Fertilization Potential of Human Sperm Is Correlated with Endogenous Platelet-Activating Factor Content

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Purpose: Platelet-activating factor (PAF) is a potent signaling phospholipid that is found in mammalian sperm and has a positive correlation with fertility. Whereas PAF is present in human sperm, there are no relational reports on its content and the cells fertilization potential. Therefore, the study objective was to determine if PAF content in capacitated-induced sperm is related to fertilization potential as determined by the sperm penetration assay (SPA).

Methods: Endogenous sperm lipids were measured for PAF content by a specific radioimmunoassay following insemination of zona pellucida-free hamster ova. Data were analyzed by regression analysis and Student's *t* test.

Results: Regression analysis revealed a positive and significant relation ($R^2 = 0.806$; $P < 0.05$) between PAF content in human sperm and SPA outcome (pass: ≥ 5.0 ; fail: < 5.0 , penetrations/ova). Patients that passed (22.61 ± 5.21 picomoles/ 10^6) the SPA had significantly ($P < 0.01$) higher PAF levels in their sperm than patients that failed (12.91 ± 1.76 picomoles/ 10^6 cells) the test.

Conclusions: PAF content in capacitated-induced sperm has a significant and positive relationship with fertilization potential. Fertilization potential may be predicted by measuring PAF levels in capacitation-induced human sperm. Determining PAF content in capacitated human sperm may be a beneficial diagnostic tool for the infertility specialist.

KEY WORDS: Fertilization potential; human sperm; platelet-activating factor; SPA.

INTRODUCTION

Male fertility requires the production of an adequate number of normal mature sperm with sufficient motility and the ability to undergo capacitation and the acrosome reaction in order to bind and penetrate the zona pellucida for fertilization. Defects in any of these necessary characteristics can lead to male infertility. Tests to evaluate sperm function (i.e. capacitation and/or the acrosome reaction) are now enabling us to better understand the concept of male factor and unexplained infertility. One of the more

important of these sperm function tests is called the sperm penetration assay. Yanagamachi *et al.* (1) first demonstrated the ability of human sperm to bind and penetrate the zona pellucida-free hamster egg. This observation has led to the development of the concept that, by measuring the ability of zona-free hamster eggs to accept (bind and penetrate) human sperm (sperm penetration assay) one could determine the functional competence (i.e. fertilization capacity or potential) of human sperm.

A number of endogenous factors have been found to regulate the fertilization potential of the sperm, for example platelet-activating factor (2). Platelet-activating factor [1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine] is a unique and novel signaling phospholipid that has pleiotropic biological properties in addition to platelet activation. Since its

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discovery in the early 1970s, this novel compound has been implicated in a variety of reproductive functions including fertilization, implantation, and parturition. The exact mechanism is uncertain, yet PAF's importance in normal fertility is significant. Platelet-activating factor (PAF) plays a significant role in mammalian reproduction (3). PAF is present in human sperm (4), and its content has a positive relationship with pregnancy outcome following conventional in vitro fertilization (5).

Although PAF content in human sperm, processed for use for in vitro fertilization, is correlated with pregnancy outcome, the relationship between PAF activity in human sperm with fertilization and pregnancy potential is not fully understood. Therefore, the objective of the present study was to determine the relationship of PAF content in human sperm processed (i.e. capacitation induced) with fertilization potential as determined by the sperm penetration assay.

MATERIAL AND METHODS

Specimen Collection and Processing

Human semen samples (with Institutional Review Board approval), obtained from men undergoing routine analysis, were collected by masturbation after 48–72 h abstinence. Sperm were isolated from the seminal fluid by 90% silane-coated silica particle suspension (Promotor, CERES Fertility, San Diego, CA) washing ($400\times g$; 12 min) and incubated in test yolk buffer (TYB; Refrigeration Medium; Irvine Scientific, Santa Ana, CA) for 48–72 h at 4°C , to induce capacitation. Following incubation, the TYB was decanted and 4 mL of human tubal fluid (HTF; CERES Fertility) were added to each specimen and incubated for 30 min at 37°C . Sperm were centrifuged ($300\times g$; 8 min) and resuspended in HTF to a final sperm suspension volume of 0.5–1.0 mL.

Sperm Penetration Assay

Cryopreserved hamster ova (CERES Fertility) were thawed per manufacturer's instructions. Zona pellucida (zona) were removed from the ova by 0.1% trypsin digestion (Sigma Chemical Company, St. Louis, MO) digestion. Zona-free hamster ova were coincubated with 3×10^5 normal, motile sperm and incubated at 37°C in HTF (100 μL) under sterile mineral oil for 3.5 h. Immediately following insemination, the remaining sperm suspensions were quick frozen in 20% glacial acetic acid (in phosphate buffered saline)

and stored at -45°C . Following incubation, ova were washed free of bound nonpenetrating sperm cells and evaluated for penetrations under phase contrast light microscopy.

Lipid Extraction

Endogenous lipids were extracted by the method of Bligh and Dyer (6). Briefly, chloroform and methanol (3:2; vol/vol) were added to the sperm suspensions and mixed for 1-min every 10 min over a 1-h period. Following extraction, the samples were centrifuged ($2000\times g$, 5 min) and lipids were obtained from the organic phase following the addition of chloroform and distilled water (1.1:0.9; vol/vol). The organic phases were evaporated to dryness under a gentle stream of nitrogen, dissolved in assay buffer (0.1% sodium azide, 0.05% Tween 20 in 50 mM sodium citrate, pH 6.3), and stored at -20°C .

Radioimmunoassay

Endogenous PAF levels were determined by a PAF specific radioimmunoassay [^{125}I], according to manufacturer's instructions (PerkinElmer Life Sciences, Inc., Boston, MA). Briefly, primary antibodies were added to tubes containing the extracted sperm lipid samples. Samples were then mixed and incubated for 15 min at room temperature. Secondary antibodies and tracer were added, mixed and incubated 24 h at room temperature. Following centrifugation ($2000\times g$; 30 min), the supernatants were decanted and the tubes blotted and counted. The standard curve was calculated by regression analysis (logit value of normalized percent bound vs. log of ng PAF assayed). Content of extracted PAF is expressed as picomoles/ 10^6 sperm.

Radioimmunoassay Performance

PAF-radioimmunoassay sensitivity is approximately 0.1 picomoles; intra-assay (multiple replicates in a single assay) reproducibility was $3.53 (\pm 0.420)$ picomoles; inter-assay (multiple replicates in multiple assays) reproducibility was $2.88 (\pm 0.134)$ picomoles.

Statistical Analysis

Data were analyzed by Student's *t* test. Statistical calculations were performed using SigmaStat for Windows, version 2.03 (Jandel Scientific Corporation, San Rafael, CA).

Fig. 1. PAF content in human sperm.

RESULTS

Ninety-five spermatozoa samples were assayed for the presence of PAF, and PAF was detected in all specimens. The mean ($\pm$ SEM) concentration of PAF in noncapacitated sperm for all samples was found to be $4.31 (\pm 0.97)$ picomoles/ 10^6 cells (Fig. 1). The PAF content in the capacitated-induced (i.e. PromotorTM-isolated and TYB incubated) spermatozoa population ranged from a low of 0.90 picomoles/ 10^6 cells (this patient failed the SPA test) to a high of 86.31 picomoles/ 10^6 cells (this patient passed the SPA test). The mean ($\pm$ SEM) concentration of PAF in the capacitated-induced sperm for all samples was found to be $14.75 (\pm 1.76)$ picomoles/ 10^6 cells (Fig. 1).

Regression analysis revealed a significant relation ($R^2 = 0.806$; $P < 0.05$) between PAF content in the capacitated-induced spermatozoa and the sperm penetration assay. The mean ($\pm$ SEM) PAF content in capacitated-induced spermatozoa was $22.61 (\pm 5.21)$ picomoles/ 10^6 cells in subjects that passed (≥ 5.0 penetrations per ova) the sperm penetration assay and $12.91 (\pm 1.76)$ picomoles/ 10^6 cells for subjects that failed (< 5.0 penetrations per ova) the sperm penetration assay. The amount of PAF in the capacitated-induced spermatozoa from subjects that passed the sperm penetration assay was significantly higher ($P < 0.01$) than that in the capacitated-induced spermatozoa from the subjects that failed the sperm penetration assay (Fig. 1).

DISCUSSION

In the present study, PAF content in capacitated-induced spermatozoa had a positive and significant

relationship with the sperm penetration assay. Whereas these findings are comparable (i.e. patients that passed the sperm penetration assay had significantly higher PAF content than those that failed; patients that became pregnant had significantly higher PAF content than those that did not become pregnant) to what we previously reported for sperm processed for use for in vitro fertilization (5), the PAF content per million cells does differ. A plausible explanation for the differences in PAF content between sperm used for in vitro fertilization and the sperm penetration assay is the nature of the sperm processing method for each as well as the harvesting of cells for extraction. In the in vitro fertilization study, sperm were induced to capacitate via the swim-up (in modified HTF for 3 h at 37°C) technique where the sperm used for the sperm penetration assay via incubation in test yolk buffer (TYB) for 48–72 h at 4°C . The sperm penetration assay-spermatozoa were collected at the time the hamster ova were inseminated while the in vitro fertilization-spermatozoa were collected following fertilization checks (~ 15 h postinsemination). Therefore, the different PAF levels found between the different sperm groups (i.e. in vitro fertilization and sperm penetration assay) are most probably a result of (A) sperm PAF synthesis rate is dependent upon sperm medium (HTF vs. TYB) and/or different temperature conditions (37°C vs. 4°C); (B) enzymes present in sperm may have degraded the PAF over time and also be temperature sensitive; (C) the in vitro fertilization-spermatozoa utilized the PAF over time; (D) a combination of all (most likely).

PAF-acetylhydrolase is present in seminal plasma and is believed to have a role as a decapacitation factor (7). Removal of acetyl hydrolase during the capacitation process (either by dilution as the sperm migrate

naturally through the female reproductive tract or by removal of seminal plasma through sperm washing procedures) may promote PAF synthesis and/or utilization which, in turn, would allow for an increase in sperm motility (8–10). PAF is released by sperm cells in the presence of capacitating media and induces capacitation (11). Patients with abnormal acetylhydrolase levels and/or activity may have impaired fertility.

PAF plays a significant role in the fertilization process, enhancing fertilization rates of mouse and rabbit oocytes (12,13). Enhanced embryo development has also been reported in rabbit oocytes that were fertilized *in vitro* with PAF exposed sperm (14). Exogenous PAF has also been reported to enhance fertilization potential of human sperm (15).

PAF-antagonists inhibit the motility, acrosome reaction, and hamster oocyte penetration in exposed sperm (9,15). Additionally, these antagonists inhibit fertilization (9,16). These data indicate the presence of a PAF-specific receptor in sperm, which has been confirmed by immunofluorescence microscopy (17,18). Therefore, PAF would appear to bind to cell surface receptors on sperm, initiating the formation of inositol triphosphate and diacylglycerol, and increasing intracellular calcium (2,19).

Sperm prior to any assisted reproductive technique and most functional assays must be first processed (e.g. silica particle suspension-washing or swim-up). This processing of sperm insures that the most normal motile population is used to help facilitate functionality. PAF content in processed human sperm has a positive relationship with fertilization potential. Measurement of PAF activity levels in sperm prior to assisted reproduction may be beneficial in predicting pregnancy outcome. Additional studies are warranted to elucidate the reproductive significance of PAF activity in sperm and PAF's role in the establishment of pregnancy.

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