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A Review of the Effect of Platelet-Activating Factor on Male Reproduction and Sperm Function

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Ten to 15% of reproductive age couples in the United States are not able to achieve a successful pregnancy and are considered infertile. Infertility affects men and women equally. Male fertility requires the production of an adequate number of morphologically normal spermatozoa with sufficient motility and the ability to undergo hyperactivation, capacitation, and the acrosome reaction in order to penetrate the oocyte's cumulus oophorus and bind to the zona pellucida for fertilization. Defects in any of these necessary events will lead to infertility. A number of endogenous factors are implicated in the regulation of spermatozoan fertility potential, including platelet-activating factor (PAF; Figure 1).

Benveniste et al (1972) first identified PAF 30 years ago when they found that it was a potent mediator of rabbit platelet aggregation in immunoglobulin E–stimulated basophils. Since then, numerous investigators have demonstrated that PAF is a unique signaling phospholipid that has pleiotropic biologic properties in addition to platelet activation (Hanahan, 1986; Braquet et al, 1987). PAF exists endogenously as a mixture of molecular species with structural variants of the alkyl moiety. The C-16 species is predominant in human sperm (Sanwick et al, 1992). Arrata et al (1978) used ^{31}P nuclear resonance spectroscopy to suggest a role for phosphate esters in male infertility. Levine et al (1987) subsequently used ^{31}P nuclear resonance spectroscopy to demonstrate that PAF concentrations were higher in fertile men than in infertile men and that PAF was absent in semen samples from vasectomized men.

PAF clearly plays a significant role in reproductive physiology. It influences ovulation, fertilization, preimplantation embryo development, implantation, and parturition (Harper, 1989). Although the exact mechanism or mechanisms for PAF action remain unclear, its importance for normal reproductive function does not. Ultimately, PAF may serve as a biomarker for normal sperm function.

PAF Synthesis and Metabolism

Phospholipase A_2 is present in human spermatozoa. It is calcium-dependent and catalyzes the formation of 1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso-PAF) from alkyl-acyl-glycerophosphocholine, an inert structural cell membrane component (Bennet et al, 1986). Lyso-PAF is biologically inactive. It can be acetylated by acetyl transferase using acetyl-coenzyme A (CoA) as an acetate donor to form 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphorylcholine (PAF). Lyso-PAF may also be acetylated by a CoA-independent arachidonyltransacylase to form alkyl-acyl-glycerophosphocholine. Acetylhydrolase is the primary enzyme responsible for inactivating PAF by the removal of the acetate group from the sn-2 position, resulting in the reformation of lyso-PAF. The metabolic pathway for PAF synthesis is presented in Figure 2.

Acetyltransferase and acetylhydrolase are both present in mammalian spermatozoa and seminal fluid (Gujarati et al, 1987). Consequently, both the enzymes necessary for PAF activation and deactivation are present in spermatozoa and seminal fluid. Letendre et al (1992) suggested that acetylhydrolase might itself act as a sperm decapacitation factor. This is based on the observation that capacitation occurs in human spermatozoa without exogenous mediators following sperm removal from seminal fluid. In fact, the data suggest that the elimination of acetylhydrolase during normal capacitation promotes PAF synthesis, which results in increased sperm motility and improved sperm–egg interactions (Roudebush et al, 1990, 1993; Hellstrom et al, 1991; Angle et al, 1993).

PAF may indeed be a biomarker for capacitation. Much of the conflicting data regarding the presence and concentration of PAF may be attributed to the use of noncapacitated spermatozoa. Further, some laboratory procedures may inadvertently decrease PAF concentrations. PAF can become tightly bound to nonsiliconized borosilicate glassware. Finally, Takamura et al (1996) demonstrated that media additives containing egg yolk or human

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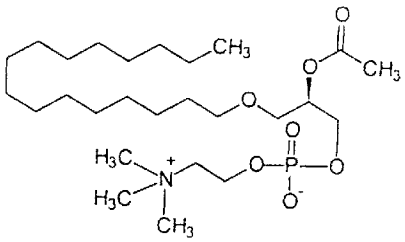

[1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine]

Figure 1. Platelet-activating factor (PAF).

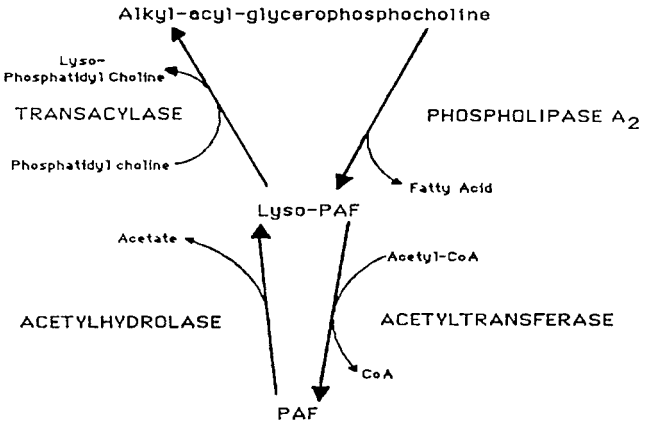

Figure 2. Platelet-activating factor (PAF) synthesis pathway.

serum contain acetylhydrolases that ultimately reduce PAF concentrations.

Insulin-like growth factor I (IGF-I) is a mitotic polypeptide that stimulates glucose and sulfate uptake. The impact and role of IGF-I on female reproductive functions is well documented. However, information concerning the impact of IGF-I on male reproductive function is sparse. IGF-I is an important factor for germ cell development and sperm maturation (Hoeflich et al, 1999). IGF-I is also important with regard to PAF activity. IGF-I will attenuate the intracellular calcium response to PAF in cultured rat mesangial cells (Inishi et al, 1994). Additionally, PAF will induce the production of IGF-I binding proteins in human adenocarcinoma cells (Giannini et al, 1996). Yilmaz et al (1999) demonstrated that scrotal circumference and percentage of normal spermatozoa are related to blood serum IGF-I concentration in yearling Angus bulls. They found that PAF concentrations in yearling Angus bulls (divergently selected for blood serum IGF-I) have a significant and positive relationship with circulating IGF-I concentrations. Sperm-derived PAF levels are significantly higher in bulls with higher IGF-I concentrations (1.9 pM/10^6 cells) than in bulls with lower IGF-I concentrations (0.6 pM/10^6 cells). Spermatozoa from high IGF-I bulls have a greater than threefold higher PAF content than spermatozoa from low IGF-I bulls (Roudebush et al, 2001a).

PAF Receptor

Detectable concentrations of PAF, along with reported observations that PAF antagonists inhibit sperm motility, prevent the acrosome reaction, and reduce hamster oocyte penetration, suggest the existence of PAF receptors (Kuzan et al, 1990; Sengoku et al, 1992, 1993; Angle et al, 1993). Reinhardt et al (1999) and Roudebush et al (2000) used immunofluorescent microscopy to demonstrate that the presence and distribution of PAF receptors on the sperm plasma membrane were predominantly concentrated over the midpiece and equatorial regions (Reinhardt et al, 1999; Roudebush et al, 2000). This pattern of PAF receptor distribution is very similar to the pattern of inositol triphosphate receptors documented by Naaby-Hanson et al (2001).

Spermatozoa undergo several postejaculatory modifications, resulting in the attainment of hyperactivated motility, capacitation, and the acrosome reaction. These changes are related to intracellular calcium fluctuations (Yanigimachi, 1994; Suarez and Dai, 1995). Naaby-Hanson et al (2001) utilized colocalization with immunoelectron microscopy and immunofluorescence to demonstrate that the greatest concentrations of calreticulin-containing vesicles and inositol 1,4,5-triphosphate receptors were located in the equatorial and neck regions of acrosome-reacted human spermatozoa. They suggest that these calcium ion storage locations are critical regulators of capacitative calcium entry during capacitation and the acrosome reaction. Additionally, Lax et al (1997) demonstrated the subcellular distribution of protein kinase C in bovine sperm and their regulation by calcium and phorbol esters. Data from Levine et al (unpublished), Roudebush et al (1997), and Purnell et al (2001) using fluorescent spectrophotometry and/or microscopy demonstrated that PAF acts via a specific G-protein receptor-mediated inositol triphosphate-diacylglycerol pathway in nonreproductive cells, in preimplantation embryos, and in spermatozoa to increase intracellular calcium levels.

Roudebush et al (2000) used immunofluorescent microscopy to demonstrate that the sperm PAF receptor exhibited a significantly altered distribution pattern between normal and abnormal spermatozoa. Importantly, there was a significant difference in fluorescence intensities between normal and abnormal spermatozoa at the midpiece. Notably, they also reported that there were also clear differences in PAF concentration, PAF receptor concentration, and messenger RNA (mRNA) for PAF receptor concentrations between fertile and infertile human spermatozoa (Purnell and Roudebush, 2001b).

Over the past few years, several investigators clearly documented the presence of RNA in ejaculated human

sperm as well as the presence of specific transcriptional gene products. The presence of RNA and its transcriptional activity may be directly related to sperm function. Purnell and Roudebush (2001a) demonstrated that RNA concentrations were lower in motile spermatozoa than in nonmotile spermatozoa.

Motile spermatozoa contain a greater concentration of PAF receptor mRNA than do nonmotile spermatozoa. Interestingly, there is an inverse correlation between actual PAF concentration and sperm motility. This would indicate that nonmotile spermatozoa contain more PAF than motile spermatozoa because they are not able to utilize it (Purnell and Roudebush, 2001b).

The amount of PAF receptor mRNA in abnormal spermatozoa is significantly higher than that found in normal spermatozoa (Roudebush et al, 2001a). Sequence analysis of PAF receptor mRNA between normal and abnormal specimens demonstrated some striking differences. First, sequence alignment between the 2 samples resulted in only 82% homology. Second, when compared to a complete PAF receptor mRNA sequence (human leukocytes; GenBank [www.ncbi.nlm.nih.gov/web/genbank] Accession D10202 D90433), the mRNA from the normal sample had 92% homology, whereas the mRNA from the abnormal sample had only 83% homology (Roudebush et al, 2001b). These differences may be the result of errors in transcription.

Endogenous PAF and Its Effect on Sperm Function

PAF is localized to spermatozoa and is not present in seminal secretions (Kumar et al, 1988; Minhas et al, 1991). PAF is found in the spermatozoa from many mammalian species, including the rabbit, mouse, bull, and boar (Kumar et al, 1988; Kuzan et al, 1990; Parks et al, 1990; Mook et al, 1998; Roudebush and Diehl, 2001). PAF and its receptor are also present in various non-human primate species (Roudebush and Mathur, 1998; Roudebush et al, 1999, 2002).

PAF concentration in boar spermatozoa is positively correlated with the fertility status (Roudebush and Diehl, 2001). The amount of PAF detected in spermatozoa obtained from a high-fertile group ranged from 1.9 to 11.3 pM/10^6 cells. The level of PAF in a low-fertile group ranged from 0.92 to 4.96 pM/10^6 cells. Sperm-derived PAF levels are significantly higher in individual boars with a high-farrow rate, those with a greater number of piglets born, and those with a greater number of piglets born alive.

PAF is present in squirrel monkey spermatozoa (Roudebush and Mathur, 1998). PAF concentrations are significantly greater during the breeding season than during the nonbreeding season. Mguruma et al (1993) and Ohshihe et al (1994) suggest that PAF metabolism is affected by androgens, estrogens, and progesterone. Androgenic hormones play an important role in male fertility and are significantly decreased during the nonbreeding season. PAF concentrations in rhesus monkey sperm are directly correlated with sperm motility and forward progression (Roudebush et al, 2002). Further, stress has a negative impact on PAF concentrations in rhesus monkey spermatozoa. PAF levels in rhesus spermatozoa are significantly lower in stressed males than in nonstressed males (Diaz et al, 1999).

The concentration of PAF in human spermatozoa was originally found to be inversely related to sperm quality (Angle et al, 1991). However, Roudebush and Purnell (2000) reported that PAF content in human spermatozoa processed for use for in vitro fertilization (IVF) correlates positively with motility indices and pregnancy rates. This contradiction may be related to when PAF content was assayed, specifically with regard to whether or not the spermatozoa had undergone capacitation. Roudebush and Purnell (2000) reported PAF concentrations in 39 sperm samples for human patients undergoing IVF without micromanipulation. They demonstrated a significant positive relationship between sperm density and PAF concentration as well as a positive correlation between PAF concentration and implantation rate and pregnancy outcome.

Exogenous PAF and Its Effect on Sperm Function

In a review of their work, Naz and Minhas (1995) report that the addition of PAF to human spermatozoa 1) increases sperm motility; 2) enhances sperm penetration of cervical mucus; and 3) improves sperm penetration assay results. They also report that the addition of PAF to murine and rabbit spermatozoa increased IVF rates. Finally, they demonstrated that the addition of PAF to murine species did not affect reproductive efficiency, nor did it have a detrimental effect on embryo development in vitro or in vivo.

To successfully fertilize an oocyte, spermatozoa must be able to penetrate the outer layers investing the oocyte, including the cumulus cells and the zona pellucida. Several investigators have examined the effect of exogenous PAF on human sperm motility (Ricker et al, 1989; Jarvi et al, 1991; Krausz et al, 1994).

Treatment of human sperm samples with increasing concentrations of exogenous PAF (3.69×10^{-7} to 3.68×10^{-13} M) for 5 minutes resulted in an increase in motion parameters (Ricker et al, 1989). Videomicroscopy techniques were used to assess initial motility, and then the samples were divided into 4 groups on the basis of motion. The greatest increase in motility was noted in the group that displayed the lowest initial motility. The mean swimming speed for this particular group went from 41.7 plus or minus 0.37 μm/s to 63.9 plus or minus 0.27 μm/s, an increase of about 53%. Motility was not affected by treatment with lyso-PAF, suggesting that the changes ob-

served in motility were due to the action(s) of PAF on the spermatozoa.

Jarvi et al (1991) reported that exposure to exogenous PAF concentrations of 0.5–100 nM resulted in significant increases in the linear velocity of human spermatozoa. The greatest increase in linear motion was observed in spermatozoa treated with 50 nM PAF. These investigators also reported a 45% increase in linear velocity as a result of treatment with lyso-PAF, in contradiction to the observations of Ricker et al (1989). The increase that occurred as a result of stimulation with PAF and lyso-PAF lasted for 3 hours. Velocity readings returned to control values after 4 hours. When albumin was absent, no increase in sperm motility was observed, even in the presence of PAF. They noted that the discrepancy in results with lyso-PAF might have been related to their use of albumin concentrations greater than those used by Ricker et al (Jarvi et al 0.3%; Ricker et al 0.025%). Finally, Krausz et al (1994) report that 64% of sperm samples exposed to 10 nM PAF for a maximum of 4 hours had up to a threefold improvement in motility. An inverse correlation was found to exist between PAF-induced increases in motility and basal motility. The percentage of acrosome-reacted spermatozoa also increased as a result of incubation with 10 nM PAF for 1 hour. A brief exposure of spermatozoa to exogenous PAF will significantly enhance sperm motility and hyperactivation (Roudebush et al, 2001b). The brief exposure of spermatozoa to PAF will significantly improve motion parameters (eg, track speed and lateral head amplitude). Lateral head amplitude is an excellent gauge of hyperactivation, a key indicator of capacitation.

Incubation of human spermatozoa with PAF caused an increase in the acrosome reaction (Angle et al, 1993; Lee et al, 1997). Spermatozoa treated with PAF fertilized oocytes at a higher rate than those treated with lyso-PAF or high ionic strength medium (Roudebush et al, 1993; Fukuda et al, 1994). Unpublished personal data in a murine system examining the effect of PAF and/or partial zona dissection on IVF rates revealed that murine spermatozoa incubated with PAF were significantly more likely to demonstrate evidence of polyspermy than spermatozoa not exposed to PAF. Finally, we demonstrated a greater rate of blastocyst formation in embryos that resulted from PAF-treated spermatozoa (Roudebush et al, 1993).

Wild and Roudebush (2001) reported that pretreatment of human spermatozoa used for intrauterine insemination significantly increased pregnancy rates. In a comparison of 60 men with normal semen analyses undergoing sperm preparation for intrauterine insemination, 30 had sperm specimens that were exposed to 15 minutes of PAF (10^{-7} mol/L). In the PAF-exposed group, 14 of 30 women become pregnant (positive fetal heart tones), and in the unexposed group, 5 of 30 women become pregnant ($P < .05$). Those patients whose spermatozoa were pretreated with PAF exhibited a 46.7% pregnancy rate compared with a 16.7% pregnancy rate for untreated specimens ($P < .05$).

Summary

Eight to 10 million couples in the United States are infertile. Male infertility is the primary diagnosis in approximately 25% of these couples and is a contributing factor in an additional 20% of these couples. There are several prerequisites (ie, production of normal, motile sperm capable of undergoing capacitation and the acrosome reaction) that must be met for male fertility. Defects in any of these will result in infertility.

PAF is present in spermatozoa and is positively correlated with fertility. Spermatozoa have a specific PAF receptor, as demonstrated by reports that the receptor is localized to the midpiece and equatorial region. Abnormal spermatozoa demonstrate a different pattern of PAF receptor locations, and PAF antagonists inhibit sperm motility and fertilization rates. Further, exogenous exposure to PAF enhances sperm motility, forward progression, fertilization, and implantation and pregnancy rates.

The exact mechanism of PAF action on spermatozoa is uncertain; however, it plays a critical role in normal sperm function. PAF appears to mediate sperm motility by inducing the formation of inositol triphosphate and diacylglycerol and by increasing intracellular calcium (Lapetina, 1982; Ahmed et al, 1994; Roudebush et al, 1997). The reproductive significance of PAF activity in spermatozoa and fertility, including the role of PAF in the establishment of pregnancy, requires further study.

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