Br. J. Pharmac. (1986), **87**, 287–289

Kadsurenone distinguishes between different platelet activating factor receptor subtypes on macrophages and polymorphonuclear leucocytes

Günter Lambrecht & Michael J. Parnham[1]

A. Nattermann & Cie. GmbH, Pharmacology Research, P.O. Box 35 01 20, D-5000 Cologne 30, W. Germany

The effects of the antagonist kadsurenone on the platelet activating factor (Paf)-induced chemiluminescence of guinea-pig peritoneal macrophages and on pig peripheral blood leucocyte aggregation were compared. Linearity and slopes of unity of the Schild plots confirmed the competitive nature of the antagonism by kadsurenone. pA_2 values indicated a 91 fold lower affinity of kadsurenone for leucocyte Paf receptors than for those in macrophages. It is concluded that these two types of Paf receptors are not identical and are provisionally designated Paf_1 and Paf_2 receptors, respectively.

Introduction Platelet activating factor (1-O-alkyl-2-0-acetyl-sn-glycero-3-phosphoryl choline; Paf, Paf-acether or AGEPC) is released from a number of different inflammatory cells and amongst its other inflammatory actions, it is a potent stimulator of polymorphonuclear leucocyte (p.m.n.l.) functions (Goetzl *et al.*, 1980; Shaw *et al.*, 1981; O'Flaherty & Wykle, 1983). The binding of Paf to platelet receptors and its human p.m.n.l. aggregating and degranulating actions have recently been shown to be antagonized by the plant-derived compound, kadsurenone (Shen *et al.*, 1985). We have shown that Paf also stimulates the oxidative burst in guinea-pig macrophages (Hartung *et al.*, 1983; Parnham & Leyck, 1983) and initiated the present study on the effects of kadsurenone on pig p.m.n.l. aggregation and guinea-pig macrophage chemiluminescence in order to compare the antagonistic properties of kadsurenone on the two cell types. We found that kadsurenone discriminates between the Paf receptors of these cells.

Methods *Guinea-pig peritoneal macrophage chemiluminescence (CL)* Male Pirbright white guinea-pigs (200–300 g, Hagemann, Extertal, W. Germany), kept under constant light and temperature and food and water *ad libitum*, each received an i.p. injection of 1.5 ml heat-killed *Corynebacterium parvum* (7 mg ml^{-1}, Deutsche Wellcome, Bergwedel) and were killed with CO_2 8 days later. Cells were harvested by peritoneal lavage with 100 ml phosphate buffered saline (PBS), pH 7.4, centrifuged (300 *g*, 10 min) and resuspended, after counting in a Coulter counter, to 5×10^6 cells ml^{-1} in balanced salt solution (BSS), pH 7.3 (Mishell & Dutton, 1967) containing 2% bovine serum albumen. Suspensions consisted of 80% macrophages (Pappenheim staining) with a viability of 90% (Trypan blue). Aliquots (200 µl) of cell suspensions were preincubated for 10 min at 37°C with or without kadsurenone (200 µl in BSS) and the CL enhancer luminol (100 µl, final conc. 10^{-5} mol l^{-1}), stimulated by addition of synthetic hexadecyl-*rac*-Paf (100 µl in BSS) and the course of CL generation measured in a six-channel Berthold Biolumat LB 9505 coupled to an Apple IIa computer. Peak CL responses were recorded. Dose-response curves to Paf in the presence and absence of increasing concentrations of kadsurenone were performed on the same cell suspension.

Pig peritoneal blood leucocyte aggregation Venous blood (approx. 2 l) was obtained by exsanguination of pigs from a slaughterhouse and mixed (10:1 v/v) with 0.9% NaCl containing sodium citrate (4.5 g l^{-1}) and heparin (12,500 u l^{-1}). P.m.n.l. (after dilution of blood 1:5 in PBS) were prepared by density centrifugation (400 *g*, 20 min; Ficoll-Paque, Pharmacia) and subsequent sedimentation (1 h, room temp.) on 1% dextran/0.9% NaCl (dextran mol. wt. 250,000, Roth, W. Germany), remaining erythrocytes being lysed (5 min) in ice cold buffer (NH_4Cl, 8.3 g l^{-1}, $KHCO_3$, 1 g l^{-1}; EDTA, 37 mg l^{-1}). Suspensions consisted of 90–95% p.m.n.l. with a viability of 90%. After washing (10 min, 300 *g*), p.m.n.l. (2×10^7 ml^{-1} PBS containing 0.5% bovine serum albumen) were kept at room temperature before preincubating 250 µl aliquots with 250 µl BSS and 25 µl kadsurenone (a gift from Dr T-Y Shen of Merck, Sharp & Dohme, U.S.A.) in PBS for 5 min at 37°C. Aggregation was triggered by addition of Paf (20 µl in PBS) and recorded in a Payton aggregometer. Peak responses

[1] Author for correspondence.

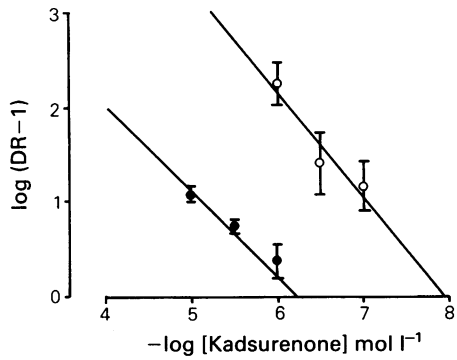

Figure 1 Least squares fitted Schild plots for antagonism by kadsurenone of Paf (10^{-10}–10^{-4} mol l^{-1})-induced chemiluminescence (CL) from guinea-pig activated peritoneal macrophages (○) and aggregation of pig peripheral blood p.m.n.l. (●). Each point is the mean of values obtained from 3–5 (for CL) and 5 (for aggregation) separate experiments; s.e.mean shown by vertical lines. Ordinate scale: log (DR − 1) where DR = dose-ratio measured from the displacement of the Paf dose-response curve by kadsurenone. Abscissa scale: negative logarithm of the molar concentration of kadsurenone. The slopes of both regression lines do not differ significantly from − 1.

were recorded and dose-response curves to synthetic hexadecyl-*rac*-Paf in the presence and absence of increasing concentrations of kadsurenone performed on the same cell suspension.

pA_2 calculations For both assays, dose-ratios (DR) for kadsurenone-induced shifts in the dose-response curves to Paf were estimated, Schild plots (Arunlakshana & Schild, 1959) constructed, and slopes determined from least-squares estimates of the resultant regression lines, using an IBM personal computer programme (EPISTAT). To determine pA_2 values, a second approach was used in which the slopes of the regression lines were constrained to − 1 because this is consistent with the competitive theory which connects pA_2 with $-\log K_B$, the dissociation constant of the antagonist-receptor complex (Tallarida *et al.*, 1979).

Results Paf produced concentration-related p.m.n.l. aggregation and macrophage CL responses with EC_{50} values of 17.89 ± 5.69 nmol l^{-1} (mean ± s.e.mean, $n = 11$) and 1.72 ± 0.65 nmol l^{-1} ($n = 5$), respectively. Maximal responses were obtained on p.m.n.l. at a Paf concentration of 6.72 ± 4.40 μmol l^{-1} and on macrophages at > 100 μmol l^{-1}. Kadsurenone caused a parallel shift to the right of the dose-response curve to Paf on both cells, with no depression of its maximal responses.

Figure 1 shows Schild plots for the action of kadsurenone on the two cell types. Both regressions are linear and the mean slopes of the lines for both preparations are not significantly different from unity: macrophage CL = − 1.10 ± 0.10 (mean ± s.e.mean, $n = 12$); p.m.n.l. aggregation = − 0.91 ± 0.04 ($n = 15$). These findings are consistent with a competitive antagonism between Paf and kadsurenone. Therefore, the calculated pA_2 values can be considered to be a representation of the affinity of kadsurenone for Paf receptors. The mean pA_2 values ± s.e.mean were: macrophage CL = 8.12 ± 0.15 ($n = 12$); p.m.n.l. aggregation = 6.16 ± 0.07 ($n = 15$).

Discussion Shen *et al.* (1985) have reported pA_2 values for kadsurenone of 6.28 with Paf-induced rabbit platelet aggregation and 6.32 with Paf-induced human p.m.n.l. aggregation. These values correspond closely to the value of 6.16 obtained by us with Paf-induced pig p.m.n.l. aggregation, suggesting that the receptors for Paf on these three cell types are similar in structure. The pA_2 value for kadsurenone of 8.12 obtained with guinea-pig macrophage CL indicates that kadsurenone has about a 100 fold higher affinity for macrophage Paf receptors, suggesting that these receptors differ from platelet and neutrophil receptors. The EC_{50} values for Paf obtained in the present study on p.m.n.l. and macrophages indicate that Paf has an approximately 10 fold greater potency at macrophage receptors. These findings point to a Paf receptor heterogeneity, and we propose that pig and human p.m.n.l. and rabbit platelet Paf receptors (Paf_1 receptors) as opposed to guinea-pig macrophage Paf receptors (Paf_2 receptors) represent sub-types of receptors of this inflammatory mediator.

For a final classification of Paf receptors, it would be helpful to know if this parallel extends to other Paf antagonists and agonistic Paf analogues, respectively.

We thank Ms C. Bittner and Ms S. Kindt for excellent technical assistance, Dr W. Rücker for assistance with computer programming and Dr H.-H. Lautenschläger for synthesizing hexadecyl-*rac*-Paf. We also thank Dr T.-Y. Shen of Merck, Sharp and Dohme, U.S.A. for a gift of kadsurenone. This study was partially supported by a project grant from the BMFT (Bundesministerium für Forschung und Technologie).

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

GOETZL, E.J., DERIAN, C.K., TAUBER, A.J. & VALONE, F.H. (1980). Novel effects of 1-O-hexadecyl-2-acyl-*sn*-glycero-3-phosphorylcholine mediators of human leucocyte function: Delineation of the specific roles of the acyl sub-

stituents. *Biochem. biophys. Res. Commun.*, **94**, 881–888.

HARTUNG, H.-P., PARNHAM, M.J., WINKELMANN, J., ENGLBERGER, W. & HADDING, U. (1983). Platelet activating factor (PAF) induces the oxidative burst in macrophages. *Int. J. Immunopharmac.* **5**, 115–121.

MISHELL, R.I. & DUTTON, R.W. (1967). Immunization of dissociated spleen cell cultures from normal mice. *J. exp. Med.*, **126**, 423–442.

O'FLAHERTY, J.T. & WYKLE, R.L. (1983). Biology and biochemistry of platelet activating factor. *Clin. Rev. Allergy*, **1**, 353–367.

PARNHAM, M.J. & LEYCK, S. (1983). Inflammatory and macrophage-stimulating actions of PAF-acether in normal and immunologically challenged rodents. In *Platelet Activating Factor*. INSERM Symposium No. 23. ed. Benveniste, J. & Arnoux, B. pp. 187–193. Amsterdam: Elsevier Science Publishers.

SHAW, J.O., PINCKARD, R.N., FERRIGNI, K.S., McMANUS, L.M. & HANAHAN, D.J. (1981). Activation of human neutrophils with 1-0-hexadecyl/octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (platelet activating factor). *J. Immunol.*, **127**, 1250–1255.

SHEN, T.Y., HWANG, S.-B., CHANG, M.N., DOEBBER, T.W., LAM, M.-H.T., WU, M.S., WANG, X., HAN, G.Q. & LI, R.Z. (1985). Characterization of a platelet-activating factor receptor antagonist isolated from haifenteng (*Piper futokadsura*): Specific inhibition of *in vitro* and *in vivo* platelet activating factor-induced effects. *Proc. natn. Acad. Sci. U.S.A.*, **82**, 672–676.

TALLARIDA, R.J., COWAN, A. & ADLER, M.W. (1979). pA_2 and receptor differentiation. A statistical analysis of competitive antagonism. *Life Sci.*, **25**, 637–654.

(Received October 28, 1985.
Accepted November 18, 1985.