Br. J. Pharmac. (1987), **91**, 799–802

Bronchial and vascular effects of Paf in the rat isolated lung are completely blocked by WEB 2086, a novel specific Paf antagonist

*Jorge Casals-Stenzel, **Jurgen Franke, ***Tilman Friedrich & †[1]Jurgen Lichey

*Boehringer Ingelheim KG, 6507 Ingelheim and **Department of Nuclear Medicine, ***Department of Cardiopulmonology and †Department of Cardiopulmonology and Society of Immunology and Asthma Research (GIA), Klinikum Steglitz, Free University of Berlin, 1 Berlin 45, F.R.G.

1 The effect of the platelet-activating factor (Paf) antagonist, WEB 2086, on Paf-induced increase of pulmonary artery perfusion pressure (Pp), bronchial inflation pressure (Pi) and wet-to-dry lung weight ratios (W/D) was investigated in the rat isolated lung.

2 Lungs were perfused with Krebs-Ringer solution (KRS) as controls or with KRS containing WEB 2086 (0.1, 1.0, 10.0 or 100 $\mu g\,ml^{-1}$) and then injected with a bolus of 20 μg Paf.

3 A dose-related inhibition of the Paf-induced increase of Pp, Pi and W/D was observed, being almost maximal for the 10.0 $\mu g\,ml^{-1}$ and complete for the 100 $\mu g\,ml^{-1}$ doses of WEB 2086 when compared to controls.

4 It is concluded that WEB 2086 is a highly effective and specific Paf antagonist in the pulmonary vasculature and bronchial tract.

Introduction

Platelet-activating factor (Paf) is an ether phospholipid with profound biological activities in both the airways and pulmonary vasculature (Voelkel *et al.*, 1982; Hamasaki *et al.*, 1984; Lichey *et al.*, 1984). An observed increase in bronchial and pulmonary artery resistance elicited by Paf is a major feature of IgE-mediated anaphylaxis (Pinckard *et al.*, 1979). Thus, Paf might be considered a putative mediator, e.g. in hypersensitivity lung reactions. The inhibition of these effects by a specific Paf antagonist would add further evidence to this hypothesis.

Recently, a variety of Paf antagonists became available, for which specific activity was claimed. In this study, we have examined WEB 2086, a thieno-triazolo-diazepine derivative (Casals-Stenzel *et al.*, 1986) for its ability to antagonize Paf-induced effects on bronchial inflation and pulmonary artery perfusion pressures in the isolated perfused lung of the rat.

Methods

Preparation of isolated lungs

Adult female Wistar rats (250–300 g) were anaesthetized by intraperitoneal administration of 60 mg sodium pentobarbitone (Nembutal, Abbott, Ingelheim, F.R.G.). Lungs were isolated according to the method described by Hauge (1968), with some modifications. Briefly, after tracheostomy, the chest was opened during positive-pressure ventilation. After being freed from the surrounding tissue, the lungs were left *in situ*. A plastic inflow cannula was inserted into the pulmonary artery through an incision in the wall of the right ventricle. The pulmonary outflow was collected via an outflow cannula placed in the left atrium. The whole animal was enclosed in a humidified chamber that was kept at constant temperature. The time elapsing between the arrest of the animal's circulation and the begining of the lung perfusion was less than 7 min.

Ventilation

The animals were ventilated via a tracheal cannula after anaesthesia and before isolating the lungs at a constant tidal volume (3–5 ml) and a constant stroke rate (30 min^{-1}). Ventilation was done by a Starling pump (Braun-Melsungen, Melsungen, F.R.G.) using humidified room air. Thus, a peak inspiratory pressure between 12 and 15 cmH_2O was achieved at the beginning of the experiments. Inflation pressure was

[1] Author for correspondence

measured from a sidearm of the tracheal cannula with a Statham P23AA transducer and monitored on a multichannel recorder (Schwartzer, München, F.R.G). End-expiratory pressure was kept at 3 cmH_2O by a water seal.

Perfusion

After isolating the lung circulation, 1.0 ml of saline with 500 u of heparin was injected into the pulmonary artery. Thereafter, perfusion was started by a roller pump (Heidolph, F.R.G.) in an open non-recirculating system with constant volume inflow (0.05 ml $min^{-1} g^{-1}$ body wt.). Perfusion pressure was measured via a sidearm of the pulmonary artery cannula with a Statham P23AA transducer. Before injecting the pharmacological agents, the lung preparations were allowed to equilibrate for 15 min under constant perfusion and ventilation. The perfusion media used in the equilibration period were identical to those used in the respective experiments as described in the protocol. Non-perfused areas were noted by a visible, incomplete washout of blood. Such lungs were discarded.

Experimental protocol

Seven series of experiments were performed, using 6 to 9 animals for each series. The total measuring period after the equilibration time was 10 min. In series 1–4, lungs were perfused for 7 min with Krebs-Ringer solution (KRS) containing the Paf antagonist WEB 2086 at four different concentrations (0.1, 1.0, 10.0 and 100 $\mu g\ ml^{-1}$ respectively). Thereafter, 20 μg of Paf was injected as a bolus of 0.3 ml in saline BSA solution (2.5 mg BSA ml^{-1}) via a second sidearm of the pulmonary cannula.

Series 5–7 served as control experiments: in series 5, lungs were perfused with KRS only. After 7 min Paf (20 μg) was injected and perfusion continued for a further 3 min. In series 6, lungs were perfused for the whole observation period of 10 min with KRS but without Paf. In series 7, lungs were perfused for 10 min with KRS containing WEB 2086 (100 $\mu g\ ml^{-1}$) only.

Wet-to-dry lung weight ratios

At the end of the experiments lungs were removed from the preparation, and their wet weight measured. They were then immediately transferred to an 80°C oven, where they remained until a constant dry weight was achieved.

Statistical analysis

Results were expressed as the means of ΔPp, ΔPi and W/D ± s.e.mean. The two-tailed Wilcoxon-Mann-Whitney test was used to assess the statistical significance of the results. Significance was accepted when $P < 0.05$.

Materials

Synthetic platelet-activating factor (Paf) (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was purchased from Bachem (Bubendorf, Switzerland), WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno-(3,2-f) (1,2,4)-triazolo-(4,3-a)(1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone) was obtained from Boehringer Ingelheim KG (F.R.G.), and bovine serum albumin from Behring (Marburg, F.R.G.).

Results

Pulmonary artery perfusion pressure

The increase in pulmonary artery perfusion pressure (ΔPp) after a bolus injection of 20 μg of Paf was significantly lower in the WEB 2086 pretreated lungs than in controls (series 5). This holds true for all but the lowest concentration used ($P < 0.01$). The reduction of ΔPp was dose-related, almost complete in group 3 rats and complete in series 4 animals (Figure 1).

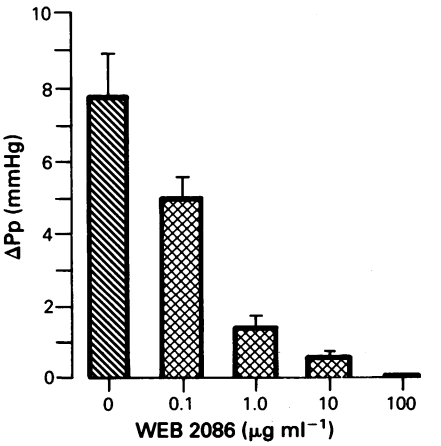

Figure 1 Inhibition of Paf-induced increase of pulmonary artery perfusion pressure (ΔPp) by WEB 2086. Each column represents the mean increase in Pp (expressed in mmHg) of 6 isolated Krebs-Ringer perfused rat lung preparations after a bolus injection of 20 μg Paf. Cross-hatched columns: pretreatment with different concentrations of WEB 2086; hatched column: without pretreatment.

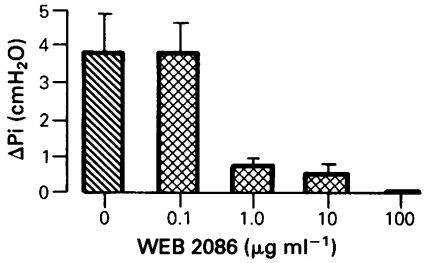

Figure 2 Inhibition of Paf-induced release of bronchial inflation pressure (ΔPi) by WEB 2086. Each column represents the mean increase in Pi (expressed in cmH_2O) of 6 isolated Krebs-Ringer perfused rat lung preparations after a bolus injection of 20 μg Paf. Cross-hatched columns: pretreatment with different concentrations of WEB 2086; hatched column: without pretreatment.

Bronchial inflation pressure

There was also a significant ($P < 0.01$), dose-related reduction of bronchial inflation pressure increase (ΔPi) in the animals in series 2–4 when compared to controls (series 5) (Figure 2).

Wet-to-dry lung weight ratios

Wet/dry weight ratios (W/D) were significantly lower in the rats in series 3 and 4 than in controls (series 5).

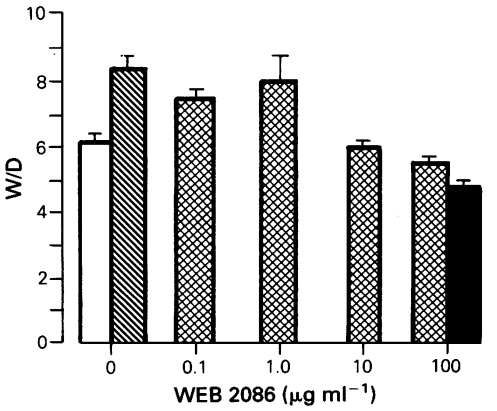

Figure 3 Inhibition of Paf-induced increase of wet-to-dry lung weight ratios (W/D) by WEB 2086. Each column represents the mean W/D of 6 isolated Krebs-Ringer perfused rat lung preparations. Crossed hatched columns: pretreatment with different concentrations of WEB 2086 and bolus injection of 20 μg Paf; hatched column: bolus injection of 20 μg Paf without pretreatment; open column: perfusion with Krebs-Ringer solution only; solid column: perfusion with Krebs-Ringer solution containing 100 μg ml^{-1} WEB 2086 only.

Series 6 (perfusion with KRS only and without injecting Paf) and series 7 (perfusion with KRS containing 100 μg ml^{-1} WEB 2086 and without injecting Paf) animals were not significantly different from the treated animals in series 3 and 4 (Figure 3).

Discussion

Recent studies have shown that Paf may be involved in various respiratory and cardiovascular diseases such as bronchial asthma (Denjean *et al.*, 1983; Cuss *et al.*, 1986) and 'adult respiratory distress syndome' (ARDS), respectively (Doebber *et al.*, 1985; Terashita *et al.*, 1985). It is suggested that in these disorders, after activation of phospholipase A_2, Paf may act either directly or via the arachidonic acid cascade resulting in enhanced biosynthesis of prostanoids. This sequence of reactions can lead to systemic hypotension, pulmonary artery hypertension, bronchoconstriction and pulmonary oedema (Halonen *et al.*, 1981; Mojarad *et al.*, 1983), characteristics of diseases like bronchial asthma and ARDS. Most recently, it has been shown that Paf induces not only immediate bronchoconstriction but also sustained non-specific hyperresponsiveness, a hallmark of bronchial asthma (Cuss *et al.*, 1986).

Thus, a specific Paf antagonist capable of blocking this biochemical key step would further clarify the pathophysiological role and therapeutic potential of Paf in humans.

Our study shows that the Paf antagonist WEB 2086 can reduce or inhibit completely the increase in pulmonary perfusion pressure, bronchial inflation pressure and the formation of lung oedema after a bolus injection of Paf in a dose-related manner. The rat isolated lung preparation was run as an open, platelet-free system to prevent production and re-entry of secondarily released mediators and thus to relate the pulmonary effects only to the amount of Paf injected.

At a concentration of 10 μg ml^{-1} WEB 2086 was able to block almost completely all parameters measured (ΔPp 93.6%; ΔPi 86.8%; W/D 100%). Thus, in our experimental set-up, WEB 2086 (molecular weight 456), in an almost equimolar dose, is a highly effective antagonist of Paf. Using the Paf antagonists SRI-441 in guinea-pigs (Farley *et al.*, 1986), SRI 63-072 and 63-119 in rats (Handley *et al.*, 1986), a much higher antagonist:agonist ratio was necessary to block effectively the Paf-induced bronchoconstriction *in vivo*. In an experimental set-up more comparable to our own, the Paf antagonist L-652,731 was used to block the Paf-induced increase of pulmonary perfusion pressure, bronchial inflation pressure and of wet/dry lung weight ratio in the guinea-pig isolated lung (Cox *et al.*, 1986). The dose of

this antagonist was also several orders of magnitude higher than the administered dose of Paf.

Thus, as far as we know, WEB 2086 is the most effective and specific Paf antagonist under the conditions studied, offering promising properties for further pathophysiological and clinical studies.

We thank Dr K.-H Weber, Boehringer Ingelheim KG for the supply of WEB 2086.

References

CASALS-STENZEL, J., MUACEVIC, G. & WEBER, K.-H. (1986). WEB 2086, a new and specific antagonist of platelet activating factor (Paf) in vitro and in vivo. *Naunyn-Schmiedebergs Arch. Pharmac.*, **334,** (Suppl.) R44.

COX, C.P., SATA, T., LIU, L.-W. & SAID, S.I. (1986). L-652, a specific antagonist of platelet-activating factor (Paf), prevents Paf-induced lung injury in guinea-pigs. *Am. Rev. Respir. Dis.*, **133,** (Suppl.) A278.

CUSS, F.M., DIXON, C.M.S. & BARNES, P.J. (1986). Effect of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet*, **ii,** 189–192.

DENJEAN, A., ARNOUX, B., MASSE, R., LOCKHART, A. & BENVENISTE, J. (1983). Acute effects of intratracheal administration of platelet-activating factor in baboons. *J. appl. Physiol.*, **55,** 799–804.

DOEBBER, TH.W., WU, M.S., ROBBINS, J.C., CHOY, B.M., CHANG, M.N. & SHEN, T.Y. (1985). Platelet activating factor (Paf) involvement in endotoxin-induced hypotension in rats. Studies with Paf-receptor antagonist kadsurenone. *Biochem. Biophys. Res. Commun.*, **127,** 799–808.

FARLEY, C., MELDEN, M.K., VAN VALEN, R.G., DEACON, R.W., ANDERSON, R.C., LEE, M.L., SAUNDERS, R.N. & HANDLEY, D.A. (1986). In vivo inhibition by SRI 63-119 in Paf-induced hemoconcentration and bronchoconstriction in the guinea-pig. *Fedn. Proc.*, **45,** 855.

HALONEN, M., PALMER, J.D., LOHMAN, I.C., McMANUS, L.M. & PINCKARD, R.N. (1981). Differential effects of platelet depletion on the physiologic alterations of IgE anaphylaxis and acetyl glyceryl ether phosphorylcholine infusion in the rabbit. *Am. Rev. Respir. Dis.*, **124,** 416–421.

HAMASAKI, Y., NOJARAD, M., SAGA, T., TAI, H.H. & SAID, S.I. (1984). Platelet-activating factor raises airway and vascular pressures and induces edema in lungs perfused with platelet-free solution. *Am. Rev. Respir. Dis.*, **129,** 742–746.

HANDLEY, D.A., DEACON, R.W., TOMESCH, J.C., KOLETAR, J.M. & SAUNDERS, R.N. (1986). Pharmacological profiles of a novel Paf antagonist: SRI 63–441. *Fedn. Proc.*, **45,** 685.

HAUGE, A. (1968). Role of histamine in hypoxic pulmonary hypertension in the rat. *Circulation Res.*, **22,** 371–383.

LICHEY, J., FRIEDRICH, T., FRANKE, J., NIGAM, S., PRIESNITZ, M., & OEFF, K. (1984). Pressure effects and uptake of platelet-activating factor in the isolated rat lung. *J. appl. Physiol.*, **57,** 1039–1044.

MOJARAD, M., HAMASAKI, Y. & SAID, S.I. (1983). Platelet-activating factor increases pulmonary microvascular permeability and induces pulmonary edema. A preliminary report. *Bull. Eur. Physiopath. Resp.*, **19,** 253–256.

PINCARD, R.N., FARR, R.S. & HANAHAN, D.J. (1979). Physiochemical and functional identity of rabbit platelet-activating factor (Paf) released in vivo during IgE anaphylaxis with Paf-released in vitro from IgE senstitized basophils. *J. Immunol.*, **123,** 1847–1857.

TERASHITA, Z.-I., IMURA, Y., NISHIKAWA, K. & SUMIDA, S. (1985). Is platelet activating factor (Paf) a mediator of endotoxin shock? *Eur. J. Pharmac.*, **109,** 257–261.

VOELKEL, N.F., WORTHEN, S., REEVES, J.T., HENSON, P.M. & MURPHY, R.C. (1982). Non-immunological production of leukotrienes induced by platelet-activating factor. *Science*, **218,** 286–288.

(Received January 5, 1987.
Revised March 25, 1987.
Accepted April 21, 1987.)