J. Pharm. Pharmacol. 1988, 40: 501–502
Communicated February 26, 1988

COMMUNICATIONS

Effects of an extract of feverfew on endothelial cell integrity and on cAMP in rabbit perfused aorta

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Abstract—Extracts of feverfew inhibit platelet aggregation and secretion of granular contents from platelets and other cells. They also modify the interaction of platelets with collagen substrates: feverfew extracts inhibit both platelet spreading and formation of thrombus-like platelet aggregates on the collagen surface. We have now investigated the effect of an extract of feverfew on the vessel wall using rabbit aortas that were perfused with a physiological salt solution in-situ. Addition of feverfew extract to the perfusion medium protected the endothelial cell monolayer from perfusion-induced injury and led to a reversible increase in the cAMP content of aorta segments. The results indicate that feverfew may have a vasoprotective effect in addition to its effects on platelets.

Leaves taken from the plant feverfew (*Tanacetum parthenium*) are sometimes used as a remedy for migraine and arthritis (Johnson et al 1985; Editorial 1985). Crude extracts of feverfew inhibit platelet aggregation (Makheja & Bailey 1982) and also inhibit secretory activity in platelets, polymorphonuclear leucocytes (Heptinstall et al 1985) and mast cells (Hayes & Foreman 1987) and it is possible that such activities are relevant to the medicinal properties of the plant. Feverfew contains sesquiterpene lactones (parthenolide and parthenolide-like compounds) that have an α-methylenebutyrolactone unit as an integral part of their structure, and these are the compounds that appear to be responsible for the anti-platelet effects of extracts of feverfew (Groenewegen et al 1986; Heptinstall et al 1987a). Furthermore, it is likely that parthenolide and parthenolide-like compounds inhibit platelet behaviour via neutralization of cellular sulphydryl groups (Heptinstall et al 1987a, b). Recently we have shown that feverfew extract inhibits platelet deposition on collagen substrates, and we have proposed that feverfew may have antithrombotic potential (Loesche et al 1987). We have now examined the effect of a feverfew extract on the integrity of the endothelial cell layer and the cAMP content of segments of rabbit perfused aortas.

Materials and methods

Feverfew extract. Feverfew (*Tanacetum parthenium*) was grown in the Department of Botany, University of Nottingham. The leaves were dried in air, powdered and extracted with chloroform (20 mL g^{-1} dried leaves). After drying the extract under nitrogen, the residue was dissolved in the same volume of phosphate-buffered saline, pH 7·3; any insoluble material was then removed by filtration. The solution was kept at 4°C up to one week without loss of activity (Heptinstall et al 1985).

Perfusion experiments. Chinchilla rabbits (2·5–3·0 kg) were fasted for 18 h before the experiment. Under pentobarbitone narcosis and artificial respiration the thoracic aorta was isolated and all collateral arteries were ligated. Individual segments (length, 1 cm) of the descending thoracic aorta were cannulated, washed with medium 199 (Gibco, USA) to remove blood and perfused with medium 199 or with medium 199 that contained feverfew extract (0·1 mL extract per mL medium) for up to 4 h. The procedure involves continuous recycling of a small volume of medium (3–4 mL) through each segment at a pressure of 100 mmHg. The perfusate was kept at 37°C and was equilibrated with 5% CO_2/95% O_2, and the flow rate was 10 mL min^{-1}. Several (3–4) individual segments were simultaneously perfused in order to compare the results obtained in the same animal.

Morphology of the endothelial cell monolayer. After 1 and 4 h of perfusion the aortic segments were washed with medium 199, then with 5% glucose, impregnated with 0·1% silver nitrate and fixed overnight with 2·5% glutardialdehyde under a pressure of 100 mmHg. The fixed vessels were cut longitudinally, washed with phosphate-buffered saline and then processed for scanning electron microscopy as described elsewhere (Davies & Bowyer 1975). After coating the specimen with a thin layer of gold (30 nm) the luminal surface was examined with a Phillips-500 scanning electron microscope at a beam voltage of 25kV. Two indices were used to assess the integrity of the endothelial cell layer: the endothelial cell injury index and the intercellular border injury index. The former is the number of sloughing and argyrophylic endothelial cells per mm^2; the latter is the number of endothelial cells with abnormal borders (widening, borders with craters, stomata).

cAMP determination. After perfusion for different lengths of time the segments were cut into small pieces, frozen in liquid nitrogen, weighed and then homogenized in ethanol containing 1 mM hydrochloric acid. The homogenate was kept overnight at 4°C, then centrifuged and the supernatant was evaporated to dryness under reduced pressure. The amount of cAMP in the samples was determined using a radioimmunoassay test kit from Amersham International.

Results and discussion

Perfusion of rabbit aorta with medium 199 for 4 h led to an

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increase in the endothelial cell injury index. This was prevented by feverfew extract. In contrast, the intercellular border injury index did not change with time of perfusion and was unaffected by the extract (Table 1). In a previous investigation we found

Table 1. Effects of feverfew extract on endothelial cell morphology in rabbit perfused aorta. Mean ± s.d. of 3 experiments.

	Time of perfusion (h)	Endothelial cell injury index (cells mm^{-2})	Intercellular border injury index (cells mm^{-2})
Control	1	2·5 ± 0·5	51 ± 10
Feverfew	1	2·6 ± 0·3	53 ± 9
Control	4	16·3 ± 2·1	54 ± 6
Feverfew	4	2·3 ± 0·6	48 ± 4

that compounds that elevate intracellular cAMP protect the endothelial cell monolayer of blood vessels from perfusion-induced injury (Voyno-Yasenetskaya et al, unpublished data). Consequently we examined the cAMP content of aorta segments that had been perfused in the absence and presence of feverfew extract. In the presence of feverfew extract the cAMP content of aorta increased two-fold reaching a maximum 15 min after commencing perfusion, and then slowly returned to normal (Fig. 1).

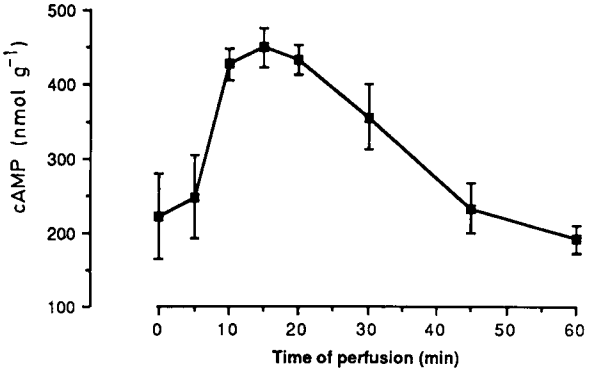

FIG. 1. cAMP content of aortic segments that were perfused with medium 199 containing feverfew extract for several lengths of time. The cAMP content (mean ± s.d. of 5–6 separate experiments) is given in nmol g^{-1} tissue wet weight. During perfusion with the medium alone there was no change in the cAMP content.

Since the protective effects of feverfew extract on endothelial cell injury and its effects on intracellular cAMP do not follow the same time-scale, it is not clear whether these two effects of the extract are related. Neither do we know whether the effect on cAMP is via stimulation of the adenylate cyclase or by inhibition of the cAMP phosphodiesterase, and whether such effects are related to the ability of feverfew extract to interact with cellular sulphydryl groups.

These effects of feverfew extract on the vessel wall were observed at concentrations of the extract similar to those that inhibit platelet behaviour in-vitro, including platelet spreading and aggregate formation on immobilized collagen (Loesche et al 1987). We believe that these effects are brought about by parthenolide and other parthenolide-like materials present in the extract (Heptinstall et al 1987b) and it will be interesting to find out whether these compounds are also responsible for the effects of the extract on endothelial cell integrity and cAMP.

The results of the present and previous papers are consistent with feverfew being of value as an antithrombotic agent as well as being of value in migraine and arthritis.

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