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Review

THE EFFECTS OF AN AUTOCRINE LOOP MEDIATED BY PLATELET-ACTIVATING FACTOR (PAF) IN HEC-1A CELLS ARE REVERTED BY UTEROGLOBIN.

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Key words : platelet-activating factor. uteroglobin, HEC-1, endometrial carcinoma
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Platelet-Activating Factor and HEC-1A cells

Platelet-Activating Factor (PAF), a pro-inflammatory phospholipid, is produced in high amount by endometrial cells both in basal conditions and following different stimulations[1), 2)]. In the same tissue, specific PAF receptors have been demonstrated[3)]. Several studies have suggested a role for PAF in implantation[4), 5)] although more recent evidences indicate a prominent role of this phospholipid in angiogenesis[6)].

We have studied PAF production, expression of PAF receptors and the role of PAF/PAF receptor system in growth processes in the human endometrial adenocarcinoma cell line HEC-1A, which was established by Kuramoto et al, in 1972[7)].

Early studies performed by our group, demonstrated that HEC-1A cells are able to synthesize PAF[8), 9)] at least through the remodelling pathway of PAF synthesis. Indeed, the activity of PAF acetyltransferase, the main enzyme involved in PAF synthesis through the remodelling pathway together with phospholipase A2 (PLA2)[10)], can be measured both in basal conditions as well as following stimulation with the calcium ionophore A23187[9)] and P[8)]. Of interest, a high proportion of newly synthesised PAF was found in the external medium indicating that HEC-1A cells secrete the phospholipid[9)]. The mass spectrometry pattern of PAF-like material produced by HEC-1A cells is identical to that of deuterated standard PAF, demonstrating that it is bioactive.

To demonstrate the presence of specific PAF receptors in HEC-1A cells, binding studies were performed using [^{3}H]-PAF as tracer. Results of these studies are consistent with the presence of two binding sites for PAF, one with high affinity (1.7 nM) and low capacity and the other with low affinity (9.7 μM) and high capacity[9)].

We evaluated the effect of PAF on some intracellular signal transdution pathways related to cell proliferation. First, we evaluated the effect on intracellular calcium concentrations ($[Ca^{2+}]_i$) in Fura-2 loaded HEC-1A cells. We found that PAF determines an increase in $[Ca^{2+}]_i$ in HEC-1A cells with an EC50 of 5.6 nM, consistent with interaction with the high affinity site detected with binding studies. On the other hand, the effect appears to be mediated by specific

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receptors, because the PAF receptor antagonist L659,989 dose-dependently inhibited the effect of PAF on $[Ca^{2+}]_i$ increase. PAF-induced increase in $[Ca^{2+}]_i$ appears to be due to both phospholipase C-mediated release from intracellular stores as well as to influx from extracellular medium, as demonstrated by experiments conducted in calcium-free medium and using BAPTA to chelate intracellular calcium[9]. Next, we determined the effect of PAF on protein tyrosine phosphorylation. We found that PAF induces time- and concentration-dependent increase of tyrosine phosphorylation of several proteins in a molecular weight range of 42-150 kDa[11], suggesting the activation of tyrosine kinases by PAF in these cells. The prominent effect observed on tyrosine phosphorylation in a protein band of 42-44 kDa prompted us to verify whether it corresponds to proteins of the mitogen-activated protein kinase (MAPK, also known as extracellular-signal regulated kinases, ERKs) family which migrate at this molecular weight. These kinases era important intermediary enzymes in converting extracellular signals into intracellular responses linked to cell proliferation[12] which have been shown to be stimulated by PAF in several cell types[13, 14]. By the use of specific anti-ERK antibodies, we demonstrated that PAF induces a time- and dose-dependent increase in the phosphorylation of p42 and p44 ERKs[11]. Immunokinase assay demonstrated that the phospholipid also stimulates the activity of the two enzymes[11], suggesting that PAF can stimulate pathways linked to proliferation in these cells. We also evaluated the effect of PAF on the expression of the early oncogene c-fos. We found that PAF induces a time- (15-60 min) and dose- (1nM-10 μM) dependent increase of c-fos mRNA expression. Again, this effect was completely abolished by the PAF receptor antagonist L659,989 suggesting the involvement of specific PAF receptors in this effect. In addition, we found that PAF-induced c-fos mRNA expression was strongly inhibited in PKC depleted cells either in basal conditions and after PAF stimulation[11], indicating an involvement of PKC isoenzymes on this PAF effect. Conversely, the effect of PAF on c-fos mRNA expression was not affected by treatment with general inhibitors of tyrosine kinases, such as genistein or the erbastatin analogue methylhydroxycinnamate[11]. On the other hand, our group has demonstrated that both genistein and methylhydroxycinnamate inhibit proliferation of HEC-1A cells by inducing cell cycle arrest and apoptotosis[15] and thus lack of interference of these two compounds with PAF-stimulated proliferative pathways is not surprising.

Consistent with the result on c-fos mRNA expression and activation of ERKs, PAF dose-dependently stimulated thymidine uptake in HEC-1A cells[9, 11]. The EC50 for this effect was 0.7 μM, thus suggesting the involvement of the low affinity binding site detected in our binding studies. Interestingly, we found a dramatic inhibitory effect on thymidine uptake by the treatment with the PAF receptor antagonist L659,989[9]. This results suggested us the possibility that PAF, produced and released into the medium by HEC-1A cells, could stimulate autrocrinally their proliferation, and that the PAF antagonist is able to interrupt such effect by competitive interaction with the same receptors. To gain insight on this hypothesis, we evaluated the effect of L659,989 on cell number during 5 days of treatment. We found that cell growth curve was strongly reduced starting from the first day of treatment with 10 μM L659,989, indicating that the antagonist is able to inhibit HEC-1A cell growth. We next asked whether such effect could be due to an impairment of the cell cycle progression. To answer this question, HEC-1A cells were synchronized with aphidicolin, a potent non-specific inhibitor of DNA polymerase α and subsequently washed and allowed to re-enter the cell cycle. Indeed, 18 h after aphidicolin release, the synchronized cells were back in G_0/G_1 phase of the cell cycle. Conversely, L659,989-treated cells were partially accumulated in G_2/M phase. Similarly, after 36 h in the presence of the PAF antagonist cells were still in G_0/G_1 phase instead that in S phase as in control cells[9].

We also showed evidence for a possible involvement of the IGF system in the effect of PAF. Indeed, we found that PAF stimulates the release of IFG binding proteins (IGFBPs) in the culture medium of HEC-1A cells[16], indicating that the IGF system may be also involved in the proliferative effect of PAF.

Overall, our results indicate the existence of an

autocrine loop mediated by PAF in HEC-1A cells that is involved in supporting the growth of these cells.

Uteroglobin and HEC-1A cells

Uteroglobin (UG) is a secretory protein of 15.8 kDa, initially discovered in the rabbit uterine fluid during early pregnancy and subsequently found in several extrauterine tissues. UG is highly expressed in human endometrium where it appears to be involved in maintaining pregnancy and facilitating labour[17]. One of the main effects of UG is inhibition of PLA2[18], an enzyme involved both in prostaglandin and PAF synthesis. UG has also been shown to inhibit PAF acetyltransferase activity[19]. Through inhibition of these enzymes, UG may regulate cell proliferation, maintaining a low proliferative rate in cancer cells, as demonstrated in lung tumor cells, where overexpression of UG gene reverts the malignant phenotype[20] or in prostate tumors, where lack of expression of UG is associated to a more favourable prognosis[21]. In agreement with these data, UG-knock out mice revealed a high incidence of malignancies (Murkeijee A, personal communication). In this light we evaluated whether lack of UG expression could be responsible for the PAF-mediated autocrine proliferative loop observed in HEC-1A cells.

We observed that UG mRNA transcripts and protein could not be detected in HEC-1A cells. Therefore, we transfected HEC-1A cells with an expression vector containing UG cDNA. We selected the transfected clones, and we used some of them to evaluate growth rate as well as acetyltransferase and PLA2 activity. Cells transfected with the empty vector (mock) were used as control, in order to assess the specificity of the effects of UG transfection. The effect of UG expression in HEC-1A cell growth was assessed by two different approaches, i.e. monolayer cloning assay and growth in soft agar. In both assays the transfection with UG determined a dramatic effect on proliferation[22]. In particular, colony formation in soft agar was totally suppressed in UG-transfected cells, indicating the development of a less malignant phenotype. Flow cytometric analysis of DNA content in cells synchronized with aphidicolin demonstrates that this effect is due to a slower progression in the cell cycle. In particular, 24 h after aphidicolin removal about 45% of wild type and mock-transfected cells were back in G0/G1 phase of the cell cycle, whereas most of UG transfected cells were still in G2/M phase[22]. UG transfected cells started replenishing G0/G1 only 12 h later.

To gain insights on whether this effect was due to interruption of PAF-mediated autocrine loop, we evaluated PLA2 and acetyltransferase activity in UG-transfected, mock-transfected and wild-type HEC-1A cells. The activity of both enzymes was strongly reduced in UG-transfected cells, suggesting that UG can disrupt the PAF proliferative loop in HEC-1A cells by reducing PAF synthesis (Fig. 1).

In summary, HEC-1A cells synthesize and secrete PAF which, through binding to specific receptors, promotes by activation of several signal transduction pathways, including PTK, MAPK and PKC, cell proliferation. This autocrine loop may be stopped by UG, through inhibition of PAF synthesis (Fig. 1).

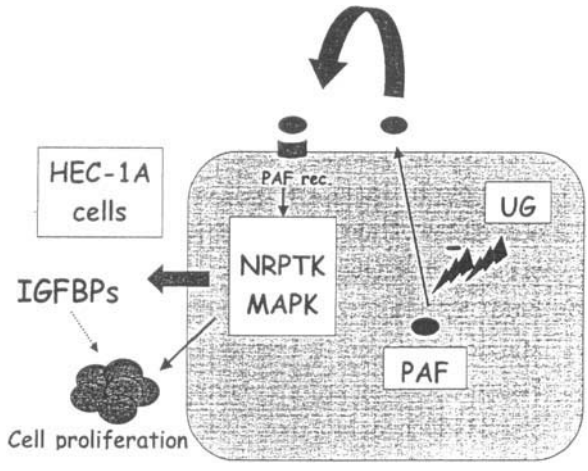

Fig. 1: PAF-mediated autocrine loop in HEC-1A cells.
In wild-type HEC-1A cells [lacking expression of uteroglobin (UG)], PAF, synthesized and released by the cells in the external medium, by interacting with its specific surface receptors activates a cascade of signalling events [non-receptorial tyrosine kinase (NRPTK), mitogen activated protein kinase (MAPK) and protein kinase C (PKC)] leading to proliferation of the cells. PAF also induces the release of IGF binding proteins (IGFBPs) which may also be involved in such proliferative effects. Re-expression of UG by transfection of the cells disrupts PAF synthesis by inhibiting PLA2 and acetyltransferase activity, resulting in interruption of the autocrine loop and decreased proliferative and malignant potential of the cells.

References

1) Alecozay AA, Casslen BG, Riehl RM, Deleon FD, Harper MJ, Silva M, Nouchi TA, Hanahan DJ: Platelet-activating factor in human luteal phase endometrium. Biol Reprod. 1989, 41: 578-86.
2) Alecozay AA, Harper MJ, Schenken RS, Hanahan DJ: Paracrine interactions between platelet-activating factor and prostaglandins in hormonally-treated human luteal phase endometrium *in vitro*. J Reprod Fertil. 1991, 91: 301-12.
3) Kudolo GB, Harper MJ: Characterization of platelet-activating factor binding sites on uterine membranes from pregnant rabbits. Biol Reprod. 1989, 41: 587-603.
4) Spinks NR, O'Neill C: Antagonists of embryo-derived platelet-activating factor prevent implantation of mouse embryos. J Reprod Fertil. 1988, 84: 89-98.
5) Acker G, Braquet P, Mencia-Huerta JM: Role of platelet-activating factor (PAF) in the initiation of the decidual reaction in the rat. J Reprod Fertil. 1989, 85: 623-9.
6) Ko HM, Seo KH, Han SJ, Ahn KY, Choi IH, Koh GY, Lee HK, Ra MS, Im SY: Nuclear factor kappaB dependency of platelet-activating factor-induced angiogenesis. Cancer Res. 2002, 62: 1809-14.
7) Kuramoto H, Tamura S, Notake Y: Establishment of a cell line of human endometrial adenocarcinoma *in vitro*. Am. J. Obestet. Gynecol. 1972, 114: 1012-9
8) Baldi E, Bonaccorsi L, Finetti G, Luconi M, Muratori M, Susini T, Forti G, Serio M, Maggi M: Platelet-activating factor in human endometrium. J Steroid Biochem Mol Biol. 1994, 49: 359-63
9) Maggi M, Bonaccorsi L, Finetti G, Carloni V, Muratori M, Laffi G, Forti G, Serio M, Baldi E: Platelet-activating factor mediates an autocrine proliferative loop in the endometrial adenocarcinoma cell line HEC-1A. Cancer Res. 1994, 54: 4777-84.
10) Snyder F, Fitzgerald V, Blank ML: Biosynthesis of platelet-activating factor and enzyme inhibitors. Adv Exp Med Biol. 1996, 416: 5-10.
11) Bonaccorsi L, Luconi M, Maggi M, Muratori M, Forti G, Serio M, Baldi E: Protein tyrosine kinase, mitogen-activated protein kinase and protein kinase C are involved in the mitogenic signaling of platelet-activating factor (PAF) in HEC-1A cells. Biochim Biophys Acta. 1997, 1355: 155-66.
12) Stork PJ, Schmitt JM: Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. Trends Cell Biol. 2002, 12: 258-66.
13) Franklin RA, Mazer B, Sawami H, Mills GB, Terada N, Lucas JJ, Gelfand EW: Platelet-activating factor triggers the phosphorylation and activation of MAP-2 kinase and S6 peptide kinase activity in human B cell lines. J Immunol. 1993, 151: 1802-10.
14) Honda Z, Takano T, Gotoh Y, Nishida E, Ito K, Shimizu T: Transfected platelet-activating factor receptor activates mitogen-activated protein (MAP) kinase and MAP kinase kinase in Chinese hamster ovary cells. J Biol Chem. 1994, 269: 2307-15.
15) Muratori M, Nicoletti I, Vannelli GB, Luconi M, Macorsini E, Serio M, Forti G, Maggi M: Genistein induces a G2/M block and apoptosis in human uterine adenocarcinoma cell lines. Endocrine-related Cancer. 1997, 4: 203-18.
16) Giannini S, Maggi M, Cresci B, Finetti G, Bonaccorsi L, Luconi M, Rotella CM, Forti G, Serio M, Baldi E: Platelet-activating factor enhances production of insulin-like growth factor binding proteins in a human adenocarcinoma cell line (HEC-1A). Gynecol Oncol. 1996, 61: 333-40.
17) Peri A, Dubin NH, Dhanireddy R, Mukherjee AB: Uteroglobin gene expression in the rabbit uterus throughout gestation and in the fetal lung. Relationship between uteroglobin and eicosanoid levels in the developing fetal lung. J Clin Invest. 1995, 96: 343-53.
18) Miele L, Cordella-Miele E, Mantile G, Peri A, Mukherjee AB: Uteroglobin and uteroglobin-like proteins: the uteroglobin family of proteins. J Endocrinol Invest. 1994, 17: 679-92.
19) Camussi G, Tetta C, Bussolino F, Baglioni C: Antiinflammatory peptides (antiflammins) inhibit synthesis of platelet-activating factor, neutrophil aggregation and chemotaxis, and intradermal inflammatory reactions. J Exp Med. 1990, 171: 913-

27.

20) Zhang Z, Kundu GC, Panda D, Mandal AK, Mantile-Selvaggi G, Peri A, Yuan CJ, Mukherjee AB: Loss of transformed phenotype in cancer cells by overexpression of the uteroglobin gene. Proc Natl Acad Sci U S A. 1999, 96: 3963-8.

21) Weeraratna AT, Cajigas JA, Schwartz A, Enquist EG, Manyak MJ, Patierno SR: Loss of uteroglobin expression in prostate cancer: relationship to advancing grade. Clin Cancer Res. 1997, 3: 2295-300.

22) Peri A, Bonaccorsi L, Muratori M, Luconi M, Baldi E, Granchi S, Pesciullesi A, Mini E, Cioppi F, Forti G, Serio M, Miele L, Maggi M: Uteroglobin reverts the transformed phenotype in the endometrial adenocarcinoma cell line HEC-1A by disrupting the metabolic pathways generating platelet-activating factor. Int J Cancer. 2000, 88: 525-34.

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