ISSN 2693-2504

The Functional Role of Platelet-Activating Factor in Spermatozoa Physiology

Journal of Bioscience & Biomedical Engineering

Mini Review

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Submitted : 31 Mar 2021 ; Published : 14 Apr 2021

Introduction

Platelet-activating factor (alkylacetylglycerolphosphocholine; PAF) is a potent signaling phospholipid which has been found in numerous cell types in every physiological system studied to date. In reproduction, PAF is found to have a variety of roles, for example: in ovulation, sperm function, and early preimplantation development. The goal of this mini review is to highlight PAF's synthesis, mechanism of action and its functional role in sperm physiology. PAF functions via a G protein coupled receptor mediated pathway, which ultimately increases intracellular calcium levels to enhance sperm motility required for fertilization. Exogenous PAF was also found to increase fertilization potential of spermatozoa in cases of non-male factor infertility. Finally, the mini review explores various lifestyle factors that could potentially affect PAF levels and fertility.

Synthesis of Platelet activating factor (PAF)

Platelet activating factor is a phospholipid mediator that belongs to a family of biologically active, structurally related alkyl phosphoglycerides [30]. PAF is continuously produced by platelets, endothelial cells, macrophages, monocytes, and neutrophils in low quantity [24], as well as by keratinocytes where it functions for cutaneous inflammation [25]. PAF is also found to be present in human sperm where it is found to have a positive relationship with sperm motility [27]. PAF is produced by two distinct pathways namely: de novo pathway, which utilizes 1-alkyl-2-acetyl-sn-glycerol and CDP-choline as substrates of a DTT-insensitive phosphocholine transferase (PAF-PCT), and the remodeling pathway which requires the production of 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (lysoPAF) via the enzyme phospholipase A2 [26].

The de novo pathway is mainly responsible for the physiological levels of PAF [24], which is kept low under normal conditions. The de novo pathway starts with the transfer of an acetyl residue onto a phospholipid, alkyl-lyso-glycerophosphate by the enzyme, acetyltransferase to form alkyl-acetyl-glycerophosphate [24]; Followed by the hydrolysis of alkyl-lyso-glycerophosphate to alkyl-acetyl-glycerol by the enzyme, phosphohydrolase [24]. The final step involves the transfer of CDP-choline to alkyl-acetyl-glycerol by choline phosphotransferase to form the bioactive form of PAF [28]. The remodeling pathway is initiated by the activation of phospholipase A2, which acts on 1-O-alkyl-2-arachidonoyl glycerophosphocholine to yield 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso-PAF) and free arachidonic acid [30]. This is then followed by the transfer of an acetyl residue to lyso-PAF to produce PAF [24]. In sperm, the enzymes lyso-PAF-acetyltransferase and PAF-acetylhydrolase have been shown to be necessary for PAF activation and deactivation respectively [24].

Mechanism of action of PAF on sperm

Human spermatozoa contain varying amounts of PAF, and it has been implicated to function as an autocrine mediator in the aspects of gamete and embryo physiology [31]. PAF functions via a G-protein coupled receptor mediated signaling event [30] that affects intracellular calcium levels [32]. Kazou, *et al.* [33] reported in mouse models that PAF enhances the acrosomal reaction required for sperm capacitation in the presence of calcium ions, further supporting the proposed PAF mechanism of action. There are PAF receptors on sperm cells [34], and they could be potential targets for therapeutics to improve sperm capacitation and subsequent fertilizing ability. PAF binds to special cell surface G-protein coupled receptors present on sperm cells inducing the formation of inositol triphosphate (IP3) and diacylglycerol (DAG), resulting in an increase of intracellular calcium levels [35]. These findings support the importance of calcium (Ca^{2+}) entry for sperm capacitation in the presence of PAF.

Endogenous PAF in sperm

Since its discovery, PAF has been positively linked with increased fertility numerous times. Roudebush, *et al.* [1] found that boars with high fertility have a significant amount more PAF than boars with low fertility. Roudebush, *et al.* [4] reported that PAF levels were significantly higher ($P < 0.01$) during the breeding season of squirrel monkeys with a mean of 3.58 ng/10(6) spermatozoa in comparison to the nonbreeding season which had mean of 0.76 ng/10(6). Sengoku, *et al.* [2] demonstrated how an antagonist of PAF, called CV-3988, decreased the fertilization ability of human sperm in vitro by both decreasing sperm penetration ability towards hamster oocyte as well as its acrosome reaction. Interestingly, they not only found that PAF increased the penetration rates of human spermatozoa in a sperm penetration assay, but it also reversed the suppressive effects of CV-3988 on human sperm, aside from the penetration effects and the acrosome reactions.

Given the positive effects PAF has on sperm motility, research into ways to improve endogenous levels of PAF could be useful in the treatment of infertility associated with low sperm motility. Marie, *et al.* [29] were able to stimulate the production of PAF by > 10-fold via the de novo pathway, by adding 2mM of oleic acid to Ehrlich ascite cells. This reveals an avenue to potentially increase the endogenous production of PAF.

Effects of exogenous PAF on sperm

Ricker *et al* [3] demonstrated how PAF significantly increased sperm motility by the addition of synthetic PAF at 3.69×10^{-13} – 3.69×10^{-7} mol/L. In contrast the addition of lyso-PAF, which has no PAF biological activity, had no effect on the motility of sperm. Odeh, *et al* [8] went on further to conclude that a lower concentration of PAF enhances motility of stallion spermatozoa, whereas a higher concentration of PAF induces the acrosome reaction.

The PAF effect on sperm to increase fertility was even more evident when Grigorious and colleagues [5] set up a study with fifty-two couples with unexplained infertility. The sperm of each male was treated with an exogenous mixture of PAF that totaled a concentration of 10^{-7} mol/L in sperm-washing medium. The sperm was prepared using the direct swim-up technique and a maximum of six IUI cycles with or without PAF treatment were given per couple. The clinical pregnancy rate after a maximum of six IUI cycles showed a significant difference with PAF treated sperm having a rate of 23.07% while non-treated PAF sperm was only 7.92% [5].

Other research - while agreeing with PAF's positive effect on motility, acrosome reaction, and fertility - are more critical on its safety. Unsal, *et al* [6] study focused on PAF and a synthetic dimethylxanthine derivative, called Pentoxifylline effect on sperm DNA damage. Using a sperm chromatin dispersion (SCD) test they qualified sperm with large or medium sized halos as non-fragmented whereas sperm with small or no halos as significantly fragmented. Sperm that was treated with a baseline had a median SCD rate of 6%. Sperm that were treated with PAF had a median SCD rate of 10%. Sperm that were treated with pentoxifylline had a median SCD rate of 15%. It was concluded that even though DNA damage was higher in samples treated with pentoxifylline compared to PAF, both groups had increased DNA damage. It was still noted that PAF seemed to be more innocent when choosing viable sperm cells and in achieving sperm motility in vitro fertilization laboratory.

Therapeutics of PAF

Wild and Roudebush explored the effects of exogenous PAF on intrauterine insemination (IUI) outcomes [38]. The study involved collecting normal semen specimens from 60 men, preparing to undergo IUI. PAF was exposed to half of the samples collected, while the other half was not exposed. There was a 46.7% pregnancy rate among the group exposed to PAF, compared to the 16.7% pregnancy rate from the unexposed group [38]. The study revealed that PAF treatment improved performance in motile sperm only and subsequent IUI pregnancy outcomes [38].

A similar study was performed by Stavroula, *et al.* [39] to outline the possible effects of sperm treatment with exogenous PAF on IUI in cases with mild male factor infertility. The study included 92 couples with mild male factor infertility, and each couple had 4 IUI cycles with or without exogenous PAF treatment. The results showed a comparable pregnancy rate of 12.24% vs 11.11% between the cases with and without PAF treatment respectively [39]. It concluded that exogenous PAF does not improve clinical outcomes in cases of mild male factor infertility [39]. Since the positive role of PAF is a receptor mediated event, suggests that this sub-population of infertile males have a defect in their respective PAF-receptors. This can be confirmed by the study of Levine, *et al.* [42], which demonstrated that abnormal spermatozoa have a different pattern of PAF receptor locations, which differ from its usual localization to the midpiece and equatorial regions of normal motile spermatozoa.

Jarvi, *et al.* [40] performed another study to illustrate the potential for PAF in conjunction with albumin could be used to improve sperm motility. Human spermatozoa were incubated with PAF, lyso-PAF or lysophosphatidylcholine concentrations, and varying concentrations of albumin, from 0% to 1.2%. Motility was then evaluated at different time periods from 5 to 240 minutes by computer motion assisted analysis. The results of the study illustrated that 50µM of PAF and 100µM of lyso-PAF, when supplemented with 0.3% albumin increased sperm linear velocity by 41% and 44% respectively, and the curvilinear velocity by 17% and 21% respectively. However, in the absence of albumin, neither PAF nor lyso-PAF induced any increase in sperm motion, when compared to control [40]. Given these findings, the study poses a potential use for PAF and its metabolite in concert with albumin, as a potential treatment for asthenozoospermia [40].

Lifestyle changes that affect PAF

There is little to no research associated with how lifestyle changes affect PAF directly; however, due to research that significantly correlates PAF with sperm fertility it can be

indirectly associated. Jóźków, *et al* [9] looked at how several aspects of lifestyle can affect fertility beginning with three sports: cycling, running, and mountaineering. They found that cycling is the most troublesome and is associated with abnormal morphology and reduced motility of sperm due to the mechanical impact sustained from sitting on a saddle, gonadal overheating, wearing tight clothes, and hypogonadism [10,11]. High intensity running was found to decrease sperm density, motility, and morphology [12]. Mountain trekkers were found to have lower sperm concentration, motility, and higher percent of abnormal and immature spermatozoa due to exposure to altitudes higher than 2000m [13].

Other examples of lifestyle factors that affect fertility is smoking, alcohol consumption, obesity, testicular heat, and vitamin intake. Smoking has been shown to have a strong correlation with % DNA fragmentation index (DFI) which is the most statistically robust test to predict fertility [14,15,16,17,18]. A DFI score of > 30% indicates infertility. Ramalu-Hansen, et al [19] found that heavy smokers have a 19% lower mean sperm concentration, and a 29% lower total sperm count than nonsmokers. Heavy alcohol consumption was found to have a significant correlation with infertility, while moderate alcohol consumption was found to have moderate impact on fertility [20]. Obesity was found to be present in infertile men more frequently than in fertile men [21]. It was also found that azoospermia and oligozoospermia occur more in obese men than non-obese men [22]. Those who tend to be in environments where the heat of their testicle is more than normal people, like individuals who use the hot tub in abundance, tend to have a significant increase in DNA fragmentation which can lead to infertility. Vitamin C and Vitamin E intake; however, was shown to decrease DNA damage from 22.1% to 9.1% in 64 males with >15% damage [23].

Future directions

The lack of available research on the direct effect of lifestyle changes on PAF opens up many avenues in future research projects. Currently, an indirect link is made, by correlating lifestyle behavior with PAF through sperm fertility. As more research is done to potentially correlate direct lifestyle behavior with PAF, it will be interesting to see how individuals can better increase their endogenous PAF concentration. Future projects can assess how exercise, nutrition, or general behavioral lifestyle can increase or decrease PAF concentration which can then lead to increased or decreased sperm fertility. This can allow for individuals struggling with specific fertility problems to have different avenues to fix these issues in a natural manner that goes beyond pharmaceutical products.

References

1. Roudebush W. E. (2001) Role of platelet-activating factor in reproduction: sperm function. *Asian journal of andrology, 3*(2): 81–85.
2. Sengoku, et al. (1993) "Andrology: Effects of Platelet Activating Factor on Human Sperm Function in Vitro." OUP Academic, 1 Sept. 1993, academic.oup.com/humrep/article-abstract/8/9/1443/633245?redirectedFrom=fulltext.
3. Ricker DD, Minhas BS, Kumar R, et al. (1989) The effects of platelet-activating factor on the motility of human spermatozoa. *Fertil Steril, 52*(4): 655-658.
4. Roudebush, W. E., & Mathur, R. S. (1998) Presence of platelet-activating factor in squirrel monkey (Saimiri boliviensis) spermatozoa: seasonal differences. *American journal of primatology, 45*(3): 301–305. https://doi.org/10.1002/(SICI)1098-2345(1998)45:3<301::AID-AJP6>3.0.CO;2-#
5. Grigoriou, Odysseas, et al. (2005) "Effect of Sperm Treatment with Exogenous Platelet-Activating Factor on the Outcome of Intrauterine Insemination." F*ertility and Sterility, 83*(3): 618–621. Crossref, doi:10.1016/j.fertnstert.2004.07.980.
6. Unsal, E., Turan, V., Aktuna, S., Hurdag, C., Bereketoglu, G., Canillioglu, Y., Baltacı, A., Ozcan, S., Karayalcin, R., Batırbaygil, H., & Baltacı, V. (2016) Effects of pentoxifylline and platelet activating factor on sperm DNA damage. *European journal of obstetrics, gynecology, and reproductive biology,* 197: 125–129. https://doi.org/10.1016/j.ejogrb.2015.12.016
7. George, J., & Abel, P. (2008) Pentoxifylline. In xPharm: The Comprehensive Pharmacology Reference (pp. 1-18). *Elsevier* Inc.. https://doi.org/10.1016/B978-008055232-3.63921-2
8. Odeh, A. I., Dascanio, J. J., Caceci, T., Bowen, J., & Eng, L. A. (2003) Effect of platelet-activating factor (PAF) on stallion sperm motility, capacitation and the acrosome reaction. *Reproduction (Cambridge, England), 126*(5): 605–613.
9. Jóźków, P., & Rossato, M. (2017) The Impact of Intense Exercise on Semen Quality. *American Journal of Men's Health,* 654–662. https://doi.org/10.1177/1557988316669045
10. "Sperm Characteristics of Endurance Trained Cyclists." *International Journal of Sports Medicine, , 2004, 25*(4): 247–251. Crossref, doi:10.1055/s-2004-819933.
11. Kipandula, W., & Lampiao, F. (2015) Semen profiles of young men involved as bicycle taxi cyclists in Mangochi District, Malawi: A case-control study. *Malawi medical journal: the journal of Medical Association of Malawi, 27*(4): 151–153.
12. Jensen, C. E., Wiswedel, K., McLoughlin, J., & van der Spuy, Z. (1995) Prospective study of hormonal and semen profiles in marathon runners. *Fertility and sterility, 64*(6): 1189–1196.
13. Verratti, V., Berardinelli, F., Di Giulio, C., Bosco, G., Cacchio, M., Pellicciotta, M., Nicolai, M., Martinotti, S., & Tenaglia, R. (2008) Evidence that chronic hypoxia causes reversible impairment on male fertility. *Asian journal of andrology, 10*(4): 602–606. https://doi.org/10.1111/j.1745-7262.2008.00346.x
14. Mona Bungum (2012) "Sperm DNA Integrity Assessment: A New Tool in Diagnosis and Treatment of Fertility", *Obstetrics and Gynecology International,* vol. 2012, Article ID 531042, 6 pages, 2012. https://doi.

org/10.1155/2012/531042
15. Giwercman, A., Lindstedt, L., Larsson, M., Bungum, M., Spano, M., Levine, R.J. and Rylander, L. (2010) Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case–control study. *International Journal of Andrology,* 33: e221-e227. https://doi.org/10.1111/j.1365-2605.2009.00995.x
16. K. Oleszczuk, A. Giwercman, M. Bungum (2011) Intra-individual variation of the sperm chromatin structure assay DNA fragmentation index in men from infertile couples, *Human Reproduction, 26*(12): 3244–3248, https://doi.org/10.1093/humrep/der328
17. Smit, M., Dohle, G.R., Hop, W.C.J., Wildhagen, M.F., Weber, R.F.A. and Romijn, J.C. (2007) Clinical correlates of the biological variation of sperm DNA fragmentation in infertile men attending an andrology outpatient clinic. *International Journal of Andrology*, 30: 48-55. https://doi.org/10.1111/j.1365-2605.2006.00710.x
18. Venkatesh, S., Singh, A., Shamsi, M.B. et al. (2011) Clinical Significance of Sperm DNA Damage Threshold Value in the Assessment of Male Infertility. *Reprod*. Sci. 18: 1005–1013. https://doi.org/10.1177/1933719111401662
19. Ramlau-Hansen, C. H., Thulstrup, A. M., Aggerholm, A. S., Jensen, M. S., Toft, G., & Bonde, J. P. (2007) Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Human reproduction (Oxford, England), 22*(1): 188–196. https://doi.org/10.1093/humrep/del364
20. Alvarez S. (2015) Do some addictions interfere with fertility?. *Fertility and sterility, 103*(1): 22–26. https://doi.org/10.1016/j.fertnstert.2014.11.008
21. Sharpe R. M. (2010) Environmental/lifestyle effects on spermatogenesis. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 365*(1546): 1697–1712. https://doi.org/10.1098/rstb.2009.0206
22. Sermondade, N., Faure, C., Fezeu, L., Shayeb, A. G., Bonde, J. P., Jensen, T. K., Van Wely, M., Cao, J., Martini, A. C., Eskandar, M., Chavarro, J. E., Koloszar, S., Twigt, J. M., Ramlau-Hansen, C. H., Borges, E., Jr, Lotti, F., Steegers-Theunissen, R. P., Zorn, B., Polotsky, A. J., La Vignera, S., … Czernichow, S. (2013) BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Human reproduction update, 19*(3): 221–231. https://doi.org/10.1093/humupd/dms050
23. Greco, E., Iacobelli, M., Rienzi, L., Ubaldi, F., Ferrero, S. and Tesarik, J. (2005) Reduction of the Incidence of Sperm DNA Fragmentation by Oral Antioxidant Treatment. *Journal of Andrology*, 26: 349-353. https://doi.org/10.2164/jandrol.04146
24. Ashraf, M. A. (2020) Biochemistry, Platelet Activating Factor - StatPearls - NCBI Bookshelf. NCBI. https://www.ncbi.nlm.nih.gov/books/NBK557392/
25. Travers, J. B., Clark Huff, J., Rola-Pleszczynski, M., Gelfand, E. W., Morelli, J. G., & Murphy, R. C. (1995) Identification of Functional Platelet-Activating Factor Receptors on Human Keratinocytes. *Journal of Investigative Dermatology, 105*(6): 816–823. https://doi.org/10.1111/1523-1747.ep12326581
26. Goracci, G., Balestrieri, M. L., & Nardicchi, V. (2009) Metabolism and Functions of Platelet-Activating Factor (PAF) in the Nervous Tissue. *Handbook of Neurochemistry and Molecular Neurobiology*, 311–352. https://doi.org/10.1007/978-0-387-30378-9_13
27. Roudebush, W. E., Zhu, J., Mitchell-Leef, D., Toledo, A. A., Massey, J. B., & Kort, H. I. (2003) Sperm motility is related to platelet-activating factor-acetylhydrolase content in semen. *Fertility and Sterility*, 80: 256. https://doi.org/10.1016/s0015-0282(03)01639-x
28. Woodard, D. S., Lee, T. C., & Snyder, F. (1987) The final step in the de novo biosynthesis of platelet-activating factor. Properties of a unique CDP-choline:1-alkyl-2-acetyl-sn-glycerol choline-phosphotransferase in microsomes from the renal inner medulla of rats. *The Journal of biological chemistry, 262*(6): 2520–2527.
29. Blank, M. L., Lee, Y. J., Cress, E. A., & Snyder, F. (1988) Stimulation of the de novo pathway for the biosynthesis of platelet-activating factor (PAF) via cytidylyltransferase activation in cells with minimal endogenous PAF production. *The Journal of biological chemistry, 263*(12): 5656–5661.
30. Montrucchio, G., Alloatti, G., & Camussi, G. (2000) Role of Platelet-Activating Factor in Cardiovascular Pathophysiology. *Physiological Reviews, 80*(4): 1669–1699. https://doi.org/10.1152/physrev.2000.80.4.1669
31. Pike, I. L., Ammit, A. J., & O'Neill, C. (1992) Actions of platelet activating factor (PAF) on gametes and embryos: clinical aspects. *Reproduction, fertility, and development, 4*(4): 399–410. https://doi.org/10.1071/rd9920399
32. Brackett, B., Bosch, P., McGraw, R., DeJarnette, J., Marshall, C., Massey, J., & Roudebush, W. (2004) 291PRESENCE OF PLATELET-ACTIVATING FACTOR (PAF) RECEPTOR IN BULL SPERM AND POSITIVE CORRELATION OF SPERM PAF CONTENT WITH FERTILITY. *Reproduction, Fertility and Development, 16*(2): 265. https://doi.org/10.1071/rdv16n1ab291
33. Sengoku, K., Ishikawa, M., Tamate, K., & Shimizu, T. (1992) Effects of platelet activating factor on mouse sperm function. *Journal of Assisted Reproduction and Genetics, 9*(5): 447–453. https://doi.org/10.1007/bf01204050
34. Roudebush, W. E., Massey, J. B., Elsner, C. W., Shapiro, D. B., Mitchell-Leef, D., & Kort, H. I. (2005) The significance of platelet-activating factor and fertility in the male primate: a review. *Journal of medical primatology, 34*(1): 20–24. https://doi.org/10.1111/j.1600-0684.2004.00087.x
35. Huang, Q. Y., & Li, M. J. (2007) Zhonghua nan ke xue = *National journal of andrology, 13*(6): 538–541.
36. Botitsi, E., Mavri-Vavayanni, M., & Siafaka-Kapadai, A. (1998) Metabolic fate of platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) and lyso-PAF (1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine) in FRTL5 cells. *Journal of lipid research, 39*(6): 1295–1304.
37. Chao, W., Siafaka-Kapadai, A., Hanahan, D. J., & Olson, M. S. (1989) Metabolism of platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) and lyso-PAF (1-O-alkyl-2-lyso-sn-

glycero-3-phosphocholine) by cultured rat Kupffer cells. *Biochemical Journal, 261*(1): 77–81. https://doi.org/10.1042/bj2610077

38. Robison, J., Yi, S., & Roudebush, W. E. (2014) Changes in Fertility after Exposure of Sperm to Exogenous PAF | INCIID. INCIID. https://www.inciid.org/sperm-exposure-exogenous-paf
39. Baka, S., Grigoriou, O., Hassiakos, D., Konidaris, S., Papadias, K., & Makrakis, E. (2009) Treatment of Sperm With Platelet-activating Factor Does Not Improve Intrauterine Insemination Outcome in Unselected Cases of Mild Male Factor Infertility: A Prospective Double-blind Randomized Crossover Study. *Urology, 74*(5): 1025–1028. https://doi.org/10.1016/j.urology.2009.06.023
40. Jarvi, K., Roberts, K. D., Langlais, J., & Gagnon, C. (1993) Effect of platelet-activating factor, lyso-platelet-activating factor, and lysophosphatidylcholine on sperm motion: importance of albumin for motility stimulation. *Fertility and sterility, 59*(6): 1266–1275. https://doi.org/10.1016/s0015-0282(16)55988-3
41. Levine, A. S., Kort, H. I., Toledo, A. A., & Roudebush, W. E. (2002) A review of the effect of platelet-activating factor on male reproduction and sperm function. *Journal of andrology, 23*(4): 471–476.