Platelet-activating factor in cirrhotic liver and hepatocellular carcinoma

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Supported by "La Ligue Nationale Française Contre le Cancer" (Comité de la Corrèze)

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Received: 2005-09-06   Accepted: 2005-10-09

Abstract

AIM: Platelet-activating factor (PAF) is a pro-inflammatory and angiogenic lipid mediator. Here we aimed to investigate levels of PAF, lyso-PAF (the PAF precursor), phospholipase A_2 (PLA$_2$, the enzymatic activity generating lyso-PAF), acetylhydrolase activity (AHA, the PAF degrading enzyme) and PAF receptor (PAF-R) transcripts in cirrhotic liver and hepatocellular carcinoma (HCC).

METHODS: Twenty-nine patients with HCC were enrolled in this study. Cirrhosis was present in fourteen patients and seven had no liver disease. Tissue PAF levels were investigated by a platelet-aggregation assay. Lyso-PAF was assessed after its chemical acetylation into PAF. AHA was determined by degradation of [^{3}H]-PAF. PLA$_2$ levels were assessed by EIA. PAF-R transcripts were investigated using RT-PCR.

RESULTS: Elevated amounts of PAF and PAF-R transcripts 1 (leukocyte-type) were found in cirrhotic tissues as compared with non-cirrhotic ones. Higher amounts of PAF and PAF-R transcripts 1 and 2 (tissue-type) were found in HCC tissues as compared with non-tumor tissues. PLA$_2$, lyso-PAF and AHA levels were not changed in cirrhotic tissues and HCC.

CONCLUSION: While the role of PAF is currently unknown in liver physiology, this study suggests its potential involvement in the inflammatory network found in the cirrhotic liver and in the angiogenic response during HCC.

Mathonnet M, Descottes B, Valleix D, Truffinet V, Labrousse F, Denizot Y. Platelet-activating factor in cirrhotic liver and hepatocellular carcinoma. *World J Gastroenterol* 2006; 12(17): 2773-2778

http://www.wjgnet.com/1007-9327/12/2773.asp

INTRODUCTION

Platelet-activating factor (PAF) is a potent pro-inflammatory lipid mediator that sparks a wide range of immunoregulatory actions[1]. A phospholipase A_2 (PLA$_2$)-dependent process on membrane alkyl-acyl-glycerophosphocholines generates the lyso-PAF precursor, and its subsequent acetylation results in the PAF molecule[2]. Tissue PAF concentrations are physiologically down-regulated by a specific PAF acetylhydrolase activity (AHA)[2]. PAF acts through specific PAF-receptors (PAF-R) present on the membrane of responsive cells[3]. Several experimental animal models have highlighted that the liver PAF content was elevated by various types of injury, including endotoxin exposure[4,5], ischemia-reperfusion[6], hemorrhagic shock[7], and carbon tetrachloride (CCl$_4$)-induced cirrhosis[8,9]; results of all these animal studies leading to the conclusions that PAF is a potent vasoconstrictor and systemic vasodilator. While animal models have reported the involvement of PAF in experimental cirrhosis, no clinical study has directly investigated its values in the human cirrhosis liver.

Several *in vitro* studies have highlighted the potential involvement of PAF in carcinogenesis. Thus, PAF acts on the growth of various human tumor cell lines[10,11], increases adhesiveness of tumor cells to vascular endothelia[12], enhances oncogene expression[13], and can contribute to tumor development by enhancing cell motility and by stimulating the angiogenic response[14,15]. Clinical studies have reported elevated PAF amounts in human breast[16], and colon carcinoma[17,18]. However, PAF was not found in higher amounts in lung[19], and thyroid carcinoma[20], suggesting that over-expression of PAF is not necessarily a universal event in carcinogenesis but may be tumor-specific. At present, to our knowledge,

no study has investigated PAF levels in hepatocellular carcinoma (HCC). HCC is a hypervascular tumor. Several angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiopoietins, have been reported to promote angiogenesis in HCC[21]. Of interest, PAF is known to mediate the effect of these angiogenic growth factors in several *in vitro* and *in vivo* models[22-24], suggesting a putative involvement of PAF in HCC.

To improve our knowledge concerning the role of PAF in the human liver, we investigated the levels of PAF and lyso-PAF, the enzymatic activities implicated in PAF production (i.e., PLA2) and degradation (i.e., AHA) and the presence of PAF-R transcripts in human cirrhotic and HCC tissues.

MATERIALS AND METHODS

Subjects

The procedure of the present study followed the general rules edited by the French National Ethics. Between January 1999 and December 2004, 29 consecutive patients (27 men and 2 females; mean age 62.5 years (range 30-78 years), median age 68 years) with HCC underwent resection in the Department of Surgery of Limoges' CHU. Resectability was preoperatively assessed by ultrasonography, computed tomography and/or magnetic resonance imaging. Intraoperative ultrasonography was performed in all patients. The operative procedures included 4 wedge resections, 10 segmentectomies, 7 left hepatectomies, 6 right hepatectomies and 2 total hepatectomies before liver transplantations. A curative resection, defined as an operation in which all the tumors were macroscopically resected during surgery, was performed in 21 patients (72%). The mean margin from the edge of the tumor was 7.5 ± 1.5 mm. The mean and median tumor size was 7.79 ± 4.6 cm and 8.2 cm, respectively. Twenty tumors (69%) were solitary. Cirrhosis was present in 14 patients (48%), with the cause being alcoholism in 46%, hepatitis C in 35%, hepatitis B in 5%, alcohol and hepatitis in 7%, and unknown in 7%. Two patients had liver fibrosis without cirrhosis, 6 had steatosis, and 7 had no liver disease. Histologically, there were 10 well differentiated HCC (34%), 14 moderately differentiated HCC (48%), 4 poorly differentiated HCC (14%), and 1 fibrolamellar carcinoma, according the histopathologic criteria cited by Kojiro M[25]. Thirteen HCC (44%) had a fibrous capsule that was invaded in 68%. Vascular invasion was present in 19 cases (65%) and 8 HCC presented a high mitosis index. According to pT staging[26], there were 2 pT1, 8 pT2, 13 pT3 and 6 pT4 HCCs. Specimens for the pathologic tissue and the control tissue close to the pathologic one were obtained during the surgical procedure. Specimens were frozen at -80 ℃ until used.

Platelet-activating factor assay

Tissue samples were ethanol-extracted (80% final), purified using thin layer chromatography (TLC), and assayed for PAF activity by aggregation of washed rabbit platelets as previously reported[17-20]. The aggregating activity of samples was measured using a calibration curve obtained with 2.5 to 20 pg of synthetic PAF (Novabiochem, Switzerland). Results were expressed as picograms of PAF per mg of tissue. The lipid compound extracted from blood was further characterised on the basis of its aggregating activity in the presence of 0.1 mmol/L BN 52021 (Tebu, Le Perray-en-Yvelines, France), a specific PAF receptor antagonist and its retention time during TLC.

Assay of lyso-PAF

Lyso-PAF was measured in ethanolic biopsy sample after its chemical acetylation into PAF as previously described[17-20]. Briefly, ethanolic samples were dried, mixed with 200 μL of pyridine and 200 μL of acetic anhydride, and kept overnight in the dark at room temperature. After evaporation of the reagents and removing of the traces of pyridine with chloroform, the dried samples were retrieved with 100 μL of 600 mL/L ethanol, and PAF was bioassayed as described before. The amount of lyso-PAF was established as the difference between the quantity of PAF measured before and after acetylation of the samples. Results were expressed as picograms of PAF per mg of tissue.

Acetylhydrolase assay

Frozen biopsy specimens were pulverised and homogenised in 1 mL of AHA buffer (140 mmol/L NaCl, 3 mmol/L KCl, 4 mmol/L HEPES, 22 mmol/L EDTA). After centrifugation, supernatants were used for AHA and PLA2 determinations. AHA was assessed as previously described[17-20]. Briefly, 10^5 dpm of [^{3}H]acetyl-PAF (10 Ci/mmol, NEN), 0.1 mmol/L PAF, AHA buffer (pH 8) in a final volume of 450 μL, and 50 μL of tissue extract supernatants were incubated for 30 min at 37 ℃. The reaction was stopped with addition of 100 μL of bovine serum albumin (100 g/L) and 400 μL of trichloracetic acid (200 g/L). Samples were centrifuged at 1500 g for 15 min, and supernatants were counted in a liquid scintillation counter. Results were expressed as fentomoles of PAF degraded per min per mg of tissue as means of duplicate assays. Variation between duplicates was less than 7%.

PLA2 activity assay

PLA2 levels were assessed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems Europe, Oxon, UK) and as previously described[17,18,20]. Results were expressed as international unit (IU) per mg of tissue as means of duplicate assays. Variation between duplicates was less than 6%

Reverse-transcriptase polymerase chain reaction (RT-PCR) of PAF-R transcripts

Total RNA from tissue samples extracted with RNAwiz (Ambion, Austin, TX) was reverse-transcribed and the complementary DNA (cDNA) was amplified by PCR as previously described[17,18,20]. The human PAF-R transcript 1 sense primer was 5'-GACAGCATAGAG

GCTGAGGC-3', the transcript 2 sense primer was 5'-CCTGAGCTCCCCGAGAAGTCA-3' and the antisense primer was 5'-TAGCCATTAGCAATGACCCC-3. The sense and anti-sense primers of glyceraldehyde-3-phosphate dehydrogenase (GADPH, a positive control of PCR amplification) were 5'-GGCTGAGAACGGGAAGCTTG-3' and 5'-GGATGATGTTCTGGAGAGCC-3', respectively. PCR products were electrophoresed on a 20 g/L agarose gel (Gibco, France) and visualised by ethidium bromide staining. Expected sizes of amplified products were: PAF-R transcript 1225 bp; PAF-R transcript 2269 bp; spliced variant of PAF-R transcript 2351 bp; and GAPDG, 439 bp.

Statistical analysis

Differences between groups were assessed using the Mann-Whitney *U*-test. A paired student's *t*-test was used to analyse intragroup differences. $P < 0.05$ was considered statistically significant.

RESULTS

PAF and human cirrhosis

A 4-fold increase of PAF levels was found in the cirrhotic tissue (1.61 ± 0.43 pg/mg, $n = 14$) as compared to the non-cirrhotic one (0.39 ± 0.39 pg/mg, $n = 7$) (Figure 1A). In contrast, the AHA levels (the PAF degrading enzyme) were unchanged ($P = 0.33$, Mann-Whitney *U*-test) in the cirrhotic tissue (3.93 ± 0.38 fmol/(min.mg), $n = 14$) as compared to the non-cirrhotic tissue (5.00 ± 0.53 fmol/(min.mg), $n = 7$) (Figure 1B). Similarly, PLA$_2$ levels (the enzymatic activity that generated the lyso-PAF precursor) were unchanged ($P = 0.23$, Mann-Whitney *U*-test) in the cirrhotic tissue (1.35 ± 0.17 U/mg, $n = 14$) as compared with non-cirrhotic one (1.76 ± 0.24 U/mg, $n = 7$) (Figure 1C). In agreement with these latter results, amounts of the lyso-PAF were similar ($P = 1$, Mann-Whitney *U*-test) in the cirrhotic tissue ($1\,934.0 \pm 556.9$ pg/mg, $n = 14$) as compared to the non-cirrhotic one ($1\,488.9 \pm 331.0$ pg/mg, $n = 7$) (Figure 1D).

PAF-R and human cirrhosis

The PAF-R gene produces three different species of mRNA [i.e., transcript 1 (leucocyte-type), transcript 2 (tissue-type) and an elongated form of the transcript 2]; both transcripts ultimately yield the functional PAF-R. As shown in Figure 1 (lower panel), RT-PCR experiments showed the presence of PAF-R transcript 1 but not transcript 2 in the cirrhotic tissue.

PAF and human HCC

A significant ($P = 0.0002$, *t*-test for paired data) increase of PAF levels was found in tumor tissues (1.69 ± 0.34 pg/mg, $n = 21$) as compared to the non-tumor ones (1.20 ± 0.33 pg/mg, $n = 21$) (Figure 2A). This increase was significant ($P = 0.0044$, student *t*-test for paired data) for cirrhotic patients (1.88 ± 0.41 pg/mg *vs* 1.61 ± 0.43 pg/mg for tumor and non-tumor tissues, respectively) and also ($P < 0.05$, student *t*-test for paired data) for non-cirrhotic patients (1.29 ± 0.62 pg/mg *vs* 0.39 ± 0.30 pg/
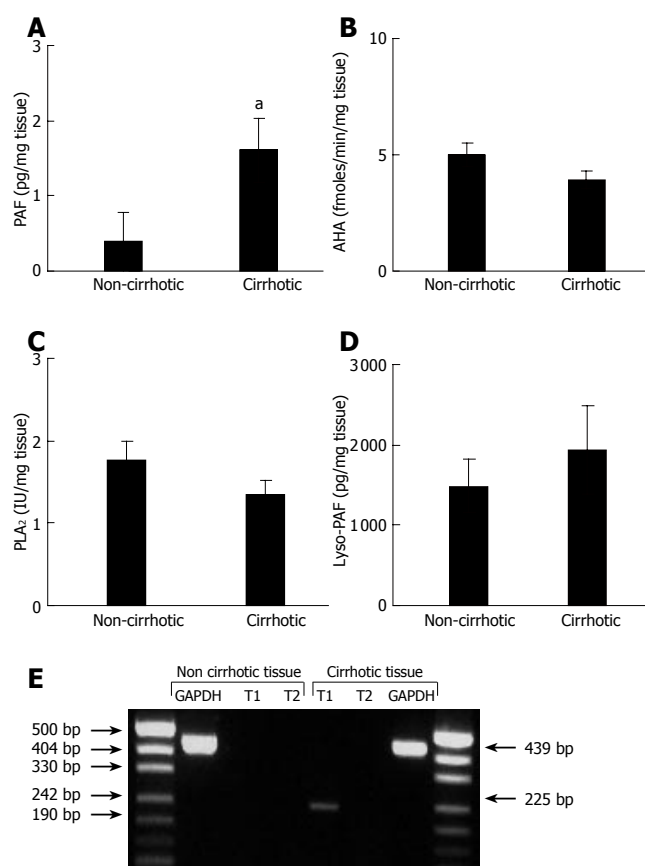

Figure 1 PAF, lyso-PAF, AHA, PLA$_2$ levels and PAF-R transcripts in cirrhotic and non-cirrhotic liver tissues. *Upper panel* shows the specimens of cirrhotic ($n = 14$) and non-cirrhotic tissue ($n = 7$) obtained during the surgical procedure. **A**: PAF determined by a platelet-aggregation assay, [a]$P < 0.05$ *vs* non-cirrhotic; **B**: AHA determined by investigating the degradation of [^{3}H]-PAF, ($P = 0.33$); **C**: PLA$_2$ assessed by enzyme-linked immunosorbent assay, ($P = 0.23$); **D**: Lyso-PAF measured after its chemical acetylation into PAF, ($P = 1.00$). Results are expressed as mean±SE. Statistical analysis was performed using the Mann Whitney *U*-test. *Lower panel* shows PAF-R transcripts determined by RT-PCR. Expected sizes of amplified products were 225 bp (PAF-R transcript 1), 269 bp (PAF-R transcript 2), 351 bp (spliced variant of PAF-R transcript 2), and 439 bp (GAPDG). Sizes of PCR products and DNA size ladder are indicated by arrows. All experiments were performed in triplicate.

mg for tumor and non-tumor tissues, respectively). For HCC patients, PAF values were not significantly different ($P = 0.52$, Mann-Whitney *U* test) according to the tumor status (1.85 ± 0.49 pg/mg *vs* 1.62 ± 0.44 pg/mg for six T1/T2 patients and fifteen T3/T4 patients, respectively). Only tumor PAF levels were affected in HCC tissue. Indeed, the enzymatic activities implicated in PAF production and degradation were not changed. Thus, the AHA levels (the PAF degrading enzyme) were unchanged ($P = 0.24$, *t*-test for paired data) in the tumor tissue (4.72 ± 0.52 fmol/(min.mg), $n = 21$) as compared to the adjacent tissue (4.29 ± 0.32 fmol/(min.mg), $n = 21$) (Figure 1B). Amounts of the lyso-PAF were unchanged ($P = 0.48$, *t*-test for paired data) in the tumor tissue ($1\,588.9 \pm 377.9$ pg/mg, $n = 21$) as compared to the non-tumor one ($1\,616.8 \pm 373.7$ pg/mg, $n = 21$) (Figure 1D). Moreover, PLA$_2$ levels (the enzymatic activity that generated the lyso-PAF) were unchanged ($P = 0.26$, *t*-test for paired data) in HCC tissue (1.28 ± 0.27 U/mg, $n = 21$) as compared with non-tumor one (1.49 ± 0.14 U/mg, $n = 21$) (Figure 1C).

PAF-R and human HCC

As shown in Figure 2 (lower panel), RT-PCR experiments indicated the presence of PAF-R transcripts 1 and 2 in HCC tissues. The spliced variant of PAF-R transcript 2 was not detected.

DISCUSSION

Elevated PAF levels were found in the cirrhotic tissue as compared to the non-cirrhotic one, confirming, for the first time in human, the results obtained in experimental models of liver cirrhosis[8,9]. Elevated levels of PAF can result from a higher PAF production or a lower PAF degradation. Arguing against the latter hypothesis, the AHA levels (the PAF degrading enzyme) were unchanged in the cirrhotic tissue as compared to the non-cirrhotic tissue. Thus, the elevated PAF levels in cirrhotic tissue might be due to an elevated PAF synthesis. Kupffer cells which are a well known source of PAF[27] might be an appropriate candidate for these elevated tissue PAF levels. Strengthening this hypothesis, Kupffer cells from cirrhotic rat was found to produce more PAF than kupffer cells of control rats[8]. Confirming a previous study[28], PLA2 levels (the enzymatic activity that generated the lyso-PAF) were unchanged in the cirrhotic tissue. In agreement with these latter results, amounts of the lyso-PAF were similar in the cirrhotic tissue as compared to the non-cirrhotic one. PAF acts as a local cell-to-cell mediator. Thus, an elevated PAF synthesis is only of relevance if PAF-R can be detected at the site of PAF production. The PAF-R gene produces three different species of mRNA (i.e., transcript 1, transcript 2 and an elongated form of the transcript 2); both transcripts ultimately yield the functional PAF-R[3]. Leucocytes produce PAF-R transcripts 1, while PAF-R transcripts 2 are found in organ extracts. Thus, PAF-R transcripts 1 and 2 are also named "leukocyte-type" and "tissue-type", respectively. RT-PCR experiments indicated the presence of PAF-R transcript 1 in the cirrhotic tissue. These PAF-R transcripts from the "leukocyte-type" fit well with the previous study that showed higher hepatic levels of PAF-R mRNA in CCl4-induced liver cirrhosis[8], with cirrhotic kupffer cells as cellular source of these PAF-R mRNA. Thus our present study concludes that PAF and PAF-R transcripts are present in the human cirrhotic liver. PAF may have pathophysiologic functions and might account, at least in part, in the inflammatory response occurring in the cirrhotic liver. On the one hand, PAF might act directly by stimulating the production of humoral mediators including peptide leukotrienes (LTC4, LTD4, LTE4)[29], prostaglandin E2 (PGE2)[30], and nitric oxide[31], which can affect cell proliferation and gene expression. On the other hand, and with respect to its potent immunoregulatory actions[1,32], PAF might act indirectly on stellate cell-immune cell interactions by promoting leukocyte chemotaxis and adherence, by influencing leukocyte activation and by affecting their cytokine secretions.

Previous clinical studies have reported the elevated PAF levels in breast and colon carcinomas[16-18], but not in lung and thyroid carcinomas[19,20], suggesting that over-expression of PAF is not necessarily a universal event
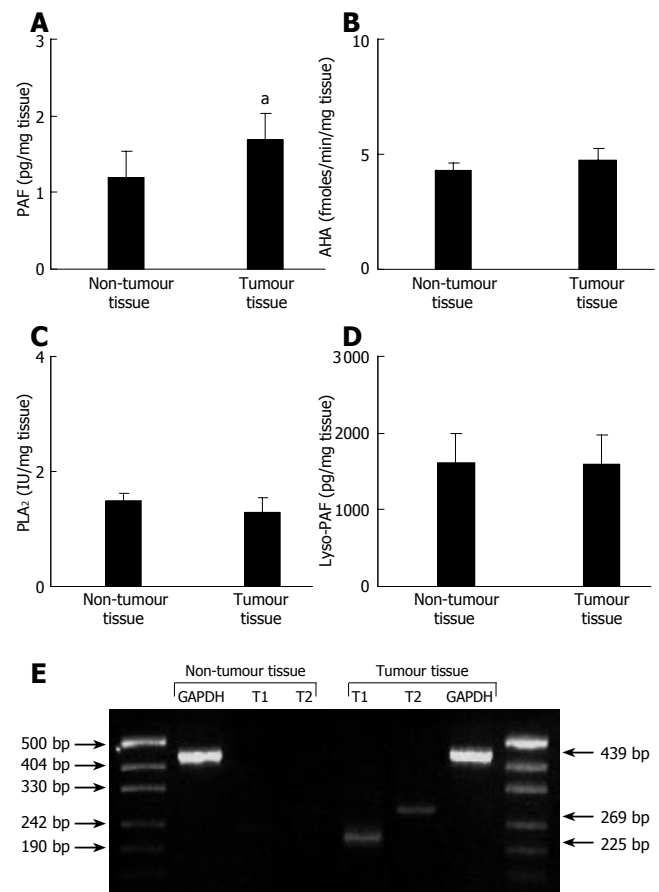

Figure 2 PAF, lyso-PAF, AHA, PLA2 levels and PAF-R transcripts in HCC and non-tumour tissues. *Upper panel* shows specimens from 21 HCC patients obtained during the surgical procedure. **A**: PAF, [a]$P < 0.05$ *vs* non-tumour tissue; **B**: AHA ($P = 0.24$); **C**: PLA2 ($P = 0.26$); **D**: Lyso-PAF ($P = 0.48$). Statistical analysis was performed using the Students' t-test for paired samples. Results are expressed as mean ± SE. *Lower panel* shows PAF-R transcripts determined by RT-PCR. Expected sizes of amplified products were 225 bp (PAF-R transcript 1), 269 bp (PAF-R transcript 2), 351 bp (spliced variant of PAF-R transcript 2), and 439 bp (GAPDG). Sizes of PCR products and DNA size ladder are indicated by arrows. All experiments were performed in triplicate.

in carcinogenesis, but may be tumor-specific. Increased PAF levels were found in HCC tissues. In contrast to colorectal cancer patients[17], tumor PAF level did not change according to the tumor status. As suggested above, the elevated PAF levels in HCC tissue might be due to elevated PAF synthesis by Kupffer cells. In contrast to that found in colon carcinoma[17], only tumor PAF levels were affected in HCC tissue. Indeed, the enzymatic activities implicated in PAF degradation (i.e., AHA) and production (i.e., PLA2) were not changed; these latter results fitting well with the data reporting no differences in phospholipids composition (including phosphatidylcholine one) between human hepatoma homogenates and normal regions outside the tumors[33]. Few clinical studies had focussed on PAF-R levels in cancer tissue. Levels of PAF-R transcripts were not modified in colorectal, lung and thyroid carcinoma[17,19,20], while PAF-R (tissue-type) levels were increased in liver metastasis of colorectal cancer[18]. PAF-R transcripts 1 (leukocyte-type) and 2 (tissue-type) were found in the HCC tissues. Kupffer cells might be the cell source for the elevated levels of PAF-R

(leukocyte-type) transcripts. Thus, transforming growth factor beta (TGF-β) and PAF stimulated PAF-R mRNA synthesis in monocytic/macrophagic cells[34,35]. Elevated levels of TGFβ[36] and PAF (Figure 2A) are found in HCC tissues. PAF-R transcript 2 (tissue-type) has also been reported to be up-regulated in HCC tissue. Such an up-regulation of PAF-R transcripts 2 has been previously reported in endometrial cells in response to estradiol[37]. Interestingly, locally elevated estrogen formation has been reported in HCC tissues[38], and estrogen enhances angiogenesis through a pathway involving PAF in a mouse Matrigel model *in vivo*[39]. Thus, whatever molecular signals implicated in these elevated PAF-R transcript levels, the current data argue for a pathophysiological role of PAF in HCC tissues.

In conclusion, PAF is a potent pro-inflammatory compound that acts as a local cell-to-cell mediator. Since PAF can generate biological responses detectable at levels of 10 fmol/L, regulating PAF levels is important since elevated levels of PAF might result in biological effects. To the best of our knowledge, this clinical study, for the first time, reports an increase in PAF and PAF-R transcripts in cirrhotic liver tissue and HCC tissue, suggesting a pathological role for PAF in liver cirrhosis and HCC. In cirrhotic tissue, PAF might act on stellate cell-immune cell interactions by influencing leukocyte activation and chemotaxis, and by altering the local cytokine network. An angiogenic role of PAF might be suggested in HCC.

REFERENCES

1 **Denizot Y**, Guglielmi L, Donnard M, Trimoreau F. Platelet-activating factor and normal or leukaemic haematopoiesis. *Leuk Lymphoma* 2003; **44**: 775-782

2 **Arai H**, Koizumi H, Aoki J, Inoue K. Platelet-activating factor acetylhydrolase (PAF-AH). *J Biochem (Tokyo)* 2002; **131**: 635-640

3 **Izumi T**, Shimizu T. Platelet-activating factor receptor: gene expression and signal transduction. *Biochim Biophys Acta* 1995; **1259**: 317-333

4 **Mustafa SB**, Flickinger BD, Olson MS. Suppression of lipopolysaccharide-induced nitric oxide synthase expression by platelet-activating factor receptor antagonists in the rat liver and cultured rat Kupffer cells. *Hepatology* 1999; **30**: 1206-1214

5 **Sakaguchi T**, Nakamura S, Suzuki S, Oda T, Ichiyama A, Baba S, Okamoto T. Participation of platelet-activating factor in the lipopolysaccharide-induced liver injury in partially hepatectomized rats. *Hepatology* 1999; **30**: 959-967

6 **Calder A,** Embrey MP. Letter: Prostaglandins and the unfavourable cervix. *Lancet* 1973; **2**: 1322-1323

7 **Silomon M,** Pizanis A, Larsen R, Rose S. Role of platelet-activating factor in hepatocellular Ca^{2+} alterations during hemorrhagic shock. *J Surg Res* 1997; **72**: 101-106

8 **Yang Y,** Harvey SA, Gandhi CR. Kupffer cells are a major source of increased platelet activating factor in the CCl4-induced cirrhotic rat liver. *J Hepatol* 2003; **39**: 200-207

9 **Yang Y,** Nemoto EM, Harvey SA, Subbotin VM, Gandhi CR. Increased hepatic platelet activating factor (PAF) and PAF receptors in carbon tetrachloride induced liver cirrhosis. *Gut* 2004; **53**: 877-883

10 **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**: 4777-4784

11 **Bussolati B,** Biancone L, Cassoni P, Russo S, Rola-Pleszczynski M, Montrucchio G, Camussi G. PAF produced by human breast cancer cells promotes migration and proliferation

12 **Mannori G**, Barletta E, Mugnai G, Ruggieri S. Interaction of tumor cells with vascular endothelia: role of platelet-activating factor. *Clin Exp Metastasis* 2000; **18**: 89-96

13 **Tripathi YB**, Kandala JC, Guntaka RV, Lim RW, Shukla SD. Platelet activating factor induces expression of early response genes c-fos and TIS-1 in human epidermoid carcinoma A-431 cells. *Life Sci* 1991; **49**: 1761-1767

14 **Montrucchio G,** Lupia E, Battaglia E, Del Sorbo L, Boccellino M, Biancone L, Emanuelli G, Camussi G. Platelet-activating factor enhances vascular endothelial growth factor-induced endothelial cell motility and neoangiogenesis in a murine matrigel model. *Arterioscler Thromb Vasc Biol* 2000; **20**: 80-88

15 **Montrucchio G,** Sapino A, Bussolati B, Ghisolfi G, Rizea-Savu S, Silvestro L, Lupia E, Camussi G. Potential angiogenic role of platelet-activating factor in human breast cancer. *Am J Pathol* 1998; **153**: 1589-1596

16 **Pitton C,** Lanson M, Besson P, Fetissoff F, Lansac J, Benveniste J, Bougnoux P. Presence of PAF-acether in human breast carcinoma: relation to axillary lymph node metastasis. *J Natl Cancer Inst* 1989; **81**: 1298-1302

17 **Denizot Y,** Gainant A, Guglielmi L, Bouvier S, Cubertafond P, Mathonnet M. Tissue concentrations of platelet-activating factor in colorectal carcinoma: inverse relationships with Dukes' stage of patients. *Oncogene* 2003; **22**: 7222-7224

18 **Denizot Y,** Descottes B, Truffinet V, Valleix D, Labrousse F, Mathonnet M. Platelet-activating factor and liver metastasis of colorectal cancer. *Int J Cancer* 2005; **113**: 503-505

19 **Denizot Y,** Desplat V, Drouet M, Bertin F, Melloni B. Is there a role of platelet-activating factor in human lung cancer? *Lung Cancer* 2001; **33**: 195-202

20 **Denizot Y,** Chianea T, Labrousse F, Truffinet V, Delage M, Mathonnet M. Platelet-activating factor and human thyroid cancer. *Eur J Endocrinol* 2005; **153**: 31-40

21 **Sugimachi K,** Tanaka S, Terashi T, Taguchi K, Rikimaru T, Sugimachi K. The mechanisms of angiogenesis in hepatocellular carcinoma: angiogenic switch during tumor progression. *Surgery* 2002; **131**: S135-S141

22 **Lemieux C,** Maliba R, Favier J, Theoret JF, Merhi Y, Sirois MG. Angiopoietins can directly activate endothelial cells and neutrophils to promote proinflammatory responses. *Blood* 2005; **105**: 1523-1530

23 **Sirois MG,** Edelman ER. VEGF effect on vascular permeability is mediated by synthesis of platelet-activating factor. *Am J Physiol* 1997; **272**: H2746-H2756

24 **Deo DD,** Axelrad TW, Robert EG, Marcheselli V, Bazan NG, Hunt JD. Phosphorylation of STAT-3 in response to basic fibroblast growth factor occurs through a mechanism involving platelet-activating factor, JAK-2, and Src in human umbilical vein endothelial cells. Evidence for a dual kinase mechanism. *J Biol Chem* 2002; **277**: 21237-21245

25 **Kojiro M**. Histopathology of liver cancers. *Best Pract Res Clin Gastroenterol* 2005; **19**: 39-62

26 **Greene FL,** Stewart AK, Norton HJ. A new TNM staging strategy for node-positive (stage III) colon cancer: an analysis of 50,042 patients. *Ann Surg* 2002; **236**: 416-421; discussion 421

27 **Chao W,** Siafaka-Kapadai A, Olson MS, Hanahan DJ. Biosynthesis of platelet-activating factor by cultured rat Kupffer cells stimulated with calcium ionophore A23187. *Biochem J* 1989; **257**: 823-829

28 **Niwa Y**, Sakane T, Ichikawa M, Kondo T, Taniguchi S. Phospholipid transmethylation and choline phosphotransferase in microsomal fraction of human diseased liver. *J Hepatol* 1986; **2**: 458-467

29 **Sakagami Y**, Mizoguchi Y, Seki S, Kobayashi K, Morisawa S, Yamamoto S. Release of peptide leukotrienes from rat Kupffer cells. *Biochem Biophys Res Commun* 1988; **156**: 217-221

30 **Gandhi CR,** DeBuysere MS, Olson MS. Platelet-activating factor-mediated synthesis of prostaglandins in rat Kupffer cells. *Biochim Biophys Acta* 1992; **1136**: 68-74

31 **Mustafa SB,** Howard KM, Olson MS. Platelet-activating factor augments lipopolysaccharide-induced nitric oxide formation

of tumor cells and neo-angiogenesis. *Am J Pathol* 2000; **157**: 1713-1725

by rat Kupffer cells. *Hepatology* 1996; **23**: 1622-1630

32   **Peplow PV**. Regulation of platelet-activating factor (PAF) activity in human diseases by phospholipase A2 inhibitors, PAF acetylhydrolases, PAF receptor antagonists and free radical scavengers. *Prostaglandins Leukot Essent Fatty Acids* 1999; **61:** 65-82

33   **Eggens I,** Backman L, Jakobsson A, Valtersson C. The lipid composition of highly differentiated human hepatomas, with special reference to fatty acids. *Br J Exp Pathol* 1988; **69**: 671-683

34   **Parent JL,** Stankova J. Transforming growth factor-beta up-regulates the gene expression of the human platelet-activating factor receptor in monocytic and B cell lines. *Biochem Biophys Res Commun* 1993; **197**: 1443-1449

35   **Shirasaki H,** Adcock IM, Kwon OJ, Nishikawa M, Mak JC, Barnes PJ. Agonist-induced up-regulation of platelet-activating factor receptor messenger RNA in human monocytes. *Eur J Pharmacol* 1994; **268**: 263-266

36   **Bedossa P**, Peltier E, Terris B, Franco D, Poynard T. Transforming growth factor-beta 1 (TGF-beta 1) and TGF-beta 1 receptors in normal, cirrhotic, and neoplastic human livers. *Hepatology* 1995; **21:** 760-766

37   **Sato S**, Kume K, Takan T, Mutoh H, Taketani Y, Shimizu T. Up-regulation of the intracellular Ca2+ signaling and mRNA expression of platelet-activating factor receptor by estradiol in human uterine endometrial cells. *Adv Exp Med Biol* 1996; **416:** 95-100

38   **Castagnetta LA,** Agostara B, Montalto G, Polito L, Campisi I, Saetta A, Itoh T, Yu B, Chen S, Carruba G. Local estrogen formation by nontumoral, cirrhotic, and malignant human liver tissues and cells. *Cancer Res* 2003; **63**: 5041-5045

39   **Seo KH,** Lee HS, Jung B, Ko HM, Choi JH, Park SJ, Choi IH, Lee HK, Im SY. Estrogen enhances angiogenesis through a pathway involving platelet-activating factor-mediated nuclear factor-kappaB activation. *Cancer Res* 2004; **64**: 6482-6488