

inhibition (not shown), and the pattern was similar to that obtained for PMA. The fact that parthenolide and feverfew inhibited PMA and U46619-responses in one way and collagen responses in another implies that the inhibitor is affecting different mechanisms important in the different responses.

To investigate whether the similarities between the effects of feverfew extract and parthenolide on [^{14}C]5-HT secretion also applied to the aggregation process, a further set of experiments was performed. The effects of feverfew extract and parthenolide for a given agonist were investigated on platelets prepared from the same blood sample. Platelet aggregation was induced by either PMA (500 ng mL^{-1}), adrenaline (100 μM) or AA (1 mM) and three different samples

of PRP were studied for each stimulant. As for [^{14}C]5-HT secretion, both feverfew extract and parthenolide caused concentration-dependent inhibition of the aggregation induced by the three different agents. For each stimulant, the patterns of inhibition found for either feverfew or parthenolide were again similar. Fig. 3 shows examples of the results obtained for PMA and AA. For AA, 50 μL of feverfew extract and 37 μM parthenolide caused complete inhibition of the aggregation response. For adrenaline, in a typical experiment, 25 μL of extract and 83 μM parthenolide completely inhibited the response (data not shown). For PMA there was only partial inhibition of aggregation at the highest concentration of feverfew extract and parthenolide used. Taking the data together we conclude that parthenolide in the feverfew extract is responsible for the biological effects of the extract, at least in platelets.

Despite the very potent inhibition of the secretory response by both feverfew and parthenolide, very little effect on the aggregation response to PMA was found. For other stimulants such as AA or adrenaline the two responses seemed to be affected with similar potency. Therefore we carried out further experiments with crude feverfew extract in which platelet aggregation was measured and subsequently [^{14}C]5-HT secretion was determined in the same sample of PRP. Platelets were activated by ADP (10 μM), collagen (6 μg mL^{-1}), adrenaline (100 μM) or PMA (500 ng mL^{-1}) and the effect of different volumes of extract was determined. Except in the case of the PMA-induced responses, inhibition of the two processes occurred in parallel. Fig. 4 shows examples of the results obtained for PMA and adrenaline. In the case of adrenaline-induced activation, inhibition of aggregation and [^{14}C]5-HT secretion occurred in parallel. This was also the case for ADP and collagen-induced activation (data not shown). When platelets were activated by PMA it can be seen clearly that while PMA-induced aggregation was barely affected (e.g. for 50 μL of extract), there was almost no secretion of [^{14}C]5-HT (4%) in that same sample of platelets.

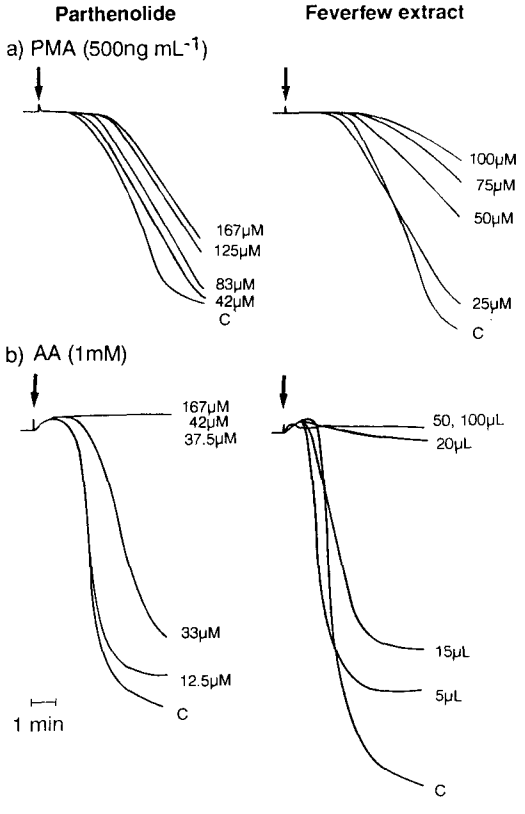

FIG. 3. Comparison of the effects of parthenolide (μM) and feverfew extract (μL) on aggregation induced by (a) PMA (500 ng mL^{-1}) or (b) AA (1 mM). For each stimulant the comparison was made on platelets from the same blood sample. Three experiments were carried out for each stimulant and a representative result is shown. The control samples are marked C.

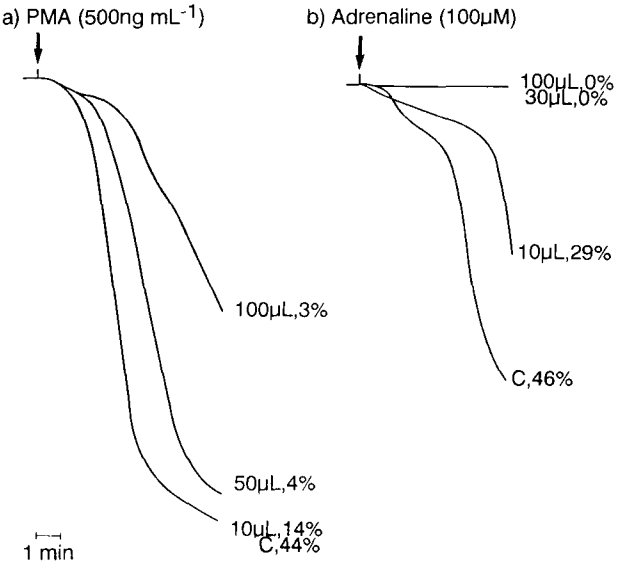

FIG. 4. The effects of feverfew extract (μL) on aggregation and [^{14}C]5-HT secretion induced by (a) PMA (500 ng mL^{-1}) or (b) adrenaline (100 μM). The amounts of [^{14}C]5-HT secretion (%) in each sample of PRP are shown at the end of each aggregation trace. The results are representative of three similar experiments.

The relative lack of effect by both feverfew extract and parthenolide on PMA-induced platelet aggregation and the marked inhibition of secretion induced by this agent, is of some interest. White et al (1974) found that aggregation induced by concentrations of PMA greater than 100 ng mL^{-1} is not dependent on secretion of 5-HT or adenine nucleotides from the storage granules. The results presented here agree with this finding in that aggregation induced by 500 ng mL^{-1} PMA occurred in the virtual absence of [^{14}C]5-HT secretion. PMA is also known to activate protein kinase C in the cell, which is thought to give rise to secretion of intracellular storage granules (Nishizuka 1984). It is therefore interesting to note that feverfew and parthenolide affect this part of the activation of platelets by PMA most markedly. Furthermore, it has been shown that parthenolide and compounds like it possess antitumour activities (Lee et al 1971) and PMA is a known tumour promoting agent. Feverfew/parthenolide may thus provide us with a tool to study biological processes and the phenomena described here warrant further investigation.

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