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Gossypol: a potent inhibitor of PAF-acether- and leukotriene-induced contractions of guinea-pig lung parenchyma strips

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Gossypol, a substance extracted from cotton plants, markedly inhibited the contractile responses of guinea-pig lung parenchyma strips stimulated with leukotriene B_4 (LTB_4), leukotriene D_4 (LTD_4) and PAF-acether but not the responses to histamine (except at high concentration). It was slightly less potent than nordihydroguaiaretic acid (NDGA) and a PAF-antagonist (BN 52021). From previously reported effects of gossypol on the cyclooxygenase and lipoxygenases, and on the similarity of the inhibition produced by NDGA and gossypol on the lung parenchyma, it is suggested that the inhibition of the myotropic activity of the lung parenchyma by gossypol is due to interactions with the formation of cyclooxygenase products within the guinea-pig lung.

Gossypol is a phenolic compound extracted from the roots, stems and seeds of the cotton plant. It was shown to be a highly effective male contraceptive (reviewed by Qian & Wang 1984). Many mechanisms have been proposed to explain its actions, especially its ability to inhibit a number of enzymes such as lactate dehydrogenase and ATP-ase (Wong et al 1972; Lyman et al 1959; Tanksley et al 1970; Finley et al 1973; Myers & Thorneberry 1966; Abou-Donia & Dieckert 1974; Breitbart et al 1984; Lee & Malling 1981). Gossypol was also shown to inhibit membrane enzymes (Reyes et al 1984; Haspel et al 1985) including enzymes involved in phospholipid metabolism (Vainio et al 1985), calcium transport (Breitbart et al 1984) and prostaglandin synthesis (Hamasaki & Tai 1985).

The aim of this work was to study further the mechanism of action of gossypol in relation to arachidonic acid metabolism in the lung. For this purpose, we analysed the effects of gossypol on the contractions of guinea-pig lung parenchymal strips (GPLP) induced by histamine, leukotriene B_4 (LTB_4), leukotriene D_4 (LTD_4) and platelet activating factor (PAF-acether), as it has been previously demonstrated that the contractions of the parenchyma to these agonists could be inhibited either by specific receptor antagonists or by inhibitors of arachidonic acid metabolism. The effects of gossypol were also compared with those of nordihydroguaiaretic acid (NDGA), a phenolic lipoxygenase inhibitor and to a novel antagonist of PAF-acether (BN 52021, an extract from *Ginkgo biloba*; Braquet 1984).

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MATERIALS AND METHODS

Male Hartley guinea-pigs, 400–500 g, were killed by cervical dislocation and bled by section of the aortic artery. The lungs and heart were immediately removed and placed in cold Krebs solution. The lung parenchyma from the margin of each lobe (3 × 3 × 30 mm) were cut in strips, transferred to a cascade superfusion system, perfused with oxygenated Krebs solution (5 mL min^{-1}; 37 °C) and equilibrated under constant tension of 1·5 g for 1 h before their responses to various agents were examined (Sirois et al 1980). Tissues were stimulated with a fixed dose of histamine ($2{\cdot}7 \times 10^{-8}$ mol) and only those responding with an increase in tension of at least 200 mg were used in subsequent experiments (approximately 90% of all strips were used). Cumulative dose-response curves to histamine, LTB_4 and LTD_4 were done by the sequential injection of increasing doses of the agonists and the responses were recorded isometrically with UC-2 Gould transducers coupled to a polygraph (Gould 8000 S).

Since PAF-acether was tachyphylactic, a fresh lung strip was used for each experiment. The contractions were expressed as percentage of tissue response to $2{\cdot}7 \times 10^{-8}$ mol (5 μg) histamine (approximate ED 70%).

The drugs used were: gossypol (Fig. 1) (Sigma, St Louis, MO), BN 52021 (a hexacyclic trilactone from the *Ginkgo biloba* tree) (IHB-Ipsen, Le Plessis

Robinson, France), nordihydroguaiaretic acid (NDGA) and histamine (Sigma, St Louis, MO), leukotriene B_4 (LTB_4)-free-acid and leukotriene D_4 (LTD_4)-methylester were supplied by Paesel, Germany and PAF-acether C16 (1-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-3-phosphorylcholine) was supplied by Bachem, Switzerland. LTD_4-methylester was hydrolysed with a potassium chloride solution. PAF-acether, LTB_4 and LTD_4 were divided into aliquots and kept frozen at −20 °C. Before each experiment, they were diluted to appropriate concentrations and used immediately. Gossypol and NDGA were dissolved in dimethylsulphoxide. All the drugs were infused at a rate of 0·1 mL min^{-1} starting 30 min before and during injections of the agonists. Final dimethylsulphoxide concentration was 0·2%.

The composition of the Krebs solution was (mM): NaCl, 118; KCl, 4·7; $MgSO_4$, 1·17; KH_2PO_4, 1·18; $CaCl_2$, 2·5; $NaHCO_3$, 25; glucose, 11. This solution was continuously gassed with 95% O_2 and 5% CO_2 (pH 7·4).

RESULTS

Effects of gossypol on the contractions of guinea-pig lung parenchyma to histamine, LTB_4 and LTD_4

In the first series of experiments, the effects of gossypol on the contractile responses of the tissue to increasing doses of histamine, LTB_4 and LTD_4 were studied. As shown in Fig. 1 (upper panel), gossypol reduced the activity of histamine. At the concentrations of 0·2 μg mL^{-1}, gossypol shifted the dose-response curve to the right and the apparent EC50 was increased by 6–7 times. Increasing the concentration of the inhibitor to 2 μg mL^{-1} did not augment the inhibition of histamine response compared with the effect of 0·2 μg mL^{-1}. However, a higher concentration of gossypol (20 μg mL^{-1}) further displaced the dose-response curve of histamine to the right by more than a log. The maximal activity of histamine on the parenchyma strips was also depressed by gossypol.

The lowest concentration of gossypol used (0·2 μg mL^{-1}) did not interfere with the contractions of the tissue to LTB_4 which induced contractions at a concentration as low as 1×10^{-12} mol (0·3 ng). The maximal activity was reached with a concentration of $0{\cdot}9 \times 10^{-9}$ mol (300 ng) (Fig. 1, centre panel). At 2 μg mL^{-1} of gossypol, the contractions induced by the low doses of LTB_4 (up to $0{\cdot}9 \times 10^{-11}$ mol, 3 ng) were completely inhibited. However higher doses of LTB_4 were only reduced by this concentration of the inhibitor and the apparent EC50 of LTB_4 increased by approximately half a log. Increasing the

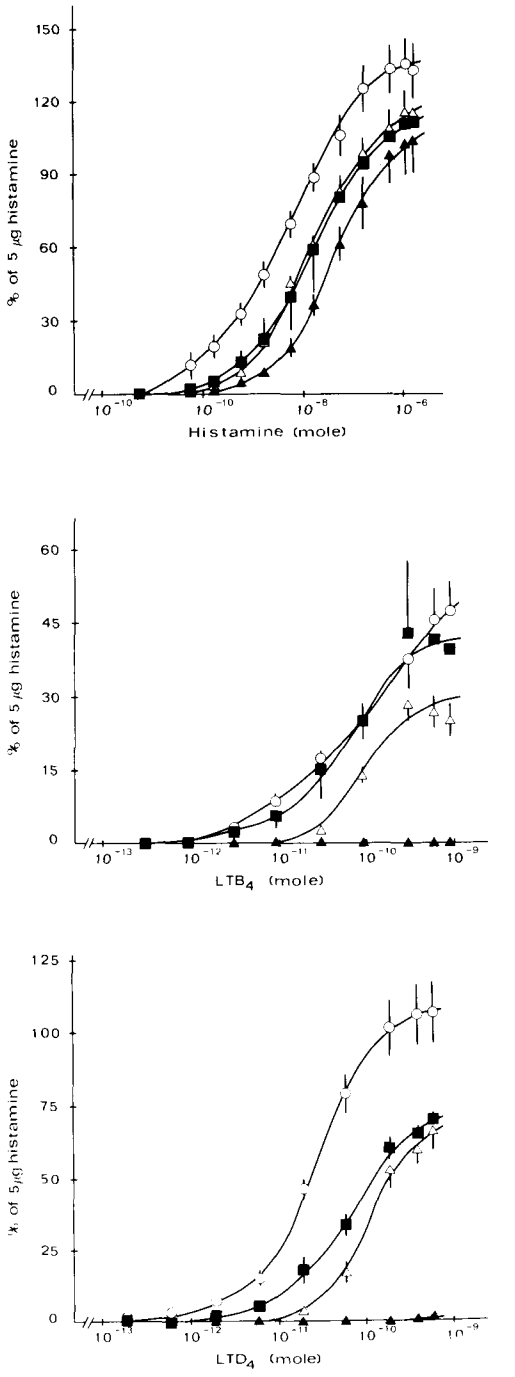

FIG. 1. Effects of gossypol on the contractions of strips of guinea-pig lung parenchyma to increasing doses of histamine (upper panel), LTB_4 (centre panel) and LTD_4 (lower panel). Each point is the mean ± s.e.m. of 4–6 experiments. Contractions are expressed as per cent of the contractile effect of 5 μg ($2{\cdot}7 \times 10^{-8}$ mol) of histamine. Key: □ control, ■ 0·2, △ 2·0, ▲ 20·0 μg mL^{-1}.

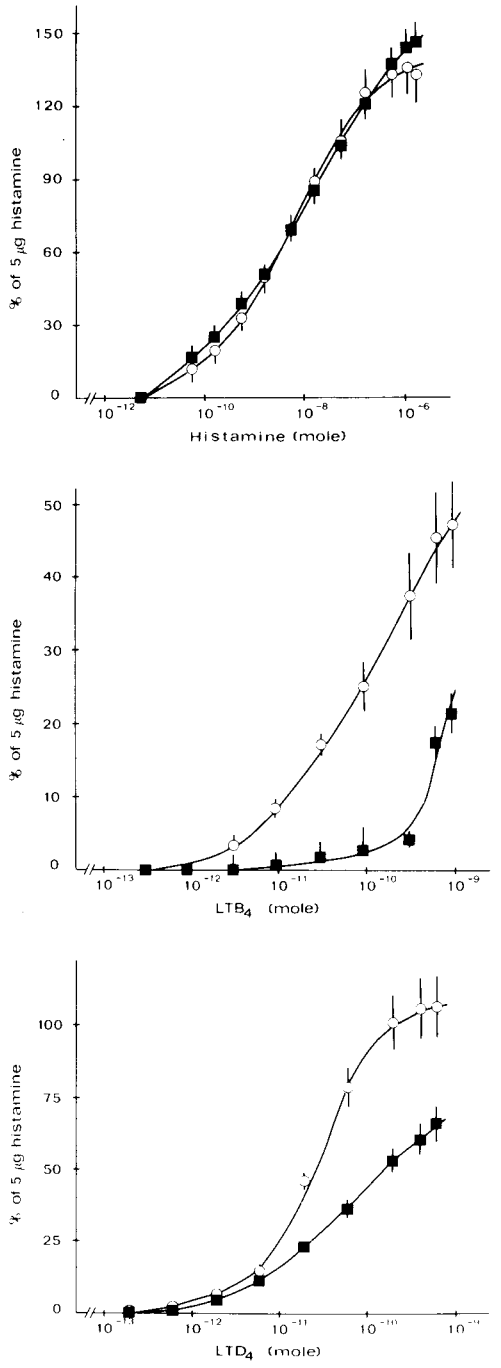

FIG. 2. Effects of nordihydroguaiaretic acid (NDGA) on the contractions of strips of guinea-pig lung parenchyma to increasing doses of histamine (upper panel), LTB_4 (centre panel) and LTD_4 (lower panel). Each point is the mean ± s.e.m. of 6 experiments. Contractions are expressed as per cent of the contractile effect of 5 μg ($2{\cdot}7 \times 10^{-8}$ mol) of histamine. Key: □ Control, ■ NDGA 3 μg mL^{-1}.

concentration of gossypol to 20 μg mL^{-1} abolished the response of the tissue to LTB_4.

Gossypol produced inhibitory effects on LTD_4-induced contractions of GPLP similar to, but more marked than, those produced on LTB_4 contractions. As shown on the lower panel of Fig. 1, the dose-response curve to LTD_4 in the presence of gossypol (0·2 μg mL^{-1}) was very depressed; the high as well as the low doses of LTD_4 were inhibited by the drug. The 2 μg mL^{-1} concentration produced slightly more inhibition of the LTD_4-stimulated tissue than the 0·2 μg mL^{-1} concentration. The 20 μg mL^{-1} concentration abolished the response to LTD_4 as it did the response to LTB_4.

Effects of nordihydroguaiaretic acid (NDGA) on the contraction of guinea-pig lung parenchyma to histamine, LTB_4 and LTD_4

In the second set of experiments, the effects of NDGA, a lipoxygenase inhibitor, on the responses of the tissue to histamine, LTB_4 or LTD_4 were studied. At the single concentration used (3 μg mL^{-1}), NDGA did not affect the contractile activity to histamine (Fig. 2, upper panel), but it strongly diminished the responses to LTB_4 and LTD_4. The inhibition of the responses to LTB_4 at doses from 1×10^{-12} to 1×10^{-10} mol was total, whereas with the larger doses of agonist, activity was much reduced (Fig. 2, centre panel). The contractions induced by small doses of LTD_4 (from 7×10^{-13} to 7×10^{-12} mol) were not affected by NDGA but the inhibitory activity became marked (approximately 50%) for doses of LTD_4 ranging from 7×10^{-12} to 7×10^{-10} mol (Fig. 2, lower panel).

Comparative effects of gossypol, NDGA and BN 52021 on PAF-induced contractions of guinea-pig lung parenchyma

The effects of gossypol on the myotropic responses of the tissue to PAF-acether (3 ng) were studied in the third set of experiments and compared with the effects of NDGA and BN 52021, a novel PAF-acether antagonist. As shown in Table 1, PAF-acether (3 ng) produced a contraction equivalent to 38% of the response to 5 μg of histamine. The lowest concentration of gossypol (0·2 μg mL^{-1}) did not affect the response to PAF-acether but 2·0 and 20 μg mL^{-1} reduced the contractions by 65 and 100%, respectively. Similarly, NDGA (3 μg mL^{-1}) decreased the contractile activity of the tissue to 3 ng of PAF-acether by 41%. For comparison, a specific PAF antagonist was tested and produced a concentration-dependent inhibition of the response to

Table 1. Effects of gossypol, nordihydroguaiaretic acid (NDGA) and BN52021 (PAF-acether antagonist) on the contractions of strips of guinea-pig lung parenchyma to PAF-acether (3 ng). Contractions are expressed as per cent of the contractile effect of 5 μg ($2·7 \times 10^{-8}$ mol) of histamine; n is the number of experiments.

	μg mL^{-1}	Contractions (% 5 μg histamine)	n
Control		37·9 ± 6·2	5
Gossypol	0·2	40·4 (+6·6)	2
	2·0	13·4 ± 3·3 (−64·6)	6
	20·0	0·0 (−100)	2
NDGA	3·0	22·4 ± 10·5 (−40·9)	4
BN 52021	0·1	29·9 ± 11·5 (−21·1)	5
	0·5	19·9 ± 8·8 (−47·5)	6
	1·0	12·7 ± 7·5 (−66·5)	4
	30·0	8·1 ± 2·2 (−78·6)	6

PAF-acether ranging from 21% at 0·1 μg mL^{-1} to 79% at 300 μg mL^{-1}.

DISCUSSION

These results showed that gossypol is a potent inhibitor of leukotrienes (B_4 and D_4) and PAF-acether-induced contractions of guinea-pig lung parenchyma. At 2 μg mL^{-1}, it produced significant inhibition of LTB_4, LTD_4 and PAF-acether but the response to histamine was not significantly affected. Higher concentrations completely abolished the responses to the lipid mediators whereas the apparent EC50 of histamine was shifted to the right by approximately 1 log. Our results also showed that NDGA (3 μg mL^{-1}) decreased the response of the tissue to lipid mediators but not to histamine. Our results also confirmed previous results (Touvay et al 1986) which showed that a novel antagonist of PAF-acether, BN 52021, produces a concentration-dependent inhibition of the myotropic activity of the tissue to PAF-acether. Significant inhibition of PAF-acether was achieved with much lower concentrations of BN 52021 than with gossypol.

Our results also showed that there was a similarity between the inhibitory effect of gossypol and NDGA on the tissue, which suggested that these phenolic compounds may share a similar mechanism of action. The myotropic activities of LTB_4, LTD_4 and PAF-acether on the parenchyma were previously shown (Piper & Samhoun 1981; Sirois et al 1985; Touvay et al 1986) to be mediated, at least in part, by the tissue generation of cyclooxygenase products, whereas the activity of histamine on the parenchyma appears to be mainly a direct effect. The inhibition of the contractile activity of the parenchyma to LTB_4, LTD_4 and PAF-acether by NDGA (3 μg mL^{-1}; 1×10^{-5} M) could be explained by its inhibitory activity towards cyclooxygenase as significant inhibitory activity against the cyclooxygenase in neutrophils has been reported (Salari et al 1984). Hamasaki & Tai (1985) have shown that gossypol is also an inhibitor of prostaglandin synthetase and lipoxygenases of rat basophilic leukaemia cells at similar concentrations. The inhibitory effects of gossypol and NDGA on contractions of the parenchyma induced by leukotrienes and PAF-acether could be due to the inhibition of the arachidonic acid metabolism in this tissue by these two substances.

However, gossypol has also been reported to inhibit several membrane-bound enzymes which may be involved in the contractile action of lipid mediators. For instance, gossypol was shown to modify the electrochemical properties and transport systems of cell membranes (Reyes et al 1984; Haspel et al 1985; Breitbart et al 1984), to inhibit phospholipase A_2 (Vainio et al 1985) and a phospholipid-sensitive, calcium-dependent protein kinase (Kimura et al 1985), as well as to interfere with calcium transport and divalent cation ATPase of plasma membranes (Breitbart et al 1984). In previous studies we have reported that inhibitors of phospholipase A_2 (Sirois et al 1982) and inhibitors of calcium fluxes (Sirois et al 1986) strongly decreased the contractile responses of the parenchyma strips to leukotrienes. The inhibitory activity of gossypol on the contractions of the parenchyma to LTB_4, LTD_4 and PAF-acether could then be related to its effects on a number of enzymes. It is also possible that gossypol might interact with the binding sites of these agonists, although the lungs bear specific receptors for these lipid mediators (Sirois et al 1983; Touvay et al 1986); this would be due to non-specific binding of gossypol to cell proteins.

In summary, our results have shown that gossypol inhibited the contractions of guinea-pig lung parenchyma strips to LTB_4, LTD_4 and PAF-acether but not to histamine (except at high concentrations). The potency of gossypol was compared with that of NDGA and a specific PAF-antagonist (BN 52021). On the basis of the previously reported effects of gossypol on the metabolism of arachidonic acid, it is suggested that it may interfere with the formation of cyclooxygenase products in the guinea-pig lung.

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