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Exposure of Preimplantation Embryos to Platelet-Activating Factor Increases Birth Rate

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***Problem*:** Platelet-activating factor (PAF) plays a significant role in fertility. Preimplantation stage embryos produce PAF (ePAF) which is required for development. PAF's mechanism of action is receptor-mediated and its presence has been reported in the developing mouse and human embryo. Exposure of preimplantation stage mouse embryos results in higher implantation rates. However, the effect of such treatment on live-birth rates and birth weights has not been reported. Therefore, the objective the study was to determine the effect of exposing preimplantation mouse embryos to PAF on subsequent birth rate and weight.

***Design*:** Two-cell stage preimplantation stage mouse embryos exposed to PAF (10^{-7} M) for 15 min prior to intraoviductal transfer.

***Methods*:** Preimplantation stage embryos were recovered from eCG/hCG primed BDF1 female mice. Embryos were exposed to synthetic PAF (10^{-7} M) for 15 min. PAF-treated embryos were transferred to the oviducts of pseudopregnant female CD-1 female mice. Superovulated and cultured BDF1 embryos not treated with PAF served as in vitro controls and naturally ovulated embryos with no collection/culture served as in vivo controls. Embryos were permitted to develop to term (18–21 days). The number of pups born per litter and litter weights subsequently were recorded.

***Results*:** A total of 160 BDF1 mouse embryos were collected, treated, and transferred (20 per CD-1 recipient) as described. There was a significant ($P < 0.05$) increase in the number of pups born to the PAF treatment group (56/80; 70%) as compared to the control group (44/80; 55%). There was also a significant difference ($P < 0.05$) in litter birth weights between the PAF (1.31 g/litter) and controls groups (1.25 g/litter). There was a significant difference ($P < 0.05$) in birth weights between the PAF treatment group and the in vivo group (1.51 g/litter). There was a significant difference in birth weights between the in vitro control and in vivo groups (1.51 g/litter). There were no observational malformaties to pups born in any group.

***Conclusions*:** Brief exposure of preimplantation stage embryos to PAF will result in a significant increase of delivery rates (pups/litter) as well as birth weights. However, the increase of birth weight was significantly below that found naturally. Additional studies are warranted to elucidate the mechanism of PAF's action in the preimplantation stage embryo and subsequent uterine development.

KEY WORDS: Mouse; embryo; platelet-activating factor; term development.

INTRODUCTION

Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF) is a unique and

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novel signaling phospholipid that plays a significant role during fertilization and the preimplantation period. Although the production of PAF by preimplantation embryos has been reported, physiologic roles of this potent mediator remain unclear. Embryonic PAF has been suggested to serve as a survival factor (1). Embryonic PAF is synthesized and released by rabbit and mouse embryos during the preimplantation phase, with maximum levels at the expanded blastocyst stage (2,3). PAF production by human embryos has been correlated with development (4,5) and pregnancy potential (6). Embryonic PAF can act as an autocrine stimulator of embryo development (7,8), which can be blocked by PAF antibodies or antagonists (8,9). PAF's mechanism of action in the embryo appears to be receptor-mediated since different PAF antagonists competitively inhibit its action (9). The mouse PAF receptor has only recently been characterized (10–12). Expression of the PAF receptor in human embryos has also been documented (13). Supplementation of culture medium with PAF improves two-cell development to the blastocyst stage in the mouse (14), apparently by stimulation of embryonic metabolism (6). The effect of exogenous PAF on in vitro mouse embryo development appears to be mouse-strain (15) and ligand-isoform (16) specific. Exposure of mouse embryos to PAF prior to embryo transfer will significantly improve implantation rates (17). However, the effect of exposing embryos to PAF prior to embryo transfer on pregnancy outcomes (i.e., birth rates and litter weight) is unknown. Therefore, the study objective was to determine the effect of exposing preimplantation mouse embryos prior to embryo transfer on number of pups born per litter and litter birth weight.

MATERIALS AND METHODS

Embryo Culture Medium

Powdered α-MEM with Earle's salts and L-glutamine was dissolved in 1000-mL tissue culture-grade water (Irvine Scientific, Santa Ana, CA) and modified with 0.075-g penicillin-G (Sigma, Chemical Company, St. Louis, MO), 0.075-g stretptomycin sulfate (Sigma), 2.1-g sodium bicarbonate (Sigma), and 1 mM calcium lactate (Calbiochem, San Diego, CA). The modified α-MEM was adjusted to a final pH of 7.2–7.4 and an osmolarity of 280+5 mOsm/kg, sterile filtered through a 0.22 μM filter unit with nylon membranes (Corning, NY) and equilibrated in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C.

Platelet-Activating Factor Preparation

A stock solution of synthetic platelet-activating factor was prepared by dissolving PAF in 100% methanol to a concentration of 10^{-6} M. A working solution of PAF was prepared by drying down 0.1 mL of the stock PAF solution under a gentle stream of nitrogen in siliconized tubes. The dried PAF was reconstituted with 1 mL of α-MEM (0.3% BSA). The final working concentration of PAF was 10^{-7}M and was used within 15 min of preparation.

Embryo Collection and Culture

Mice were housed in a specific-pathogen-free facility with a 14-h light:10-h dark photoperiod at 23°C and food and water provided ad libitum. Pronuclear-zygote (one-cell stage) embryos were collected from the excised oviducts of equine chorionic gonadotropin (eCG; 10 IU; Sigma) and human chorionic gonadotropin (hCG; 10 IU; Sigma) primed BDF/J (Jackson Laboratory, Bar Harbor, ME) female mice (4 weeks of age) were mated with fertile BDF/J male (6 months of age) mice. Interval between eCG and hCG was 48 h. Embryos (one-cell stage) were collected 16–18-h post-hCG in modified α-MEM (0.3% BSA, Sigma) under sterile-equilibrated mineral oil in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C. The one-cell stage BDF/J embryos were cultured in modified α-MEM (0.3% BSA) in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C for 24-h to the two-cell stage.

Exposure of Embryos to Platelet-Activating Factor

Embryos were exposed to the working PAF solution (1 mL) in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C for 15 min. Following PAF exposure, embryos were washed 3× in α-MEM (0.3% BSA).

Embryo Transfer

Embryos were transferred to the oviducts of eCG/hCG (2.5 IU each) primed female CD-1 female mice (8–10 weeks of age; Charles River, Wilmington, MA). Primed female mice were exposed to vasectomized CD-1 male mice (12+ weeks of age). The pseudopregnant female CD-1 mice

were anesthetized with an intraperitoneal injection of avertin (2,2,2-tribromoethanol in *tert*-amyl alcohol; Sigma; 0.2 mg/kg body weight). A small paracostal incision was made, near the dorsal midline at the level of the last rib, to expose and remove the fat pad, ovary, oviduct, and uterus. A small hole was made in the bursa over the infundibulum. Embryos (10/oviduct; 20 total per recipient) were introduced into the infundibulum and into the opening of the ampulla via a 135 μM micropipette (Stripper®, Mid-Atlantic Diagnostics, Inc., Marlton, NJ). Embryos were permitted to develop to term (18–21 days). Pups born per litter and litter weights were recorded.

Statistical Analysis

Data were analyzed by the chi-squared test (pups born per litter) and Student's t test (litter weight). Litter weights were normalized to take into account differences in litter weights due to different numbers of pups born per litter. Statistical calculations were performed with SigmaStat for Windows, version 2.03 (Jandel Scientific Corporation, San Rafael, CA).

RESULTS

A total of 160 BDF1 mouse embryos were collected, treated, and transferred (20 per CD-1 recipient) as described. There was a significant ($P < 0.05$) increase in the number of pups born in the PAF treatment group (56/80; 70%) over the control group (44/80; 55%) (Fig. 1). There was a significant difference ($P < 0.05$) in litter birth weights between PAF (1.31 g/litter) and the in vitro control groups (1.25 g/litter) (Fig. 2). There was a significant difference ($P < 0.05$) in birth weights between the PAF treatment group and the in vivo group (1.51 g/litter) (Fig. 2). There was a significant difference ($P < 0.05$) in birth weights between the in vitro control and in vivo groups (1.51 g/litter) (Fig. 2). There were no observational malformaties to pups born to any group.

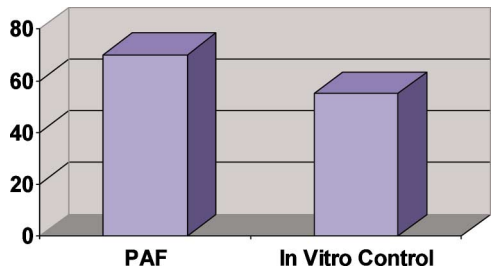

Fig. 1. Effect of PAF on delivery rates: pups born/litter.

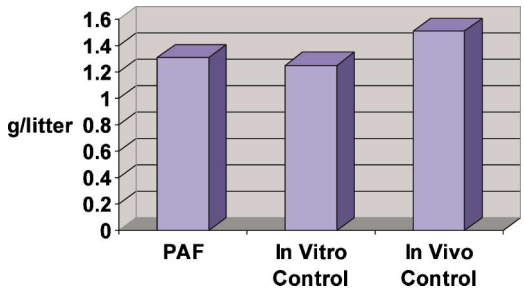

Fig. 2. Effect of PAF on litter weights.

DISCUSSION

In conclusion, this is the first report on the effect of exposing preimplantation embryos to exogenous PAF prior to embryo transfer on delivery data. Exposure of preimplantation stage embryos to PAF will result in a significant increase of delivery rates (pups/litter) as well as birth weights. However, the increase of birth weight was significantly below that found naturally.

Preimplantation stage embryos in a variety of species (e.g., human and mouse) produce and release PAF (18,19). PAF production by human embryos is related to their subsequent pregnancy potential (6). Embryos cultured in the presence of PAF have an enhanced developmental (20) and higher implantation rates upon transfer to synchronized recipients that may be due, in part, to stimulation of embryonic metabolism by embryo-derived PAF (21). PAF directly influences the oxidative metabolism of glucose and lactate in the mouse preimplantation embryo (22). Cholinephosphotransferase and acetyltransferase (the enzymes that catalyze the final step in the biosynthetic pathways for PAF production) are present in mouse preimplantation stage embryos (23).

PAF antibodies inhibit embryo development (9) and antagonists inhibit implantation (23,24) providing further evidence, albeit indirect, for the presence and requirement of embryo-derived PAF during the pre- and peri-implantation periods. Molecular evidence for the PAF receptor in human and mouse has been reported (11,12) with expression highest at the two-cell stage and lowest at the morula stage (25).

In summary, preimplantation stage embryos exposed to PAF will result in improved delivery rates

and higher litter weights. PAF binds to its receptor, which can be blocked by PAF antibodies (9) or antagonists (23,24), resulting in increased intracellular calcium levels (11), increased mitotic rates (7), enhanced growth and development (8,14) implantation (17), and delivery rates. The collective data provides further evidence that the effect of PAF on preimplantation embryo development is receptor-mediated and may involve the IP_3 system. PAF may affect preimplantation embryonic development by regulation of intracellular calcium levels via a G-protein-IP_3 signaling system.

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