Distribution of lysophospholipids and metabolism of platelet-activating factor in human follicular and peritoneal fluids

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The distribution of lysophosphatidylcholine, lyso-platelet-activating factor and platelet-activating factor (PAF) was studied in human plasma and in follicular and peritoneal fluid. In plasma, peritoneal and follicular fluids, 51%, 87% and 89%, respectively, of the total lipids were found in the protein fraction (the density > 1.21 fraction). Two forms of lysophospholipids were identified in this fraction: one of high affinity and one of low affinity for albumin. The metabolism of PAF in human follicular fluid, peritoneal fluid and plasma was also investigated. PAF-acetylhydrolase activity was found in both peritoneal and follicular fluids which induced a time-dependent hydrolysis of [³H]PAF. The half-life of PAF was estimated to be 7–12 min in plasma, 15–25 min in peritoneal fluid and approximately 2 h in follicular fluid. PAF-acetylhydrolase activity in embryo culture media supplemented with 10% serum was markedly inhibited by addition of commercial serum albumin. When 25 g albumin l⁻¹ was added, 22% of [³H]PAF was hydrolysed h⁻¹ compared with 72% in media without albumin. The concentrations of lysophosphatidylcholine measured in plasma, in follicular and peritoneal fluids were 252, 286 and 53 µmol l⁻¹, respectively. The distribution of these lysophospholipids and the metabolism of PAF in the female genital tract fluids reported in the present study provide evidence for the involvement of these biologically active lipid mediators in a variety of reproductive processes including sperm–egg interactions and embryonic development.

Introduction

Alterations in lipid metabolism are key events regulating several steps of mammalian fertilization, such as sperm capacitation and acrosome reaction as well as sperm–egg fusion (reviewed by Langlais and Roberts, 1985; Parks and Ehrenwald, 1990; Roldan and Harrison, 1990). Compositional and structural changes in proteins, sterols and phospholipids of capacitated sperm membranes are associated with increased membrane fluidity and permeability, along with increased intracellular calcium concentrations, leading to sperm membrane fusions triggering the acrosome reaction. Among those lipid modifications are sperm cholesterol efflux mediated by human female genital tract proteins (Langlais *et al.*, 1981; Langlais *et al.*, 1988) and the breakdown of sperm phospholipids into fusogenic lysolipids, resulting from activation of phospholipases associated with male gametes (Langlais and Roberts, 1985; Antaki *et al.*, 1988; Guérette *et al.*, 1988; Langlais *et al.*, 1992).

Since it is well known that lipid exchanges occur between cells and extracellular fluid compartments, it is anticipated that

the lipid composition and metabolism of female genital tract fluids may also play regulatory roles in sperm motility, capacitation and the acrosome reactions, as well as in later reproductive stages such as embryo development and implantation. However, while there have been reports on changes in cholesterol and phospholipids in oviductal fluids of cows during the oestrous cycle (Killian *et al.*, 1989), few data are available regarding lysophospholipid composition and metabolism in human oviductal, follicular and peritoneal fluids that may contribute to the composition of the milieu where sperm–egg interactions occur *in vivo*. This report presents data on the distribution and metabolism of lysophospholipids in human plasma and in follicular and peritoneal fluids; the lysophospholipids investigated in this study were lysophosphatidylcholine (LPC), lyso-platelet-activating factor (Lyso-PAF) and its precursor, platelet-activating factor (PAF).

Materials and Methods

Chemicals and reagents

All solvents and chemicals were of analytical grade. Radioactive material was purchased from Dupont, MA: ([¹⁴C]-

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palmitoyl)-lysopalmitoyl phosphatidylcholine ($[^{14}\text{C}]\text{LPC}$ NET-683, $1[^3\text{H}]\text{hexadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine}$ NET-668, alkyl- $2[^3\text{H}]\text{acetyl-sn-glycerol-3-phosphorylcholine}$ NET-910, and $1[^3\text{H}]\text{alkyl-sn-glycerol-3-phosphorylcholine}$ (a mixture of C14–C16–C18 alkyl ethers) NET-878. NaCl and EDTA were obtained from Sigma, MO, and KBr, chloroform and methanol were purchased from Fisher, NJ.

Collection of follicular fluid, peritoneal fluid and maternal blood plasma

Samples of human follicular fluid (FF) and peritoneal fluid (PF) were obtained from patients registered in an *in vitro* fertilization programme. PF from 29 women was obtained at mid-cycle during exploratory laparoscopy or during tubal ligation procedures. FF was obtained at the time of laparoscopy from 15 women undergoing oocyte recovery for IVF. Only FF and PF with minimal blood contamination were used for the study. When the volume of packed red blood cells, following centrifugation, was more than 2% of the fluid volume, the samples were not used in the present study. The potential effects of ovarian stimulation were excluded by obtaining control samples of follicular fluid at the time of exploratory laparoscopy and samples of peritoneal fluid were obtained at the time of oocyte retrieval. Fluids collected in either condition did not show significantly different lipoprotein profiles (data not shown). Blood was collected in tubes containing EDTA. Plasma was obtained following a 10 min centrifugation at 1000 *g*. PF and FF were centrifuged for 10 min at 1000 *g* to eliminate residual cells. Fluids were kept at 4°C for ultracentrifugation or at –20°C until enzymatic assays.

Density gradient ultracentrifugation

Heat-inactivated (56°C for 30 min) human plasma, PF or FF was incubated in the presence of either radioactive LPC + PAF or LPC + Lyso-PAF for 30 min at 56°C. Radioactive products (88 nmol $[^{14}\text{C}]\text{LPC}$ (57 mCi mmol^{–1}), 115 pmol $[^3\text{H}]\text{PAF}$ (56.7 Ci mmol^{–1}) and 175 pmol $[^3\text{H}]\text{lyso-PAF}$ (52 Ci mmol^{–1}) were evaporated to dryness under a stream of nitrogen in 15 ml plastic tubes (Falcon Labware, Becton-Dickinson, Mississauga, Ontario) and resuspended by sonication in 1 ml of a low density solution ($d = 1.006$) of KBr. After sonication of radioactive lipids, 2 ml of fluids (plasma, peritoneal or follicular fluid) was added with 1 ml KBr solution ($d = 1.006$) and the mixture was incubated for 30 min at 56°C. This incubation period allowed the distribution of labelled lipids in the lipoproteins and lipid-binding proteins in the fluids. Incubation at 56°C also prevented the hydrolysis of $[^3\text{H}]\text{PAF}$ by PAF-acetylhydrolase present in the fluids (Farr *et al.*, 1983). At the end of the incubation, the density of the mixture was raised to 1.21 by addition of solid KBr and submitted to density gradient ultracentrifugation according to the methods of Redgrave *et al.* (1975) and Langlais *et al.* (1988). Centrifugation was performed in cellulose nitrate tubes (Beckman Inc., Palo Alto, CA) in a SW41Ti rotor. A four-density discontinuous KBr gradient was prepared and fractions were collected in plastic Eppendorf tubes by retrieving the gradients from the bottom of the cellulose tubes. Radioactivity was measured in each fraction to determine the relative distribution of each of the three lipids in lipoproteins (high density

lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) and proteins (albumin). The lipoprotein elution profile was determined by measuring total cholesterol concentrations in the eluted fractions.

Cholesterol profile

This profile was obtained using a commercial kit, cholesterol C-system (237574, Boehringer Mannheim, GmbH) as described by Langlais *et al.* (1988). This technique allows the estimation of the total cholesterol concentration (free and esterified forms).

Protein profiles

The protein fraction ($d > 1.21$) containing albumin was further characterized using a molecular-sieve column (protein column I-125: Waters, Millipore, Milford, MA) coupled to an HPLC system to identify those proteins associated with the radioactive lipids. Before application of samples to the HPLC column, all samples were filtered on 0.2 μm filters (Millipore). A phosphate buffer was used to elute the lipid-binding proteins (albumin). The protein fraction was also studied by chromatography on a quaternary methylamine-column (QMA: J. T. Baker, NJ). Elution of proteins was achieved using a discontinuous NaCl gradient from 0 to 1 mol l^{–1}, diluted in 20 mmol Tris-HCl l^{–1}, pH 7.4. Albumin was eluted with 0.2 mol NaCl l^{–1}. A radioactive lipid fraction in its free form or with low affinity for albumin was also absorbed by both types of column and eluted by the addition of methanol, with a continuous gradient for the molecular-sieve column or with the addition of 100% methanol for the QMA column.

PAF-acetylhydrolase activity

Determination of PAF-acetylhydrolase (PAF-AH) activity was carried out using $[^3\text{H}]\text{acetyl PAF}$ as the radioactive substrate. This substrate was diluted in 100 μl of 0.1 mol Hepes l^{–1} containing 2% human albumin (Sigma), pH 7.2. The enzymatic assay was performed by incubation of 50 μl of PF, FF or plasma with 100 μl of 0.1 mol Hepes l^{–1} and 100 μl of $[^3\text{H}]\text{PAF}$ (10⁵ d.p.m.), specific activity, 56.7 Ci mmol^{–1}, in polypropylene tubes. The enzymatic reaction was performed as previously described (Letendre *et al.*, 1992).

To evaluate whether the degradation results were obtained via the activity of PAF-AH, a calcium-independent enzyme (Sturk *et al.*, 1989), and not by calcium-dependent phospholipase A₂, experiments in the presence and absence of 0.25 mol EDTA l^{–1} were performed. For this purpose, 50 μl of fluids, 100 μl of $[^3\text{H}]\text{PAF}$ and 100 μl of 0.1 mol Hepes l^{–1} plus 2% albumin and 0.25 mol EDTA l^{–1} were used as the reaction mixture. The procedure used was as described above.

LPC concentrations

Total lipids were extracted twice from fluids using a modified Folch extraction as described by Langlais and Roberts (1985). LPC was isolated by applying PF, FF or plasma lipid extracts to silica gel (silica gel 250 μm , Whatman) thin-layer chromatography (TLC) with the solvent system chloroform:methanol:water

(65:25:4). In human samples, a region migrated with an R_f value of 0.2, the same migration as standard LPC or L-PAF. LPC/L-PAF was eluted from the silica gel with methanol with a $42 \pm 5\%$ recovery as determined by addition of [^3H]LPC as an internal standard. [^3H]LPC (specific activity, 30 Ci mmol^{-1}) was prepared by enzymatic hydrolysis of [^3H]phosphatidylcholine ([^3H]choline) NET-654 (Dupont) and by purification of the [^3H]LPC by TLC. The LPC/L-PAF concentration in PF, FF or plasma was obtained via the measurement of inorganic phosphorus (Duck-Chong, 1979) in the eluates, using commercial LPC for determination of the standard curve. Corrections were made to account for losses during extraction procedures; radioactivity was assayed in the eluates before inorganic phosphorus measurement to evaluate the recovery of the internal standard ([^3H]LPC) and to adjust the LPC concentrations to values corresponding to 100% recovery of [^3H]LPC.

Results

Lipid distribution in human plasma, follicular and peritoneal fluids

The lipoprotein profile in human plasma and follicular fluid was similar to that described by Langlais *et al.* (1988); LDL, VLDL and HDL were the major cholesterol transporting lipoproteins in plasma, whereas follicular fluid contained essentially a single class of lipoprotein, namely HDL (Figs 1a, b). The distribution of radioactive LPC, L-PAF and PAF in plasma was identical for all three lipids investigated. The lipids were associated with the protein fractions (albumin) and lipoproteins, with 51, 19, 19 and 8% of the radioactivity associated with albumin, HDL, LDL and VLDL, respectively (Fig. 1a).

In follicular fluid, the lysophospholipids and PAF exhibited an identical distribution; 89% of the radioactivity was associated with the albumin fraction and the remainder with the HDL fraction (Fig. 1b).

Qualitatively, PF contained the same lipoproteins (HDL, LDL, VLDL) as are found in plasma (Fig. 1c). However, the proportion of lipoproteins in the two fluids was different. Approximately the same quantity of cholesterol was found in the HDL fraction of each fluid, whereas there was a mean five-fold decrease in LDL and a mean threefold decrease in VLDL in PF compared with plasma. The distribution of labelled LPC, L-PAF and PAF was the same for all three lipids in this fluid: 87% of the total radioactivity was found in the protein fractions and the remainder distributed between the lipoproteins (Fig. 1c).

As a high percentage of labelled lipids was associated with the protein fractions, the identification of the lipid-binding proteins was undertaken by applying the protein fractions to a molecular sieve column coupled to an HPLC apparatus. A radioactive profile of the lipids in these eluates was carried out to detect these proteins. The profile obtained for LPC shows that LPC is bound only to human albumin (Fig. 2). The total radioactivity was never recovered when this system of elution with phosphate buffer was used. Thus, it was suspected that a fraction of the lipids was not bound tightly to albumin but was present either as a soluble unbound form or as a form weakly bound to albumin. This fraction of lipid was finally eluted with the addition of methanol (Fig. 2). A methanol gradient performed after the elution of albumin was sufficient to elute the

Fig. 1. Distribution of lysophosphatidylcholine (LPC) ($\diamond$), lyso-platelet-activating factor (L-PAF) ($\circ$) and platelet-activating factor (PAF) ($\square$) in (a) human plasma, (b) human follicular and (c) human peritoneal fluids. The protein (PR) and lipoprotein fractions (HDL, LDL, VLDL) are illustrated by the cholesterol profile ($\bullet$). The distribution of labelled LPC, L-PAF and PAF was determined by measurement of radioactivity. These data represent the means of (a) six different plasma samples (SEM ± 0.115), (b) four samples of FF (SEM ± 0.04), (c) five different samples of fluid (SEM of 0.036).

form of lipid that was not bound to albumin from the column. The same results were obtained for human plasma (data not shown).

A second series of experiments was performed to establish the presence of the two forms of lipid using a quaternary methylamine column to confirm that a soluble unbound form of the lysophospholipid fraction was eluted with methanol (Fig. 3). Albumin saturated with [^{14}C]LPC was eluted with $0.2 \text{ mol NaCl l}^{-1}$ and the radioactivity eluted exactly with the albumin fraction (Fig. 3a). [^3H]Lyso-PAF and [^{14}C]LPC, in the absence of albumin, were then applied to this column and eluted; these unbound forms were eluted only in the presence of methanol (Fig. 3b). Finally, a mixture of albumin saturated with [^{14}C]LPC and of a free form of [^3H]Lyso-PAF was added to the column and eluted. This allowed the elution of a form of lipid bound to albumin and a form that was eluted with methanol (Fig. 3c). Thus, it would appear that there are two forms of lysophospholipid and PAF in the two fluids tested (FF and human plasma): a

Fig. 2. Molecular weight determination of lysophospholipid-binding proteins in human follicular fluid by HPLC. Proteins were eluted with a phosphate buffer, followed by a linear methanol gradient performed from 0% to 100% methanol. (□) [^3H]platelet-activating factor; (■) [^{14}C]lysophosphatidylcholine.

form tightly bound to albumin and a form weakly bound to albumin or possibly free in the biological fluid.

PAF-acetylhydrolase activity in human fluids, follicular and peritoneal fluids

The enzymatic activity responsible for the hydrolysis of PAF in human plasma, follicular and peritoneal fluids was resistant to freezing at -20°C for several months (data not shown). Thus, PAF-AH activity could be measured either in frozen and thawed samples or in freshly collected fluids without altering the results.

Hydrolysis of [^3H]PAF in follicular fluid was markedly lower than in plasma or peritoneal fluid (Fig. 4). The kinetics of PAF hydrolysis in PF were similar to those observed in plasma, except that the activity was significantly lower in peritoneal fluid. In a typical experiment, the percentage of [^3H]PAF hydrolysed after incubation for 15 min in plasma, PF and FF, was 65%, 39% and 10%, respectively. The estimated half-life of PAF was 7–12 min in plasma, 15–25 min in PF and 2 h in FF.

Since PAF-AH in plasma is associated with lipoproteins (Stafforini *et al.*, 1987, 1989), the specific activity of the enzyme is reflected by the PAF-AH: cholesterol ratio. The mean concentrations of cholesterol in plasma, PF and FF, were 214, 47 and 35 mg dl^{-1} , respectively; thus, the estimated specific activities of PAF-AH during a 15 min incubation in plasma, PF and FF, were 0.30, 0.82 and 0.28, respectively. The specific activity of PAF-AH in follicular fluid was not significantly different from that of plasma, and the lower activity in FF is probably the result of the absence of LDL in this fluid. However, in spite of the low concentration of lipoproteins, peritoneal fluid exhibited a 2.5-fold higher specific activity of PAF-AH compared with that of plasma. This high specific activity of PAF-AH in PF

Fig. 3. Elution of lipids associated with albumin and of lipids in a free form (or with low-affinity for albumin) from a quaternary methylamine (QMA) column. (■) [^{14}C]lysophosphatidylcholine (LPC); (▨) [^3H]lyso-platelet-activating factor (Lyso-PAF). (a) Elution of albumin-bound [^{14}C]LPC on QMA. Albumin was eluted with 0.2 mol NaCl l^{-1} . (b) Elution of free [^3H]Lyso-PAF and [^{14}C]LPC on QMA. Lipids were eluted with 100% methanol. (c) Separation of albumin-bound and weakly-bound or free forms of [^3H]Lyso-PAF and [^{14}C]LPC on QMA.

might result from altered substrate composition, the presence of enzyme activators or a local increased enzyme content in peritoneal fluid.

Incubation of [^3H]PAF in plasma, PF or FF in the presence of EDTA did not affect the rates of PAF hydrolysis (data not shown), suggesting that the PAF-AH activity in these fluids is independent of calcium and that PAF hydrolysis would not be mediated by calcium-dependent phospholipase A_2 in these fluids.

Since PAF was mostly associated with albumin in FF and PF (Fig. 2), the effect of albumin concentration on the rate of

Fig. 4. Kinetic analysis of platelet-activating factor (PAF) acetylhydrolase activating in (▲) human follicular fluid (FF); (■) human peritoneal fluid (PF); and (○) human plasma. In these fluids, 6, 23 and 30 samples were tested, respectively.

Fig. 5. Effect of albumin on platelet-activating factor (PAF) acetylhydrolase activity in Ham's F-10 supplemented with 10% human plasma. Exogenous albumin concentrations range from 0 to 25 g l⁻¹.

PAF hydrolysis in culture media was measured *in vitro*. A culture medium composed of Hams's F-10 supplemented with 10% human plasma, hydrolysed 72% of [³H]PAF during a 1 h incubation (Fig. 5). Addition of exogenous albumin markedly decreased the hydrolysis of PAF, as 25 g exogenous albumin l⁻¹ resulted in 22% of PAF hydrolysis h⁻¹.

In view of the reports on the role of PAF in embryonic growth, this increase in the half-life of PAF in culture media supplemented with albumin may be beneficial for the *in vitro* development of human embryos.

LPC concentrations in human plasma, follicular and peritoneal fluid

The analysis of inorganic phosphorus associated with the lysophospholipids isolated by thin layer chromatography revealed that the mean concentrations of LPC in human plasma (*n* = 89), follicular (*n* = 15) and peritoneal fluids (*n* = 29), were 252, 286 and 53 μmol l⁻¹, respectively (Fig. 6).

Fig. 6. Lysophosphatidylcholine concentrations in peritoneal fluid (PF), follicular fluid (FF) and human plasma. Concentrations ± SEM values were 53 ± 11, 286 ± 18 and 252 ± 9 μmol l⁻¹, respectively, in each of the fluids tested.

In the chromatographic system used in this study, LPC and L-PAF comigrate, which means that the LPC concentrations include L-PAF. As plasmalogens represent 5% of plasma choline phospholipids, it is estimated that the L-PAF contributions to the LPC concentrations reported here do not exceed 1% of the lysophospholipid concentrations.

Discussion

Follicular fluid, which surrounds the preovulatory oocyte, accompanies the female gamete at the time of ovulation and may contribute to the composition of the fluids at the locus of fertilization. Thus, human sperm-egg interactions *in vivo* may occur in a milieu composed of follicular fluid, peritoneal fluid and oviductal secretions. The relative contributions of each fluid may depend upon the time after ovulation and the site at which spermatozoa encounter the oocyte. Presumably, follicular fluid concentrations may form an increasing gradient of chemotactic and capacitation factors that will orient and drive spermatozoa towards the cumulus cells and, ultimately, to the zona pellucida, the site of the physiological acrosome reaction.

Sperm capacitation and the acrosome reaction are membrane phenomena involving exchange of lipids between the sperm plasma membrane and lipid-binding proteins in the capacitation medium. Thus, the results presented in this study concerning the composition, distribution and metabolism of LPC, L-PAF and PAF in human female reproductive tract fluids may provide further insight into the involvement of these biologically active lipids in reproduction and fertility.

The distribution of cholesterol among lipoproteins in human plasma and follicular fluid, observed in the present study, corroborate earlier reports demonstrating that HDL is the sole class of lipoprotein found in follicular fluid (Simpson *et al.*, 1980; Perret *et al.*, 1985; Langlais *et al.*, 1988). Owing to the molecular-sieve properties of the blood-follicle barrier (Shalgi *et al.*, 1973), high-molecular-weight macromolecules such as LDL and VLDL do not transudate from the blood circulation into follicular fluid. The highest density subclass of HDL particles is conveyed by follicular fluid in which the HDL-cholesterol content is approximately half of that found in plasma (Langlais *et al.*, 1988). A lipoprotein profile somewhat similar to that of

plasma was observed in peritoneal fluid which displayed comparable concentrations of HDL-cholesterol but lower concentration of LDL and VLDL, suggesting that the blood-peritoneal barrier allows the passage of very high molecular weight species.

The distribution of LPC, L-PAF and PAF among proteins and lipoproteins of female genital tract fluids was studied by incorporating radioactive phospholipids into the fluids for 30 min at 56°C, followed by separation of radioactive lipid-binding proteins and lipoproteins by density-gradient ultracentrifugation, molecular sieving on HPLC and ion exchange chromatography. Given the solubility of LPC, L-PAF and PAF in water, the radioactive forms of these lipids would readily achieve equilibrium with endogenous lipids without significantly changing their endogenous concentrations. Thus, it is considered that the elution profiles presented in this study reflect the endogenous distribution of LPC, L-PAF and PAF in human plasma, as well as in follicular and peritoneal fluids.

The labelled lysophospholipids and PAF displayed identical binding patterns in the elution profiles, which is not surprising in view of their very similar acylated molecular structures. In plasma, approximately 51% of the total radioactivity eluted in the high density ($d > 1.21$) fractions, mostly comprising albumin, while the remainder of the radioactivity was evenly distributed among HDL, LDL and VLDL. These results are in agreement with a previous report on the binding properties of LPC in human plasma (Portman and Illingworth, 1973), indicating that LPC is found not only in the $d > 1.21$ fraction (albumin) but also with HDL, LDL and VLDL which exhibit high binding affinities for LPC. The study reported here indicates that L-PAF and PAF follow the same binding distribution as that observed for LPC.

The distribution profile of LPC, L-PAF and PAF in follicular and peritoneal fluids revealed that more than 89% of the lipids were associated with the albumin fraction, and that the remainder were associated with HDL. This higher proportion of lysophospholipids associated with albumin in follicular fluid is consistent with the reduced binding capacity of low lipoprotein concentrations in this fluid resulting mainly from the absence of LDL. This distribution is also in agreement with a study indicating that LPC accumulates in human follicular fluid mainly in the albumin fraction in which LPC accounts for the major part of the phospholipid composition (Perret *et al.*, 1985). According to the distribution and endogenous concentrations of LPC in human plasma ($252 \mu\text{mol l}^{-1}$) and follicular fluid ($286 \mu\text{mol l}^{-1}$), as reported in the present study, it can be estimated that the average LPC:albumin molar ratios in plasma and follicular fluid are 0.18 and 0.34.

It was established that albumin was the major LPC-binding protein in follicular fluid by application of the $d > 1.21$ fraction on a molecular sieve coupled to HPLC. Only one peak of radioactivity eluted with a retention time identical to that of commercial serum albumin; however, some residual radioactivity was absorbed by the column and labelled lipids could be eluted only by washing the column with methanol. This suggested that LPC was bound to albumin at a high-affinity binding site, but that LPC could also exist either in a soluble free state or in association with albumin via low-affinity binding sites. These results were confirmed by another series of experiments performed with a quaternary methylamine column from which

LPC-albumin and L-PAF-albumin complexes were eluted with $0.2 \text{ mol NaCl l}^{-1}$, whereas free LPC and L-PAF were retained and eluted with methanol. Albumin has one high-affinity binding site for LPC (Klopfenstein, 1969) and several binding sites with a low affinity for LPC (Portman and Illingworth, 1973). Thus, it would appear that LPC and L-PAF are bound to follicular fluid albumin via high- and low-affinity binding sites.

The concentrations of LPC in follicular fluid probably reach maximum values at the time of ovulation, since LCAT, which generates LPC via cholesterol esterification on HDL particles, is active in human follicular fluid (Langlais and Roberts, 1985). By acting upon choline plasmalogens, LCAT is probably also responsible for the production of L-PAF in follicular fluid; the endogenous concentration of L-PAF in follicular fluid was not measured in the present study, but it is estimated that it could be similar to that of plasma, which is 125 ng ml^{-1} or $0.25 \mu\text{mol l}^{-1}$ (Leonelli *et al.*, 1989). The metabolism of LPC in human follicular fluid *in vitro* does not result in any significant hydrolysis of LPC (J. Langlais, unpublished observations) since follicular fluid does not contain any red cells which harbour the enzymes (lysophospholipases and acyltransferases) that metabolize LPC in serum.

Thus, the follicle appears to provide an environment that favours LPC accumulation, as evidenced in the present study by the high concentrations of LPC in follicular fluid which may reach concentrations as high as $600 \mu\text{mol l}^{-1}$, yielding LPC:albumin ratios close to unity.

The observation that albumin of follicular fluid carries twice as much LPC as plasma albumin, suggests that albumin of follicular fluid may have different properties *in vivo* than those observed with serum albumin *in vitro* with regard to sperm motility and sperm-egg interactions. Indeed, a report by Jarvi *et al.* (in press) indicates that both LPC and L-PAF increase linear and curvilinear velocities of human spermatozoa, and that this effect requires the presence of albumin. This result is consistent with a study showing that follicular fluid enhances sperm motility and velocity *in vitro* (Falcone *et al.*, 1991). Kyono *et al.* (1984) showed that the addition of LPC to an *in vitro* human sperm capacitation medium supplemented with albumin, significantly increases the percentage of egg penetration in the hamster zona-free penetration assay.

The high content of LPC associated with follicular fluid might also contribute to the excellent capacitation potential of this fluid by promoting efficient cholesterol efflux from the sperm surface. In this regard, it is documented that LPC potentiates the release of cellular cholesterol by apolipoprotein A1 (Stein *et al.*, 1979). Stein and coworkers proposed that there are low molecular weight protein-phospholipid complexes (less than 100 kDa) in serum, which can cross the capillary endothelial barrier, in preference to lipoproteins, and promote cholesterol removal from peripheral cells. This model could represent an additional mechanism underlying the sterol efflux from human spermatozoa mediated by follicular fluid (Langlais *et al.*, 1988) and contribute to the lipid transfer activity of human follicular fluid (Ravnick *et al.*, 1990).

Lysophospholipids present in residual follicular fluid entrapped within the cumulus cell masses surrounding the ovulated oocyte could be active mediators of a sperm capacitation gradient increasing towards the zona pellucida. As direct contact is required between albumin and spermatozoa for

capacitation *in vitro* (Dow and Bavister, 1989), it is proposed that complexes of albumin, LPC, L-PAF and PAF of follicular origin may stimulate sperm-egg interactions *in vivo*. After their encounter with these albumin-lipid complexes, male gametes in quest of an ovum would experience enhanced motility and velocity (Jarvi *et al.*, in press). Since LPC and L-PAF are known to be monocyte chemoattractants (Quinn *et al.*, 1991), it is possible that these bioactive lipid mediators could also exert a chemotactic effect on spermatozoa, attracting them through an increasing gradient, towards the surface of the zona pellucida. Upon binding to the zona, prolonged exposure of spermatozoa to albumin and apoprotein-A-LPC complexes would promote sperm cholesterol efflux, allowing capacitated gametes to undergo the acrosome reaction initiated by signals transduced by the zona pellucida.

The low LPC concentrations detected in peritoneal fluid in the study reported here may have a physiological significance. Indeed, the low concentration of LPC in PF could result in an appropriate medium for the extensive survival of spermatozoa, particularly when the male gametes reach the pouch of Douglas before ovulation. After the arrival of the oocyte and its residual follicular fluid, a gradient of albumin-LPC complexes may be formed with the highest concentrations at the surface of the egg investments and the lowest concentrations of LPC, distant from the oocyte, corresponding to LPC concentrations in peritoneal or oviductal fluids.

After fertilization, the composition and metabolism of lipids in oviductal fluids could play a regulatory role in embryonic development. Given the important function attributed to PAF in preimplantation embryo development (reviewed by Harper, 1989; O'Neill, 1991), the results presented in this study, demonstrating the association of PAF with follicular fluid albumin as well as the prolonged half-life of PAF in fluids void of lipoproteins, suggest that follicular and oviductal fluids would provide a favourable milieu for the accumulation of PAF.

The distribution of [³H]PAF among plasma proteins and lipoproteins, as observed in the present study, is in agreement with earlier reports describing the binding of PAF and L-PAF to lipoproteins (Benveniste *et al.*, 1988) and the binding of PAF to albumin (Clay *et al.*, 1990; Matsumoto and Miwa, 1985). Clay and coworkers (1990) demonstrated that PAF binds to albumin at four binding sites and suggested that the receptor active form of PAF is the albumin-PAF complex, rather than free PAF. Kudolo and Harper (1990) confirmed the ability of albumin to bind PAF with such high affinity that BSA-PAF complexes are resistant to enzymatic degradation.

Given the high proportion of [³H]PAF associated with follicular fluid albumin and the low PAF-AH activity due to the absence of LDL and VLDL in follicular fluid, as evidenced in the present study, it is suggested that follicular fluid favours PAF accumulation in the form of albumin-PAF complexes which are resistant to enzymatic degradation and act as receptor active forms of PAF. The presence of PAF in human follicular fluid has already been reported (Amiel *et al.*, 1990), with concentrations ranging from 600 to 5000 pg ml⁻¹. Thus, PAF concentrations in human follicular fluid may reach concentrations as high as 10×10^{-10} mol l⁻¹ corresponding to the biological activity of PAF.

Since oviductal fluid is also a blood transudate characterized by the presence of albumin and HDL, and by the absence of

LDL and VLDL (Ehrenwald *et al.*, 1990), it is probable that oviductal fluid, as well as follicular fluid, would favour an increase in the half-life of embryo-derived PAF and allow autocrine secretion of PAF during embryonic development.

By identifying the physiological conditions that extend the half-life of PAF during embryo development *in vivo*, some insight may be gained towards the preparation of optimal culture media for human embryos *in vitro*.

Thus, it was shown here that the addition of exogenous albumin to a culture medium supplemented with 10% serum markedly decreased the hydrolysis of PAF by serum PAF-AH. Interestingly, embryo culture media with a high albumin content, such as Menezo's B2 medium, sustain good embryo development. Because of its low PAF-AH activity, follicular fluid could be used to supplement culture media used in human IVF and embryo transfer procedures. Indeed, improved pregnancy rates with the GIFT procedure have been reported when follicular fluid is used as a sperm capacitation and gamete medium (Fakih and Vijayakumar, 1990).

Increasing our knowledge of the composition and metabolism of lipids in female genital tract fluids where sperm-egg interactions and embryonic development occur may prove rewarding in creating better conditions for human IVF procedures and also in identifying some metabolic lipid disorders which may be related to the pathogenesis of unexplained infertility.

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