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Interference of the Paf antagonist Ro 19-3704 with Paf and antigen-induced bronchoconstriction in the guinea-pig

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1 *In vitro*, Ro 19-3704, a structurally related antagonist of platelet-activating factor (Paf) inhibited selectively rabbit platelet aggregation. *In vivo*, administered intravenously, it inhibited bronchoconstriction, leukopenia, thrombocytopenia and the accompanying accumulation of platelet aggregates in guinea-pig lung microvessels induced by i.v. Paf. Administered by aerosol, Ro 19-3704 failed to inhibit bronchoconstriction, thrombocytopenia or leukopenia due to i.v. Paf.

2 Bronchoconstriction induced by Paf, in aerosol form, was blocked by Ro 19-3704 administered by the i.v. or aerosol route, which suggests that it interacts with pulmonary cells responsible for bronchoconstriction.

3 Ro 19-3704 has free radical scavenging properties, since it inhibited the production of superoxide anions by macrophages stimulated by Paf and by N-formyl-methionyl-leucyl-phenylalanine (FMLP). Ro 18-7715, another Paf antagonist and analogue of Ro 19-3704, failed to inhibit the production of superoxide anions by macrophages stimulated by FMLP at concentrations which were effective against Paf.

4 Administered intravenously, Ro 19-3704 failed to block bronchoconstriction induced by an i.v. injection of ovalbumin to guinea-pigs passively sensitized with anti-ovalbumin antiserum. Passive pulmonary anaphylaxis due to an aerosol of ovalbumin was blocked by i.v. Ro 19-3704.

Introduction

The alkyl-ether phospholipid platelet-activating factor (Paf) is a mediator of immediate hypersensitivity (Vargaftig *et al.*, 1981; Page *et al.*, 1984) and accordingly, its antagonists are potential anti-allergic substances. These antagonists can be classified into two large families: the first includes substances chemically unrelated to Paf, such as the ginkgolide BN 52021 (Braquet *et al.*, 1985; Desquand *et al.*, 1986; Lagente *et al.*, 1987), the triazolothienodiazepine WEB 2086 (Casals-Stenzel *et al.*, 1986; Pretolani *et al.*, 1987) and the pyrrolo thiazole 48740 RP (Sédivy *et al.*, 1985), and the second includes the structural analogues of Paf (Terashita *et al.*, 1983; Hadvary & Baumgartner, 1985; Handley *et al.*, 1986). One of those analogues, Ro 19-3704 (Hadvary & Baumgartner, 1985) inhibits Paf-induced aggregation of rabbit platelets at submicromolar concentrations. Accordingly, its ability to antagonize the effects of Paf was compared to that of another analogue, compound CV 3988 (Terashita *et al.*, 1983) and its interference with immediate hypersensitivity reactions in different models studied.

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Methods

Sensitization procedure

Hartley guinea-pigs of either sex (300–500 g) were actively sensitized by a s.c. injection of 0.5 ml of saline containing 10 μg of ovalbumin dispersed in

1 mg of $Al(OH)_3$ (modified from Andersson & Bergstrand, 1981). The injection was repeated after 14 days and the animals were bled 7–10 days after the second injection. Normal guinea-pigs were passively sensitized by an i.p. injection of 1 ml of serum collected from the actively sensitized animals, and were challenged with ovalbumin 10–14 days after this serum transfer. Our serum preparations were tested on the passive cutaneous anaphylaxis model (Carmo *et al.*, 1986) to ensure they contained anti-ovalbumin IgE.

In vitro *studies*

Aggregation of blood platelets Rabbit platelet-rich plasma (PRP) was prepared by centrifugation (440 *g*, room temperature, 10 min) of blood collected from the carotid artery of burgundy rabbits under pentobarbitone anaesthesia (30 mg kg^{-1} i.v.); 1/10 v/v 90 mM trisodium citrate was used as anticoagulant. The platelet concentration in PRP was between 3×10^5 and 5×10^5 μl^{-1}. PRP was pre-incubated under stirring at 37°C for 2 min in the presence of the test compound or solvent before adding the agonist. Aggregation was studied in an Elvi type 840 aggregometer at 37°C and a stirring rate of 1100 r.p.m. The rate of change of light transmission caused by platelet aggregation was recorded and the maximal rate was taken as aggregation velocity. Dose-response curves were obtained by plotting the aggregation velocity against the logarithm of the dose tested and IC_{50} values were derived from these plots.

Activation of alveolar macrophages Alveolar macrophages were prepared as described by Maridonneau-Parini *et al.* (1985). Briefly, they were obtained by eight to ten repeated lung lavages of pentobarbitone-anaesthetized guinea-pigs with 5 ml of sterile phosphate buffered saline, pH 7.4 at 37°C, containing 10 mM lignocaine. Cells were then thoroughly washed and resuspended in MEM supplemented with 20 mM HEPES to a final concentration of 3×10^6 ml^{-1} and were left at 37°C for 2 h in a Corning tube. Aliquots of the suspended macrophages were transferred to 1 ml spectrophotometer cuvette at 37°C and incubated either with the drug solvent (control responses) or with the compounds to be tested for 5 min. Cytochrome c (100 μM) was added and followed by either Paf (1 μM) or FMLP (1 μM). Production of superoxide anion was measured by the superoxide dismutase- inhibitable reduction of cytochrome c, the absorbance changes being recorded at 550 nM at 1 min intervals for 4 min in a Kontron spectrophotometer type Uvikon 810. The amounts of reduced cytochrome c were calculated using an extinction coefficient of 21.1 mM^{-1} cm^{-1} (Massey, 1959). No reduction of cytochrome c was observed when superoxide dismutase was introduced in the cell suspension at a concentration of 50 μg ml^{-1}.

In vivo *studies*

General preparation Guinea-pigs were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.p.) and prepared for recording bronchial resistance to inflation as previously described (Lefort & Vargaftig, 1978). Spontaneous breathing was suppressed with pancuronium (2 mg i.v.). The right jugular vein was catheterized for injections of drugs. Both carotid arteries were cannulated and one was used to measure arterial blood pressure with a Statham-Gould P23Db transducer and the other for blood sampling (see below). At least 1 h after surgery, bronchial reactivity was tested with 5-hydroxytryptamine (1–3 μg kg^{-1} i.v.), until three similar bronchial responses were obtained.

Bronchoconstriction caused by i.v. injections of Paf The propranolol-treated animals (1 mg kg^{-1} i.v. plus 3 mg kg^{-1} i.p.) were given an initial injection of 33 ng kg^{-1} of Paf i.v. to obtain a control response. One h later, Ro 19-3704 (200 μg kg^{-1}) or CV 3988 (66, 200 or 600 μg kg^{-1}) was administered intravenously, followed after 3 min by a new injection of 33 ng kg^{-1} of Paf. Paf was also tested at 33 ng kg^{-1} immediately after administration of Ro 19-3704 in aerosol form (3 mg ml^{-1}, 10 min); control animals were given saline containing 0.1% of BSA as an aerosol.

Bronchoconstriction caused by Paf in aerosol form Guinea-pigs prepared as above, were bilaterally vagotomized and pretreated with propranolol. A solution of Paf (330 μg ml^{-1}) in saline was placed in the reservoir of a nebulizer connected with an air compressor and the flow was directed for 2 min into the outflow tube of the respiratory pump (modified from Lefort *et al.*, 1984). Since bronchoconstriction by Paf in aerosol form is not reproducible in the same animal (Lefort *et al.*, 1984), separate groups of animals were used, in which Paf was administered by aerosol for 2 min, after an i.v. injection or an aerosol administration of the drug solvent or of Ro 19-3704.

Anaphylactic bronchoconstriction caused by administration of ovalbumin Passively sensitized guinea-pigs were prepared as above. Anaphylactic shock was triggered with a 1 min i.v. infusion of 1 mg kg^{-1} of ovalbumin or by a 1 min aerosol of ovalbumin (10 mg ml^{-1} in the reservoir of the nebulizer as above). In this case, the animals were bilaterally vagotomized. Since tachyphylaxis is observed for anaphylactic shock, separate groups of animals were

used as controls, in which i.v. injection or aerosol administration of the drug solvent was studied.

Evaluation Bronchoconstriction was evaluated according to the pulmonary resistance to inflation. The intensity of bronchoconstriction was measured at its peak, which was 2–3 min after the drug administration and the arterial blood pressure was continuously recorded on a Beckman dynograph R511.

Blood cell counts Blood (200 μl) was collected from the carotid artery for total leukocyte and platelet counting which was performed with a Coulter Counter ZBI. Counts were performed before, 1 min after the i.v. injection of Paf and 1, 3 and 6 min after the i.v. infusion of ovalbumin. In the case of Paf or ovalbumin aerosols, no marked decrease of cell counts was seen after the administration of these agonists (Lefort *et al.*, 1984; Cirino *et al.*, 1986).

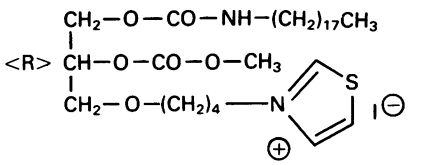
Electron microscopy studies

Paf (33 ng kg^{-1}) alone, or preceded by 200 μg kg^{-1} of Ro 19-3704 or ovalbumin (1 mg kg^{-1}) was administered into the cannulated jugular vein of normal or passively sensitized guinea-pigs. One min later, lungs were removed, small fragments were minced into slices and immediately immersed in cold 2.5% glutaraldehyde for 24 h. The specimens were then rinsed in phosphate buffer and post-fixed in 1% osmic acid, dehydrated and embedded in Epon (Luft, 1961). One micrometer sections stained with toluidine blue were used to select areas from which thin sections were prepared and stained with uranyl acetate and lead citrate. Sections were then examined in a Philips EM 300 transmission electron microscope.

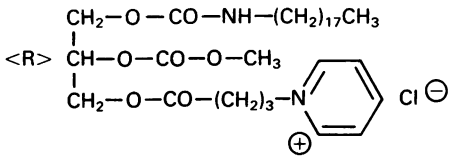
Drugs and chemicals

Ro 19-3704 (3-(4((R)-2-((methoxycarbonyl) oxy)-3-((octadecylcarbamoyl) oxy) propoxy) butyl) thiazolium iodide) and Ro 18-7715 (1-(3-(((RS)-2-((methoxycarbonyl) oxy)-3-((octadecylcarbamoyl) oxy) propoxy) carbonyl) propyl) pyridinium chloride), (Figure 1) were provided by Dr J.-M. Cassal and Dr T. Weller, F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland. CV 3988 (rac-3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl 2-thiazolioethyl phosphate) (Figure 1) was obtained from Takeda Chemical Ind., Osaka, Japan. These drugs were dissolved in ethanol and further diluted in saline containing 0.1% bovine serum albumin (BSA).

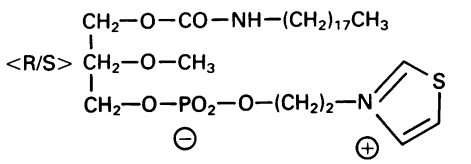
Paf (the hexadecanoic derivative purchased from Bachem, Switzerland, or provided by Prof. J.J. Godfroid, Université de Paris VII) was kept at −20°C and diluted in a 0.1% BSA-saline solution. Other



Ro 19–3704



Ro 18–7715



CV 3988

Figure 1 The structures of the three Paf antagonists, Ro19-3704, Ro18-7715 and CV 3988 used in this study.

drugs were as follows, the solvents being indicated except when 0.9% w/v NaCl solution (saline) was used: collagen in the buffer provided by Horm Chemie (Munich, FRG); sodium pentobarbitone (Nembutal, Clin-Midy, France); 5-hydroxytryptamine (5-HT), lidocaine, N-formylmethionyl-leucyl-phenylalanine (FMLP), ferricytochrome dissolved in distilled H_2O, super oxide dismutase and bovine serum albumin (BSA), (Sigma); propranolol (ICI); pancuronium (Organon, France); Eagle's Minimal essential medium (MEM) (Eurobio, France); chicken ovalbumin (ovalbumin, Worthington, New Jersey); $Al(OH)_3$ (Merck).

Data analysis

The data are expressed as means ± s.e. mean. Statistical differences between two means of data were evaluated by Student's unpaired *t* test.

Results

Inhibition by Ro 19-3704 of rabbit platelet aggregation

Ro 19-3704 dose-dependently inhibited platelet aggregation induced by Paf and the IC_{50} was related to the concentration of Paf used (Figure 2). Against 4 nM Paf, a concentration resulting in submaximal aggregation velocity (approximately 80% of the maximal), the IC_{50} was 0.07 μM for Ro 19-3704 and 3.2 μM for CV 3988. Inhibition by Ro 19-3704 was selective for Paf, since aggregation by collagen (5 $\mu g\,ml^{-1}$), ADP (1 μM) or thrombin (0.4 u ml^{-1}) was not inhibited even at a concentration as high as 10 μM.

Inhibition by Ro 19-3704 and CV-3988 of bronchoconstriction, thrombocytopenia and leukopenia induced by intravenous administration of Paf

Initially, we verified that the increase in bronchial resistance to inflation which was observed following two doses of Paf at 1 h intervals was practically the same, i.e. 10.8 $\pm$ 1.1 and 9.8 $\pm$ 1.0 cm H_2O ($n = 12$), respectively. This allowed us to study the effects of Ro 19-3704 and CV 3988 using each animal as its own control. Under these conditions, bronchoconstriction, thrombocytopenia and leukopenia due to Paf (33 ng kg^{-1}) were fully suppressed by 200 $\mu g\,kg^{-1}$ of i.v. Ro 19-3704 given 2 min beforehand (Figures 3 and 4). At the same dose, CV 3988 also inhibited bronchoconstriction and thrombocytopenia induced by Paf, and, less efficiently, the accompanying leukopenia (Figure 5).

When given by aerosol, Ro 19-3704 failed to inhibit the effects of i.v. Paf (Figure 4).

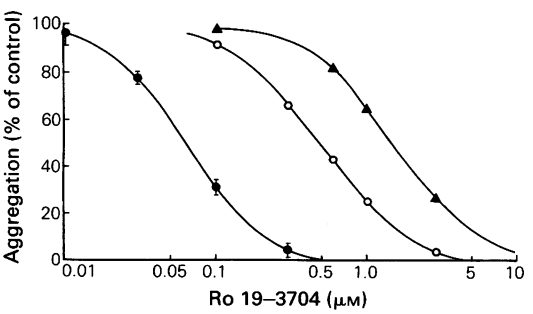


Figure 2 Inhibition by Ro 19-3704 of platelet aggregation induced by three concentrations of Paf, (●) 4 nM, (○) 30 nM and (▲) 100 nM. Rabbit PRP was preincubated with Ro 19-3704 for 3 min before the addition of Paf, 4 nM ($n = 6$, $IC_{50} = 0.1$ μM), 30 nM ($n = 2$, $IC_{50} = 0.5$ μM) or 100 nM ($n = 2$, $IC_{50} = 1.5$ μM), respectively. Vertical lines indicate s.e. mean.

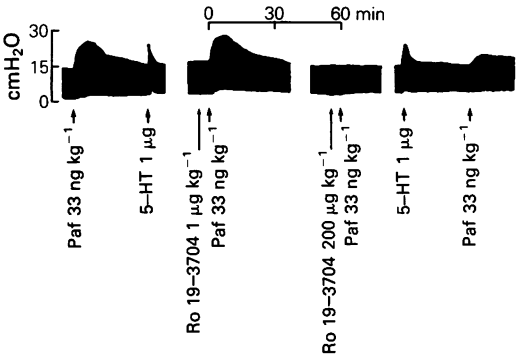


Figure 3 Inhibition by Ro 19-3704 (200 $\mu g\,kg^{-1}$, i.v.) of bronchoconstriction (cmH_2O) induced by an i.v. injection of Paf (33 ng kg^{-1}) to the guinea-pig. 5-Hydroxytryptamine (5-HT) was used as a standard bronchoconstrictor agent.

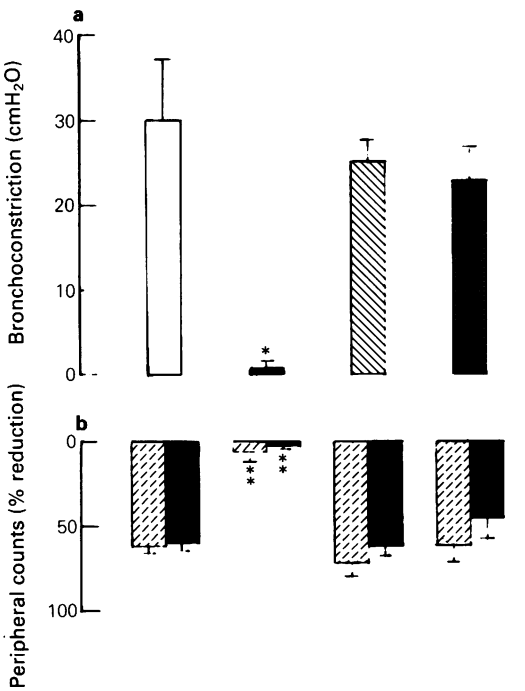


Figure 4 Inhibition by Ro 19-3704 administered intravenously or by aerosol of the bronchoconstriction (cmH_2O) (a), leukopenia and thrombocytopenia (% reduction) (b), induced by i.v. Paf (33 ng kg^{-1}) in propranolol-treated animals. (a) Control responses to Paf (open column), effect of Ro 19-3704 (200 $\mu g\,kg^{-1}$, i.v.; solid column), effect of vehicle (10 min aerosol; hatched column), effect of Ro 19-3704 (3 mg ml^{-1}, 10 min aerosol; stippled column); (b) effect of Paf on numbers of platelets (hatched columns) and polymorphonuclear leukocytes (solid columns). * $P < 0.05$, ** $P < 0.01$, $n = 4$; vertical lines indicate s.e. mean.

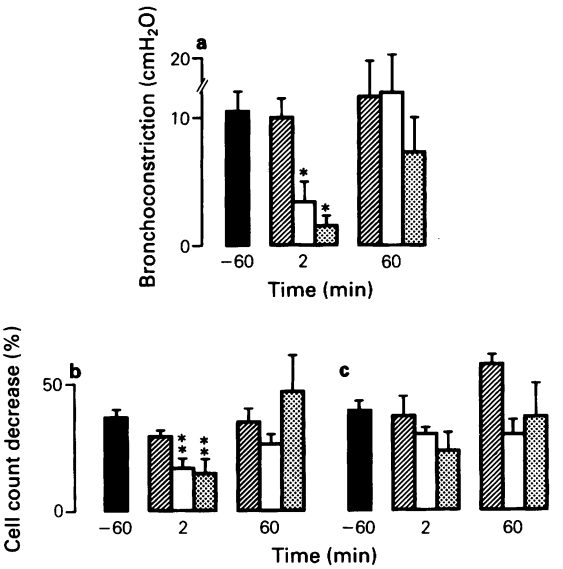


Figure 5 Effects of CV 3988 administered intravenously on the bronchoconstriction (cmH_2O) (a), thrombocytopenia (b) and leukopenia (c), (% of cell decrease) induced by an i.v. injection of Paf ($33\,ng\,kg^{-1}$) in propranolol-treated animals. The numbers on the abscissa scales indicate that Paf was administered 60 min before and 2 and 60 min after Ro 19-3704. Control responses to Paf (solid columns), effects of CV 3988 $66\,\mu g\,kg^{-1}$ (hatched columns), $200\,\mu g\,kg^{-1}$ (open columns) and $600\,\mu g\,kg^{-1}$ (stippled columns). * $P < 0.05$, ** $P < 0.01$; $n = 4$–6; vertical lines indicate s.e. mean.

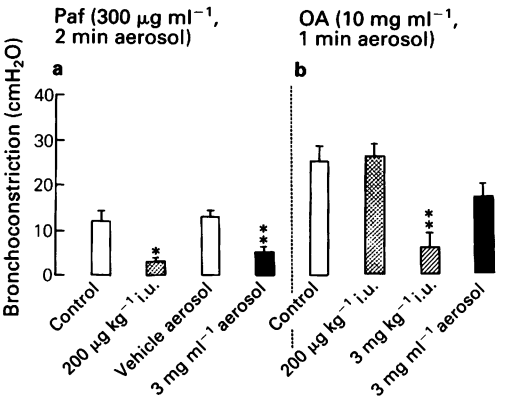


Figure 6 Inhibition by Ro 19-3704 administered intravenously or by aerosol of the bronchoconstriction (cmH_2O) induced by aerosol Paf (a) or ovalbumin (OA, b). * $P < 0.05$, ** $P < 0.01$, $n = 5$–9; vertical lines indicate s.e. mean.

Effects of Ro 19-3704 and CV 3988 on bronchoconstriction induced by Paf in aerosol form

Bronchoconstriction induced by an aerosol of Paf-acether ($330\,\mu g\,ml^{-1}$) for 2 min) was significantly reduced by Ro 19-3704 given by the i.v. route ($200\,\mu g\,kg^{-1}$) or by aerosol ($3\,mg\,ml^{-1}$, 10 min) (Figure 6). CV-3988, up to $2\,mg\,kg^{-1}$ i.v., failed to prevent bronchoconstriction induced by aerosol Paf-acether.

Inhibition by Ro 19-3704 of superoxide ion production induced by Paf and FMLP in alveolar macrophages

Guinea-pig alveolar macrophages are accessible to aerosol Paf or antigen and can be activated *in vitro* by Paf and by the leukocyte secretagogue n-formyl-methionyl-leucyl-phenylalanine (FMLP) at 10 nM–1 μM (Maridonneau-Parini *et al.*, 1985). Ro 19-3704 at 3, 10 and 30 μM induced a concentration-dependent inhibition of macrophage stimulation of Paf (1 μM), complete inhibition being obtained with 30 μM (Figure 7). Surprisingly, at 30 μM and above, Ro 19-3704 also reduced superoxide anion production induced by FMLP (Figure 7b). Since the thiazolium group of Ro 19-3704 might act as a superoxide scavenger, the pyridinium analogue Ro 18-7715, which was also effective against Paf-induced reduction of cytochrome c was tested, and shown to be totally inactive against FMLP (Figure 8a and b).

Interference of Ro 19-3704 with bronchoconstriction, thrombocytopenia and leukopenia induced by i.v. or aerosol ovalbumin administered to passively sensitized guinea-pigs

When given intravenously at a dose of up to $3\,mg\,kg^{-1}$ or by aerosol at $3\,mg\,ml^{-1}$ for 10 min, Ro 19-3704 failed to inhibit the brochoconstriction and the fall in the number of leukocytes triggered by an i.v. injection of $1\,mg\,kg^{-1}$ of ovalbumin to passively sensitized guinea-pigs (Figure 9). Since passive shock is accompanied by mild thrombocytopenia, the effect of Ro 19-3704 was not evaluated against this event.

Ro 19-3704 $200\,\mu g\,kg^{-1}$ i.v., a dose effective against bronchoconstriction induced by an aerosol of Paf, failed to inhibit the bronchoconstriction which followed a 1 min aerosol of ovalbumin ($10\,mg\,ml^{-1}$) to passively sensitized guinea-pigs. We increased the dose of Ro 19-3704 to $3\,mg\,kg^{-1}$ and this reduced bronchoconstriction significantly (Figure 6). Nevertheless, when Ro 19-3704 was administered as an aerosol, $3\,mg\,ml^{-1}$ for 10 min, a dose which blocks the effects of Paf delivered by

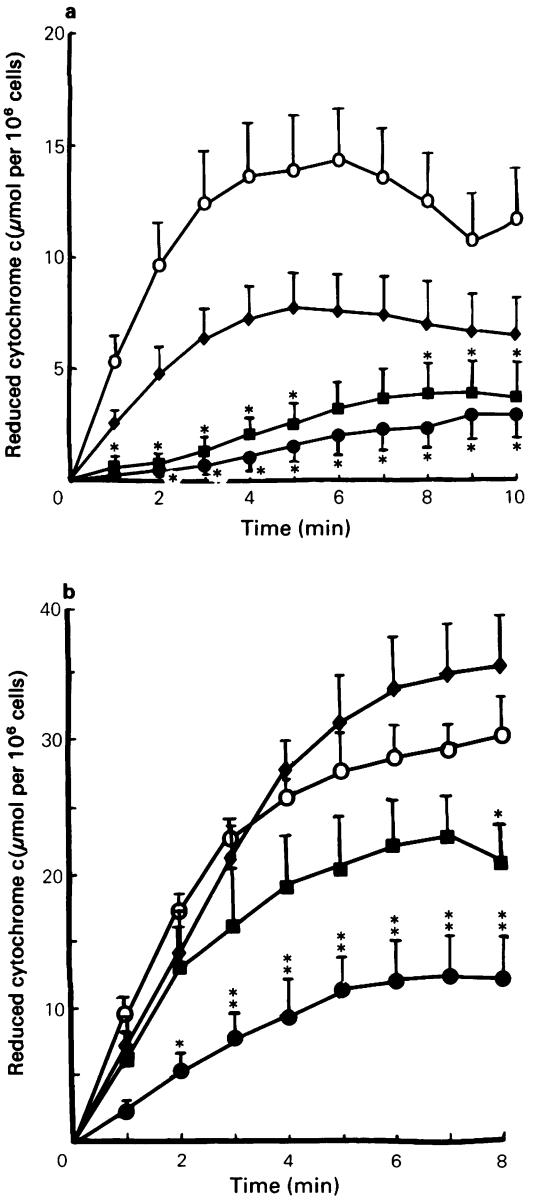


Figure 7 Inhibition by three concentrations of Ro 19-3704 of the production of superoxide anion by macrophages stimulated *in vitro* with 1 μM of Paf (a) or N-formyl-methionyl-leucyl-phenylalanine (FMLP, b). Effect of drug solvent (○), Ro 19-3704 3 μM (◆), 10 μM (■) and 30 μM (●). Results are expressed as μmol of reduced cytochrome c per 10^6 cells; vertical lines indicate s.e. mean. The spontaneous reduction of cytochrome c in the absence of the stimulating agent is represented as control in Figure 8. * $P < 0.05$, ** $P < 0.01$, $n = 6$–18 for Paf experiments; $n = 4$–20 for FMLP experiments.

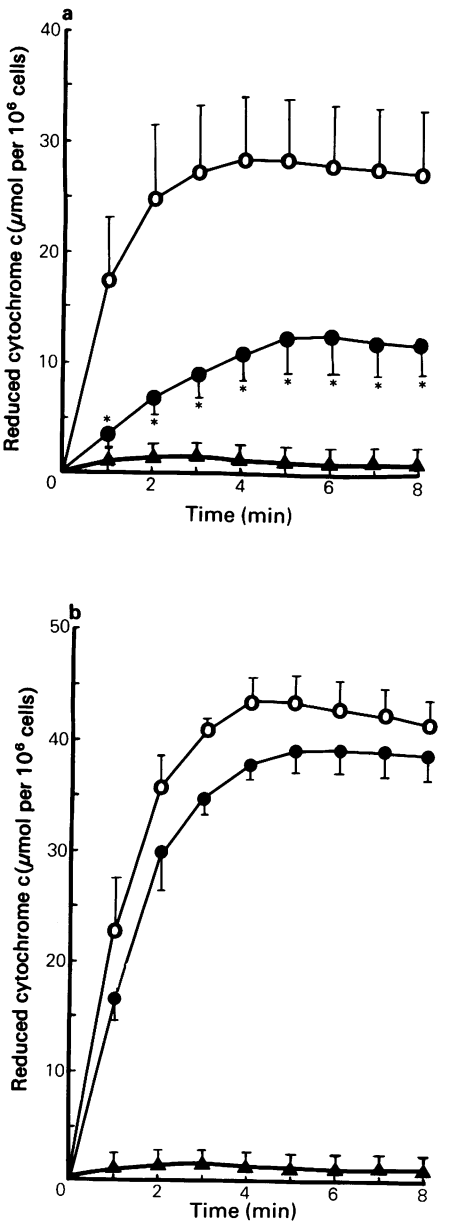


Figure 8 Effects of Ro 18-7715 on the production of superoxide anion by macrophages stimulated *in vitro* by 1 μM Paf (a) or N-formyl-methionyl-leucyl-phenylalanine (FMLP, b). Control (▲) and effect of drug solvent (○), Ro 18-7715 30 μM (●). Results are expressed as μmol of reduced cytochrome c per 10^6 cells; vertical lines indicate s.e. mean. The spontaneous reduction of cytochrome c in the absence of the stimulating agent was below the limit of measurement. * $P < 0.05$, $n = 6$–16 for Paf experiments; $n = 6$–12 for FMLP experiments.

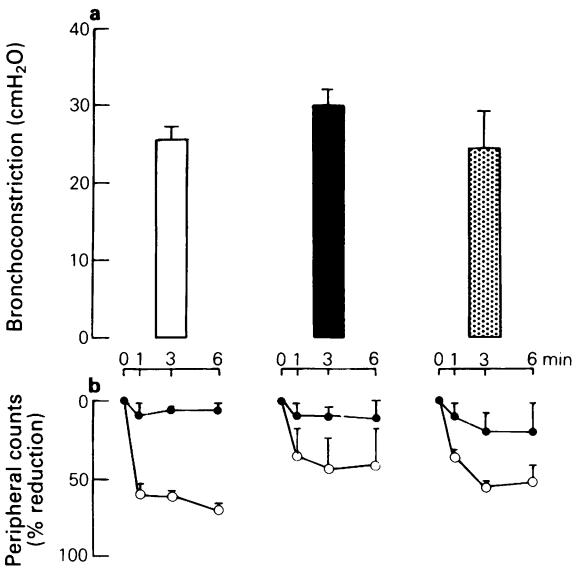


Figure 9 Interference by Ro 19-3704, administered by i.v. or aerosol route, of the bronchoconstriction (cmH_2O) (a), thrombocytopenia and leukopenia (% reduction) (b), induced by ovalbumin ($1\,mg\,kg^{-1}$, i.v.). In (a), control (open column), effect of Ro 19-3704 $3\,mg\,kg^{-1}$, i.v. (solid column), $3\,mg\,ml^{-1}$, 10 min aerosol (stippled column). In (b), (●) platelets and (○ polymorphonuclear leukocytes. $n = 3$–4; vertical lines indicate s.e. mean.

aerosol (Figure 6), only a slight, and statistically non-significant, reduction of the anaphylactic bronchoconstriction was observed. It was not possible to administer a higher concentration of Ro 19-3704 as an aerosol because it induced by itself a bronchoconstriction. The administration of Paf or of antigen by aerosol is followed by minor effects on the number of circulating blood cells (Lefort *et al.*, 1984; Cirino *et al.*, 1986) and accordingly, these parameters were not evaluated with respect to the antagonist.

Effects of i.v. Ro 19-3704 on the pulmonary effects of i.v. Paf and antigen at the microscopic level

As described previously (Lellouch-Tubiana *et al.*, 1985), the i.v. administration of Paf is followed by microthrombosis in pulmonary vasculature (Figure 10a). Ro 19-3704 suppressed these effects of Paf and as a consequence no thrombi or migrating platelets were observed in the pulmonary microvessels (Figure 10b).

Ultrastructural examination of the lungs showed the presence of large platelet thrombi in the bronchial arteries (Figure 10c) and in the alveolar capillaries after an i.v. injection of $1\,mg\,kg^{-1}$ of ovalbumin to the passively sensitized animals, with polymorphonuclear leukocytes associated with the platelets (Figure 10c). At this time, extravascular degranulated platelets were found in the vicinity of the bronchial smooth muscle (Figure 10d). The i.v. administration of Ro 19-3704 ($3\,mg\,kg^{-1}$), failed to prevent the appearance of these antigen-induced extravascular platelet aggregates.

Discussion

Ro 19-3704 is a powerful Paf antagonist, which was very effective in blocking rabbit platelet aggregation. It was also selective, since at high concentrations it failed to interfere with other platelet-stimulating substances, including arachidonate, confirming lack of anti-cyclo-oxygenase activity. The only exception to this selectivity is inhibition by Ro 19-3704 of adrenaline-induced human platelet activation (Fouque *et al.*, 1986): this is a newly described property of this and other structurally related Paf antagonists, which is not shared by other antagonists with dissimilar structures.

Ro 19-3704 and CV 3988, which was used as a reference compound in some experiments, blocked Paf-induced bronchoconstriction and the accompanying thrombocytopenia and leukopenia. Bronchoconstriction after i.v. Paf is platelet-dependent (Vargaftig *et al.*, 1980) and thus inhibition of bronchoconstriction and thrombocytopenia by both antagonists suggests that they suppress bronchoconstriction because they inhibit intravascular Paf-induced platelet activation. In agreement, Ro 19-3704 prevented the accumulation of platelet aggregates in the guinea-pig lung microvessels by Paf, as well as the other morphological effects described by Lellouch-Tubiana *et al.* (1985), such as platelet migration to the extravascular space.

Bronchoconstriction by inhaled Paf was also suppressed by Ro 19-3704 given intravenously or by aerosol. This indicates that inhaled Ro 19-3704 prevents the interaction between Paf and the cells responsible for triggering bronchoconstriction. In contrast, when Ro 19-3704 was given by aerosol, neither the bronchoconstriction nor the reduction in blood cell counts following i.v. Paf were affected. This confirms that extra-pulmonary effects (i.e., intravascular thrombocytopenia) account for bronchoconstriction and also shows that not enough Ro 19-3704 reaches the circulation upon inhalation of the concentration used.

Alveolar macrophages collected after administration of Paf by aerosol are desensitized to *in vivo*

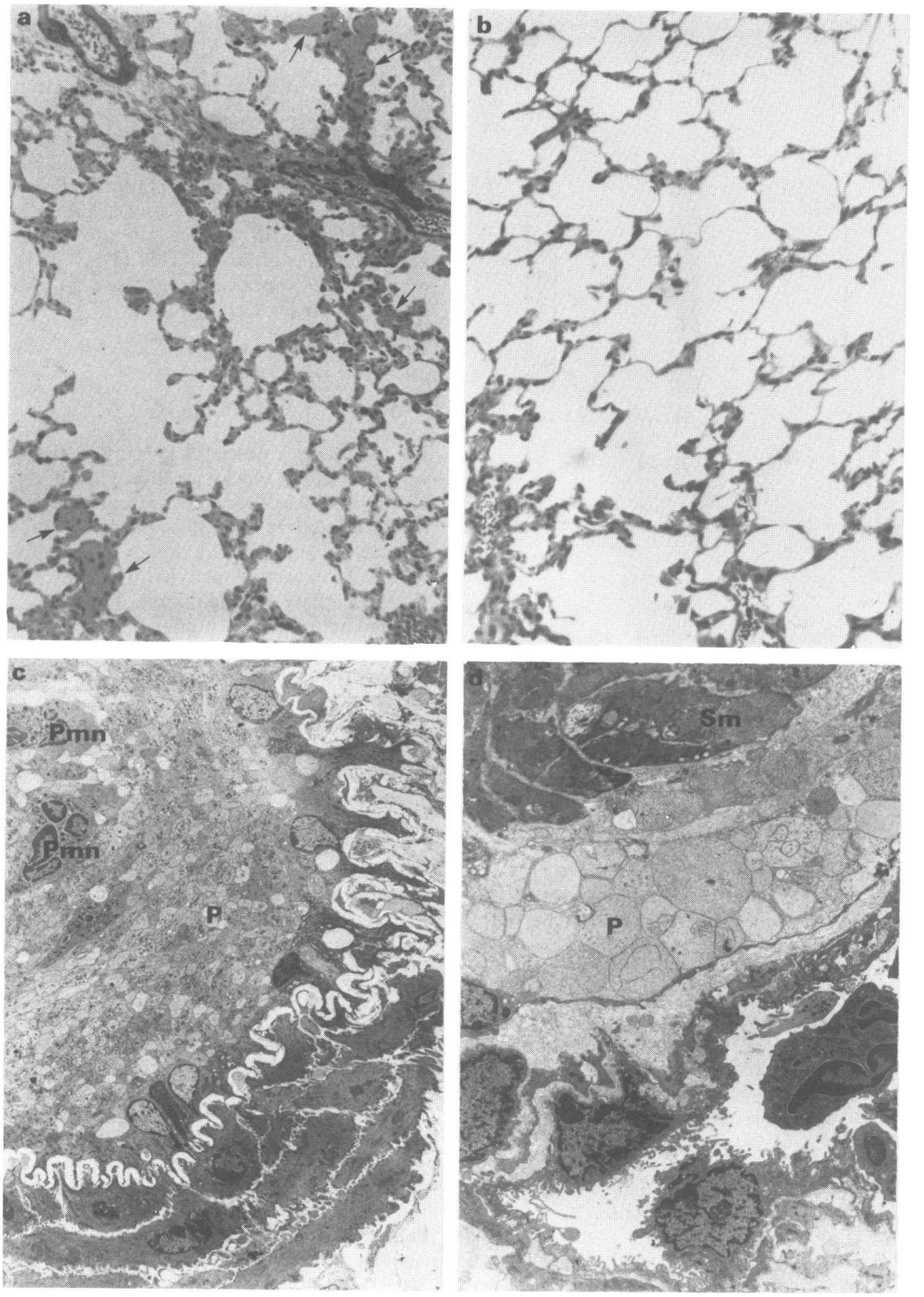


Figure 10 (a) Paf-induced intravascular pulmonary platelet aggregation. Widespread platelets are aggregated in the pulmonary microvasculature 1 min after the i.v. injection of Paf and obstruct the lumen of alveolar capillaries (⟶). Toluidine blue, × 100. (b) Platelet aggregates are not present in the pulmonary microvasculature when Ro 19-3704 is injected before the i.v. injection of Paf. Toluidine blue, × 150. (c) Electron micrograph of a bronchial arteriole 1 min after the i.v. injection of ovalbumin. The lumen is totally obstructed by a platelet thrombus (P) associated with polymorphonuclear leucocytes (Pmn). × 1100. (d) Electron micrograph of the bronchial submucosa 1 min after the i.v. injection of ovalbumin. Degranulated platelet aggregates (P) are noted at the vicinity of the bronchial smooth muscle (Sm). × 2900.

or *ex vivo* re-exposure to Paf itself (Maridonneau-Parini *et al.*, 1985). This led us to study the effect of Ro 19-3704 on the production of superoxide ions and to show that it is dose-dependently blocked by Ro 19-3704. Nevertheless, Ro 19-3704 surprisingly also inhibited the production of superoxide ions by macrophages exposed to the secretagogue tripeptide FMLP. This additional property of Ro 19-3704 is not directly related to antagonism of Paf and is probably accounted for by its ability to scavenge superoxide anions by its thiazolium group. Indeed, the other Paf antagonist Ro 18-7715, an analogue of Ro 19-3704 possessing a pyridinium group which was not expected to behave as a scavenger, failed to inhibit the production of superoxide ion by FMLP, at concentrations which were effective against Paf.

Ro 19-3704 was next evaluated as a potential anti-anaphylactic compound. Because another Paf antagonist blocks bronchoconstriction of passive anaphylactic shock (Lagente *et al.*, 1987), this model was chosen to test Ro 19-3704. Passive anaphylaxis was thought to be dependent upon the formation of antibodies of the IgE class (Detsouli *et al.*, 1985; Carmo *et al.*, 1986), but we recently found that the serum from the actively sensitized animals also contains IgG antibodies, which confer upon guinea-pig lung strips *in vitro* and *ex vivo* the ability to contract when exposed to the antigen (Desquand *et al.*, unpublished observations). When Ro 19-3704 $3\,mg\,kg^{-1}$ was administered intravenously, i.e., a dose markedly above the $200\,\mu g\,kg^{-1}$ which blocked the effects of aerosol or i.v. Paf, bronchoconstriction by i.v. ovalbumin was not modified, but bronchoconstriction by an aerosol of ovalbumin was suppressed. The fact that more Ro 19-3704 was needed to block anaphylaxis than to block the effects of Paf itself is probably not because of non-specific properties of the antagonist additional to Paf antagonism, since the doses of other antagonists needed to inhibit anaphylactic bronchoconstriction are also at least ten fold above those needed to block the effects of Paf (Lagente *et al.*, 1987; Pretolani *et al.*, 1987).

In contrast, the same concentration of Ro 19-3704 ($3\,mg\,ml^{-1}$) which antagonized (but did not suppress) bronchoconstriction due to aerosol Paf, failed to interfere with the effects of aerosol albumin. These results are consistent with the hypothesis that i.v. Ro 19-3704 reaches all the sites with which aerosol ovalbumin interacts. This was not the case when the antagonist itself was administered in aerosol form, but the concentrations of Ro 19-3704 needed to suppress the effects of intra-pulmonary antigen might not have been reached.

The systemic effects of i.v. ovalbumin are probably accounted for by both Paf-dependent and independent mechanisms. This probably explains the persistence of shock despite the administration of Ro 19-3704. This explanation is supported by the fact that anaphylactic leukopenia is not blocked by Ro 19-3704, whereas leukopenia induced by Paf is suppressed.

In summary, the novel Paf antagonist Ro 19-3704 displays anti-anaphylactic properties when administered intravenously against antigen administered in aerosol form. This suggests that local bronchopulmonary anaphylaxis is highly dependent upon the *in situ* generation of Paf and indeed, Fitzgerald *et al.* (1985) and Nucci (1986) demonstrated that when antigen was given directly into the trachea large amounts of Paf were formed, which is much less apparent when the intravascular route is used (Rotilio *et al.*, 1983).

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