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Inhibition by the anti-mitotic drug doxorubicin of platelet-activating-factor-induced late eosinophil accumulation in rats

Sandra Perez [a], Janaina Machado [a], Renato Cordeiro [a], Marco Martins [a], Radovan Borojevic [b], Patrícia Silva [a,*]

[a] *Departamento de Fisiologia e Farmacodinâmica, Instituto Oswaldo Cruz, FIOCRUZ; Av. Brasil, no. 4365, Manguinhos, CEP 21045-900, Brazil*
[b] *Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

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Abstract

Platelet-activating factor (PAF) has been shown, in the rat model of pleural inflammation, to induce the generation of an intermediate proteic factor able to cause eosinophil proliferation in vitro. This study was undertaken to investigate the effect of the anti-mitotic compound doxorubicin on PAF-induced eosinophilia in rats, in order to evaluate the contribution of local cell proliferation to this phenomenon. The late eosinophil infiltration caused by another chemoattractant leukotriene B_4 was used for comparison. We observed that local treatment with doxorubicin (20 and 40 μg/cavity), given 6 h after PAF (1 μg/cavity), suppressed the eosinophil accumulation within 24 h, whilst only the higher dose was effective when the drug was given 12 h post-PAF. An effect on chemotaxis was ruled out, since local doxorubicin (40 μg/cavity) failed to modify the eosinophil migration noted 24 h after leukotriene B_4 (0.5 μg/cavity) and the neutrophil/eosinophil infiltration noted at 6 h after PAF injection. Transfer of the pleural fluids collected 6 h after PAF from donors to recipient rats caused significant eosinophil accumulation in the recipient rats, an effect which was inhibited by the co-administration of doxorubicin (40 μg/cavity). No inhibitory effect was noted when the drug was given 6 h after the pleural fluids were transferred. We also found no change in the number of blood or bone marrow eosinophils after PAF stimulation. We conclude that doxorubicin selectively impaired the late eosinophil accumulation triggered by PAF in the pleural cavity of rats, clearly indicating that local cell proliferation seems to contribute to the development of this inflammatory response.

Keywords: Eosinophil; PAF (platelet-activating factor); Doxorubicin; Proliferation

1. Introduction

Platelet-activating factor (PAF) has been shown to have a wide spectrum of pharmacological actions and it is considered to be a potential mediator in some physiopathological reactions (Venable et al., 1993). In allergic diseases, treatment with the PAF receptor antagonist Y-24180, 4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6*H*-thieno[3,2*f*-[1,2,4,]triazolo[4,3*a*][1,4] diazepine, improves bronchopulmonary hyperresponsiveness to methacholine in asthmatic patients (Hozawa et al., 1995). Attention has been specifically focused on the ability of PAF to interfere with several functions of mature eosinophils, including the induction of cell migration (Schweizer et al., 1996), release of eosinophil cationic protein and oxygen reactive molecules (Elsner et al., 1995) and increase in the production of leukotriene C_4 (Bruijnzeel et al., 1987). PAF has been shown to be involved in the induction of DNA synthesis and in the increase of microbicidal activity in immature leucocytes (Kudo et al., 1991), and to induce proliferation of haematopoietic progenitors for basophil and eosinophil granulocytes by a mechanism dependent on the generation of interleukin 3 (Saito et al., 1993). The presence of PAF as well as PAF-acetylhydrolase was detected in some haematopoietic organs in humans and rodents (Denizot and Praloran, 1994; Denizot et al., 1995a,b), reinforcing the idea that PAF has a relevant role in the haematopoietic system.

We have previously demonstrated that intrapleural administration of PAF to rats induces a late eosinophil

* Corresponding author. Telefax: +55-21-590-9490; E-mail: patmar@gene.dbbm.fiocruz.br

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accumulation, a response which is dependent on the synthesis of an intermediate proteic factor (Silva et al., 1991). We have also shown that this factor has the ability to induce the proliferation, but not differentiation, of eosinophils in vitro by a mechanism independent of interleukin 5, interleukin 3 and granulocyte-macrophage colony stimulating factor (GM-CSF) (Perez et al., 1993). The current study was undertaken to investigate the contribution of cell proliferation to the late eosinophil accumulation elicited by PAF in the pleural cavity of rats. We used the local administration of the antimitotic compound doxorubicin, which has the ability to bind to DNA and to block its replication and transcription (Zunino and Capranico, 1990).

2. Materials and methods

2.1. Animals and induction of pleurisy

We used Wistar rats of both sexes (150–200 g) obtained from the Oswaldo Cruz Foundation breeding facilities. The reaction was produced by the intrapleural (i.pl.) injection of 0.1 ml PAF (1 μg/cavity) or leukotriene B_4 (0.5 μg/cavity) diluted with sterile 0.9% NaCl (saline) containing 0.01% bovine serum albumin. Control animals received the same volume of vehicle. At different times (1–24 h), the animals were killed with an overdose of ether and the pleural cavity was opened and washed with 3 ml heparinized saline (10 U/ml). The recovered volume was measured in a graduated syringe; in the rare cases of haemorrhage, the pleural wash was discarded. Total leucocytes were counted in a Coulter counter ZM and expressed as cells $\times 10^6$/cavity.

2.2. Blood and bone marrow leucocyte counts

Peripheral blood samples were obtained from the tail vein at 1, 6 and 24 h after the i.pl. injection of PAF. For the bone marrow analysis, the animals were killed as described above, the right femur was removed and the bone marrow was gently harvested with 10 ml of RPMI 1640 containing heparin (20 UI/ml). Total leucocyte counts were measured as previously mentioned and differential cell counts were measured in blood smears and bone marrow cytospin preparations stained with May–Grünwald and Giemsa solutions.

2.3. Protein quantification

The fluid recovered from the pleural cavity was centrifuged for 10 min at 2000 rpm and the total protein content was quantified in the supernatant, using the Biuret technique.

2.4. Pleural wash transfer

Six hours after the i.pl. injection of PAF (1 μg/cavity), the animals were killed and the pleural cavity was opened and washed with 0.5 ml of saline. The collected washes were pooled and centrifuged at 2500 rpm for 15 min at 4°C. The supernatant (0.2 ml/cavity) was injected i.pl. into recipient rats and eosinophil counts were recorded at 24 h as described above. Control recipient animals received the same volume of pleural fluids obtained from donor rats injected with saline.

2.5. Treatment

Doxorubicin (20 and 40 μg/cavity) was diluted with sterile saline and locally administered after 2, 6 or 12 h PAF administration. In the transfer assay, the drug was concomitantly given with the PAF pleural washes. Control animals were only treated with saline. All the drugs were immediately prepared before use.

2.6. Drugs

PAF-acether (1-*O*-hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) was purchased from Bachem (Switzerland) and leukotriene B_4 was from Sigma (St. Louis, MO, USA). Doxorubicin was kindly provided by Farmitalia Carlo Erba (Rio de Janeiro, Brazil).

2.7. Statistical analysis

Data are expressed as means ± standard error of mean (S.E.M.) and statistically analysed by means of the analysis of variance (ANOVA) followed by the Newman–Keuls Student's test. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effect of doxorubicin on the pleurisy induced by PAF

We confirmed that the intrapleural injection of PAF (1 μg/cavity) into naive rats caused the selective accumu-

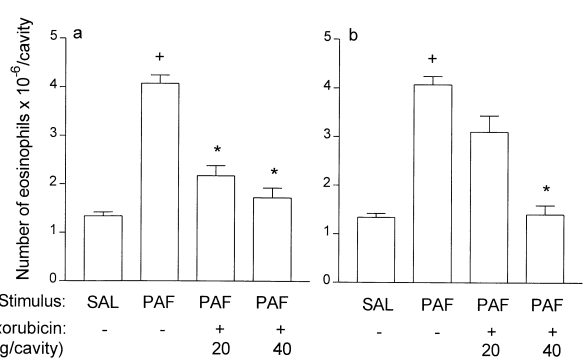

Fig. 1. Effect of local doxorubicin (20 and 40 μg/cavity) on eosinophil accumulation 24 h after PAF injection (1 μg/cavity). Doxorubicin was administered 6 (a) or 12 h (b) after PAF. Results are expressed as means ± S.E.M. from at least seven animals. $^+P < 0.05$ as compared to saline-injected rats; $*P < 0.05$ as compared to PAF-injected rats.

Table 1
Lack of effect of doxorubicin on neutrophil and eosinophil infiltration triggered by PAF

Stimulus	Treatment (μg/cavity)	Neutrophils (10^6 cells/cavity)	Eosinophils (10^6 cells/cavity)
Saline	None	0 ± 0	0.92 ± 0.27
PAF	None	0.850 ± 0.23[a]	2.21 ± 0.42[a]
PAF	Doxo	0.810 ± 0.35	2.03 ± 1.00

Doxorubicin (40 μg/cavity) was administered locally 2 h after the i.pl. injection of PAF (1 μg/cavity) and leucocyte infiltration was analysed at 6 h after pleural stimulation. Values represent the means ± S.E.M. from at least seven animals.
[a] $P < 0.05$ as compared to saline-injected rats.

Table 3
Doxorubicin is not effective in inhibiting eosinophil accumulation induced by leukotriene B_4 (LTB_4)

Stimulus	Treatment	Eosinophils (10^6cells/cavity)
Saline	None	1.26 ± 0.14
LTB_4	None	2.23 ± 0.25[a]
LTB_4	Doxo	2.72 ± 0.23

Doxorubicin (40 μg/cavity) was administered locally 6 h after LTB_4 (0.5 μg/cavity) and eosinophil accumulation was assessed 24 h after the LTB_4 injection. Values represent the means ± S.E.M. from at least seven animals.
[a] $P < 0.05$ as compared to saline-injected rats.

lation of eosinophils within 24 h, an effect which was preceded by exudation and a mixed neutrophil/eosinophil infiltration at 6 h. We showed that the local administration of the anti-mitotic drug doxorubicin (20 and 40 μg/cavity), 6 h after PAF stimulation, significantly decreased the eosinophilia noted at 24 h. Under the same conditions, only the highest dose was effective when the drug was given 12 h after PAF (Fig. 1). Conversely, the early accumulation of neutrophils and eosinophils, noted 6 h after PAF administration, was not sensitive to doxorubicin (40 μg/cavity) administered 2 h after the PAF injection (Table 1). In control experiments, local treatment with doxorubicin did not affect the basal protein content and leucocyte population of the pleural cavity 6 and 24 h following drug administration (Table 2).

3.2. Effect of doxorubicin on the eosinophil influx induced by leukotriene B_4

Although effective in the suppression of the PAF-induced late eosinophil accumulation, local treatment with doxorubicin (40 μg/cavity) did not inhibit the increase in the pleural eosinophil numbers detected 24 h after leukotriene B_4 (0.5 μg/cavity) (Table 3).

Table 2
Doxorubicin fails to modify the basal protein content and leucocyte population of the pleural cavity of naive rats

Treatment	Dose (μg/cavity)	Protein (mg/cavity)	Total leucocytes (10^6cells/cavity)	
			6 h	24 h
Saline	–	4.43 ± 0.25	10.00 ± 0.81	5.83 ± 1.06
Doxorubicin	20	3.75 ± 1.19	7.62 ± 0.96	6.01 ± 0.55
Doxorubicin	40	4.26 ± 0.42	8.94 ± 0.93	5.26 ± 0.97

The pleural protein content was analysed at 6 h and total leucocyte numbers at 6 and 4 h after drug administration. Values represent the means ± S.E.M. from at least seven animals.

3.3. Effect of doxorubicin on the eosinophilia caused by the transfer of PAF pleural washes

When the pleural washes from PAF-stimulated rats were transferred to normal recipient animals, the number of eosinophils recovered was significantly increased in the pleural fluid of recipients ($P < 0.001$). The local administration of doxorubicin into recipient rats, immediately before transfer of the 6-h of PAF pleural washes, decreased the late eosinophilia (Fig. 2). In contrast, when doxorubicin was given 6 h after the transfer, no inhibition of eosinophil accumulation was detected (Fig. 2).

3.4. Effect of i.pl. PAF on blood and bone marrow eosinophil numbers

In order to establish a potential correlation between the pleural eosinophil accumulation noted after PAF and alterations in the number of eosinophils in peripheral blood and bone marrow, we further analysed the cell content of both compartments. As indicated in Fig. 3, the number of

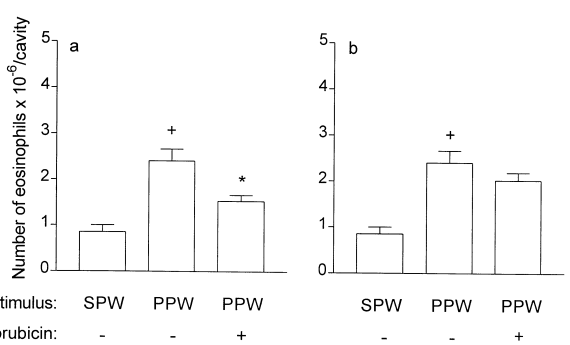

Fig. 2. Effect of local doxorubicin (40 μg/cavity) on eosinophil accumulation 24 h after the transfer of pleural washes from PAF-injected rats (PPW) or pleural washes from saline-injected rats (SPW). The drug was administered immediately before (a) or 6 h (b) after the transfer. Results are expressed as means ± S.E.M. from at least seven animals. $^{+}P < 0.05$ as compared to SPW-injected rats; $*P < 0.05$ as compared to PPW-injected rats.

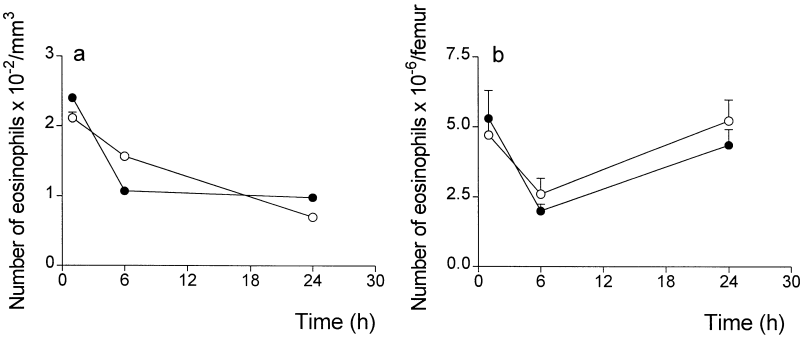

Fig. 3. Absence of changes in the eosinophil population in either peripheral blood (a) or bone marrow (b) at different times after i.pl. injection of PAF (1 μg/cavity) (•) or saline (◦) into rats. Results are expressed as means ± S.E.M. from at least seven animals.

circulating or bone marrow eosinophils remained unaltered 1, 6 and 24 h following PAF pleural stimulation.

4. Discussion

This study was undertaken to gain a better understanding of the mechanism of the pleural eosinophil accumulation induced by PAF. Recent reports have shown that PAF has the ability to increase eosinophil accumulation at the site of inflammation, enhancing their adhesion to bronchial epithelial cells by an increase in the expression of adhesion molecules (Sato et al., 1997) or through a synergistic effect on eosinophil migration elicited by eosinophil-active cytokines (Okada et al., 1997). Simultaneously, tissue eosinophil accumulation can be maintained by the local inhibition of apoptosis (Simon et al., 1997) or by the enhancement of eosinophil proliferation. The latter mechanism has been correlated with the presence of circulating eosinophil precursors in allergic airway diseases and their increased sensitivity to interleukin 5 (Denburg et al., 1995; Denburg et al., 1997; Sehmi et al., 1997), as well as with the in situ eosinophil proliferation in inflammatory tissue reactions to helminths (El-Cheikh and Borojevic, 1990; Maruyama et al., 1990).

We used a potent anti-mitotic drug which is largely used in the treatment of cancer and which is reported to inhibit replication and transcription processes by binding to DNA (Keizer et al., 1990; Richardson and Johnson, 1997). We observed that in both concentrations assayed doxorubicin inhibited pleural eosinophilia when administered 6 h after PAF, but when administered 12 h after PAF only the highest dose was able to inhibit the eosinophilia. Moreover, we questioned whether the drug was able to inhibit the chemotaxis of different inflammatory cells. Local treatment with the highest dose had no effect on the eosinophil accumulation induced by leukotriene B_4. In addition, it did not inhibit the early neutrophil and eosinophil accumulation noted 6 h after PAF administration, showing that the early eosinophil accumulation is essentially due to chemotaxis.

A previous study showed that the pleural washes collected after PAF treatment were able to induce eosinophil proliferation in hematopoietic liquid culture systems (Perez et al., 1993), indicating that the pleural fluid acquired a growth-stimulating activity for eosinophils after PAF stimulation. Since doxorubicin could also act by the local inhibition of protein synthesis, we further tested its effect on the experimental model developed by Silva et al. (1991), which showed that pleural fluid collected 6 h after PAF injection induced a late and selective accumulation of eosinophils in the pleural fluid of recipient rats. Doxorubicin inhibited the eosinophilia in the recipient rats when injected concomitantly with PAF, but not when it was injected 6 h later, indicating that the drug suppressed pleural eosinophilia in recipient rats injected with the eosinophilotactic factor generated in PAF-injected donor rats.

We also questioned whether i.pl. injected PAF had a direct effect on eosinophil numbers in blood or bone marrow, but no differences were detected at any time ranging from 1 to 24 h after stimulation. In conclusion, our results indicate that local eosinophil proliferation seems to contribute to the pleural eosinophil accumulation induced by PAF.

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References

Bruijnzeel, P.L., Kok, P.T., Hamelink, M.L., Kijne, A.M., Verhagen, J., 1987. Platelet-activating factor induces leukotriene C4 synthesis by purified human eosinophils. Prostaglandins 34, 205–214.

Denburg, J.A., Wooley, M.J., Ellis, R., Dahlback, M., O'Byrne, P.M.,

1995. Allergen-induced changes in bone marrow progenitors and airway responsiveness in dogs. Int. Arch. Allergy Immunol. 107, 239–241.

Denburg, J.A., Inman, M.D., Wood, L., Ellis, R., Sehmi, R., Dahlback, M., O'Byrne, P., 1997. Bone marrow progenitors in allergic airways diseases: studies in canine and human models. Int. Arch. Allergy Immunol. 113, 181–183.

Denizot, Y., Praloran, V., 1994. PAF and haematopoiesis: 1. 5-Fluorouracil induces PAF production in haematopoietic organs of rats. Mediators Inflamm. 3, 23–25.

Denizot, Y., Dupuis, F., Comte, L., Dulery, C., Praloran, V., 1995a. PAF and hematopoiesis: IV. Modifications of spleen and thymus PAF contents after a single dose of the chemotherapeutic drug 5-fluorouracil in mice. Cancer Lett. 27, 185–189.

Denizot, Y., Trimoreau, F., Dupuis, F., Verger, C., Praloran, V., 1995b. PAF and haematopoiesis: III. Presence and metabolism of platelet-activating factor in human bone marrow. Biochem. Biophys. Acta 16, 55–60.

El-Cheikh, M.C., Borojevic, R., 1990. Extramedullar proliferation of eosinophil granulocytes in chronic schistosomiasis mansoni is mediated by a factor secreted by inflammatory macrophages. Infect. Immun. 58, 816–821.

Elsner, J., Dichmann, S., Kapp, A., 1995. Activation of the respiratory burst in human eosinophils by chemotaxins requires intracellular calcium fluxes. J. Invest. Dermatol. 105, 231–236.

Hozawa, S., Haruta, Y., Ishioka, S., Yamakido, M., 1995. Effects of a PAF antagonist, Y-24180, on bronchial hyperresponsiveness in patients with asthma. Am. J. Respir. Crit. Care Med. 152, 1198–1202.

Keizer, H.G., Pinedo, H.M., Schuurhuis, G.J., Joenje, H., 1990. Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. Pharmacol. Ther. 47, 219–231.

Kudo, I., Kato, T., Hayashi, K., Yanoshita, R., Ikizawa, K., Huda, H., Inoue, K., 1991. Guinea pig bone marrow cells treated with platelet-activating factor generate factor(s) which affects their DNA synthesis and microbicidal activity. Lipids 26, 1065–1070.

Maruyama, H., Hig, A., Asami, M., Owashi, M., Nawa, Y., 1990. Extramedullary eosinopoiesis in the liver of *Schistosoma japonicum*-infected mice, with reference to hemopoietic stem cells. Parasitol. Res. 76, 461–465.

Okada, S., Kita, H., George, T.J., Gleich, G.J., Leiferman, M., 1997. Transmigration of eosinophils through basement membrane components in vitro: Synergistic effects of platelet-activating factor and eosinophil-active cytokines. Am. J. Respir. Cell Mol. Biol. 16, 455–463.

Perez, S.A.C., Silva, P.M.R., Martins, M.A., El-Cheikh, M.C., Cordeiro, R.S.B., Borojevic, R., 1993. Eosinophil granulocyte proliferation induced by an intermediate factor generated in the pleural cavity of rats injected with platelet-activating factor-acether. Int. Arch. Allergy Immunol. 102, 368–374.

Richardson, D.S., Johnson, S.A., 1997. Anthracyclines in haematology: preclinical studies, toxicity and delivery systems. Blood Rev. 11, 201–203.

Saito, H., Koshio, T., Yanagihara, Y., Akiyama, K., Shida, T., 1993. Platelet-activating factor-induced augmentation of production on eosinophil-lineage cells in haematopoietic precursor cells obtained from human umbilical cord blood. Int. Arch. Allergy Immunol. 102, 195–199.

Sato, M., Takizawa, H., Kohyama, T., Ohtoshi, T., Takafuji, S., Kawasaki, S., Tohma, S., Ishii, A., Shoji, S., Ito, K., 1997. Eosinophil adhesion to human bronchial epithelial cells: regulation by cytokines. Int. Arch. Allergy Immunol. 113, 203–205.

Schweizer, R.C., van Kessel-Welmers, B.A., Warringa, R.A., Maikoe, T., Raajmakers, J.A., Lammers, J.W., Koenderman, L., 1996. Mechanisms involved in eosinophil migration. Platelet-activating factor-induced chemotaxis and interleukin-5-induced chemokinesis are mediated by different signals. J. Leukocyte Biol. 59, 347–356.

Sehmi, R., Wood, L.J., Watson, R., Foley, R., Hamid, Q., O'Byrne, P.M., Denburg, J.A., 1997. Allergen-induced increases in IL-5 receptor alpha-subunit expression on bone marrow-derived CD34+ cells from asthmatic subjects. A novel marker of progenitor cell commitment toward eosinophilic differentiation. J. Clin. Invest. 100, 2466–2475.

Silva, P.M.R., Martins, M.A., Faria Neto, H.C., Cordeiro, R.S.B., Vargaftig, B., 1991. Generation of an eosinophilotactic activity in the pleural cavity of platelet-activating factor-injected rats. J. Pharmacol. Exp. Ther. 257, 1039–1044.

Simon, H., Yousefi, S., Schranz, C., Schapowal, A., Bacher, C., Blaser, K., 1997. Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. J. Immunol. 158, 3902–3908.

Venable, M.E., Zimmerman, G.A., McIntyre, T.M., Prescott, S.M., 1993. Platelet-activating factor: a phospholipid autacoid with diverse actions. J. Lipid Res. 34, 691–702.

Zunino, F., Capranico, G., 1990. DNA topoisomerase II as the primary target of anti-tumor anthracyclines. Anti-Cancer Drug Des. 5, 307–317.