Bisphenol A: A Model Endocrine Disrupting Chemical With a New Potential Mechanism of Action

Susan C. Nagel and John J. Bromfield

Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, Missouri, 65211

Humans are exposed to a multitude of exogenous chemicals, termed "endocrine disrupting chemicals" (EDCs), that can interfere with endogenous hormone action (1). EDCs originate from natural sources such as plants, fungi, and bacteria, and from a large number of manmade chemicals, most of which were not designed to disrupt hormone signaling. Because hormones often act at very low concentrations, for example estradiol functions at or below the picomolar range, EDCs can often disrupt the endocrine system at minute, environmentally relevant exposures (2). EDCs have been described to disrupt almost all types of endocrine signaling in laboratory animals and wildlife (3). Importantly, for many human health endpoints, there is strong evidence to support the hypothesis that EDCs, within the range of current human exposure, are having adverse health impacts on the general population (1). In the current issue, Veiga-Lopez et al (4) examine, for the first time, the effects of the EDC bisphenol A (BPA) on microRNA (miRNA) expression in the fetal ewe ovary.

BPA has been shown in a number of studies to act through several different receptor-mediated mechanisms of action to disrupt the endocrine system (5, 6), and, in many ways, it has become a model EDC. BPA is a xenoestrogen that binds to and activates the estrogen receptor (ER) (7). Although it has lower affinity for genomic ER than estradiol, circulating concentrations of BPA are higher than estradiol and are within a biologically active range. In addition, BPA is at least as bioactive as estradiol for a number of responses, particularly those mediated by nongenomic signaling (8). BPA is also an antiandrogen, in that it binds to the androgen receptor and blocks the normal action of androgens (9); it can also alter steroid synthesis and circulating steroid hormone concentrations (9) and disrupt peroxisome proliferator-activated receptor (11), thyroid (12, 13), and glucocorticoid signaling. In the current study, two steroid synthesizing enzymes were altered: aromatase, the primary estrogen synthesizing enzyme, and 5α-reductase, the androgen-synthesizing enzyme that converts testosterone to the higher potency dihydrotestosterone.

A major strength of the current study is that the circulating BPA concentration in treated animals was within the range of current human exposure that has been associated with disease (14). BPA is a high-production chemical used in numerous products including polycarbonate plastic, resin lining of metal food cans, some dental sealants, and thermal receipt paper. Because BPA has widespread use in many products and is readily absorbed both with internal and external exposure, it is detected in most humans, water, house dust, and many food products (14, 15). Due to its many routes and sources of exposure, humans circulate approximately 1 to 2 ng/mL of unconjugated BPA in serum (14). Circulating concentrations have been associated with many human diseases, such as infertility (oocyte number retrieved at in vitro fertilization (16), recurrent pregnancy loss (17), etc, insulin resistance, diabetes, obesity, cardiovascular disease, and hypertension in adults; and obesity and behavior in children (18). Importantly, for most of these associations causation has been demonstrated by laboratory studies in animals.

EDCs can alter the trajectory of cell differentiation and result in developmental origins of adult health and disease. The current study examines a novel mechanism by which EDCs may alter fetal development and lead to disease later in life. Hormones like estradiol and testosterone play key roles in normal development, and small changes in timing or concentration can program the fetus or neonate, result-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

Received April 22, 2013. Accepted April 26, 2013.

For article see page 1873

Abbreviations: BPA, bisphenol A; EDC, endocrine disrupting chemical; ER, estrogen receptor; miRNA, micro-RNA.

ing in lifelong consequences. EDCs like BPA have been shown to alter human development, and parental occupational exposure to BPA has been associated with a reduction in anogenital distance in boys, an androgen-sensitive endpoint (19). Compounds like BPA do not directly cause DNA mutation but induce epigenetic developmental events resulting in adult onset disease (20).

The developing ovary requires precisely orchestrated cues by steroid hormones to establish a competent female germ line, making this process vulnerable to disruption by EDCs (reviewed in Ref. 21). Perinatal BPA has been shown to impact metaphase-II oocytes by increasing aneuploidy rates in mice and monkeys; accelerate follicle development resulting in rapid depletion of the follicular reserve in sheep (22, 23); and accelerate reproductive senescence in rats (24). In addition, folliculogenesis is disrupted in mice and primates after BPA exposure, leading to multioocyte follicles, increased unenclosed oocytes, and nongrowing oocytes in later follicles (22). Recently, Lee et al (25) demonstrated perturbation in estradiol production and steroid hormone pathways in mice after BPA exposure. Although speculation exists as to the precise underlying mechanism of these effects, alterations in gene expression have been demonstrated suggesting a role for epigenetic regulators, such as altered DNA methylation and miRNA expression, eg, primordial follicle development has been shown to be dependent on miRNA-143 (26).

In the current issue, Veiga-Lopez et al investigate mechanisms of ovarian disruption by fetal exposure to BPA and altered expression of miRNAs in the ewe. BPA was administered from gestation day 30 to 90 via sc injection in corn oil. On gestation day 65 and 90, fetal ovaries were harvested, RNA was isolated and used to interrogate 742 miRNAs using a PCR array, and BPA was found to alter the normal developmental pattern of miRNA expression. On gestation day 65 relative to control-treated ovaries, 45 miRNAs were down-regulated by BPA. One important pathway altered by BPA was expression of miRNAs that regulate SOX family genes, a gene family critical in sex determination and embryonic development.

In addition to miRNAs, expression of a subset of genes was analyzed in the current study, and BPA altered expression of two key steroidogenic enzymes on gestation day 65. Expression of aromatase, the primary estrogen-synthesizing enzyme, and 5α-reductase, the androgen-synthesizing enzyme that converts testosterone to the higher potency dihydrotestosterone, was up-regulated. Aromatase expression is key to follicle development. miRNA-224, miRNA-378, and miRNA-383 regulate aromatase expression during follicle development in the adult ovary (27–29). In the current study, miRNA-383 was up-regulated in BPA-treated animals between gestation day 45 and 90. A number of miRNAs have now been investigated in follicle development, and a key pattern has emerged in the pathways that they are proposed to target: cell cycle, apoptosis and importantly aromatase activity (reviewed in Ref. 30). miRNAs generally function as negative regulators of protein synthesis by coupling with complementary mRNA sequences and either inhibiting their translation or targeting them for degradation. Due to the relatively new understanding of the role of miRNAs in gene regulation, the direct targets of many specific miRNAs or their role in early development are largely unknown. Additional studies are needed to examine whether BPA modulates miRNA expression to directly regulate aromatase expression in the fetal ovary.

There is widespread human exposure to BPA at concentrations that cause adverse effects in animals and people. Estrogens play key roles in orchestrating fetal development, and estrogenic activity during fetal life has been associated with developmental origins of adult disease in humans, eg, fetal estrogen is positively associated with breast cancer and endometriosis in adulthood (31–34). Because BPA can both bind directly to ERs and increase endogenous estrogen, via up-regulation of aromatase, it likely functions to increase the overall estrogenic activity during fetal development.

Taken together, perinatal BPA exposure has a significant impact on the developing ovary and results in decreased fertility in adulthood by increasing reproductive senescence and accelerating the rate of atresia in adulthood. Although the current study neither assessed the adult consequences of fetal exposure to BPA nor examined whether aromatase expression is targeted by any of the miRNAs altered by BPA exposure, it suggests that increased fetal aromatase expression may be an underlying mechanism via decreased expression of miRNAs.

The current study adds to a very limited number of studies on EDC modulation of miRNA expression (35–37) and suggests that altered miRNA expression may be a potential mechanism of EDC action during development.

Acknowledgements

Address all correspondence and requests for reprints to: Susan C. Nagel Ph.D., Obstetrics, Gynecology and Women's Health, M659 MSB, 1 Hospital Drive, University of Missouri, Columbia, Missouri, 65211, E-mail: nagels@health.missouri.edu.

This work was supported by the National Institutes of Health, Grants R01 ES021394, R21 ES020039, R21 HD056441 to S.C.N.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Zoeller RT, Brown TR, Doan LL, et al.** Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. *Endocrinology*. 2012;153:4097–4110.
2. **Vandenberg LN, Colborn T, Hayes TB, et al.** Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev*. 2012;33:378–455.
3. **World Health Organization** State of the science of endocrine disrupting chemicals-2012. In: Bergman Å, Heindel JJ, Jobling S, Kidd KA, Zoeller RT, eds. An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme (UNEP) and WHO. WHO Press, World Health Organization, Geneva, Switzerland 2013.
4. **Veiga-Lopez A, Luense LJ, Christenson LK, Padmanabhan V.** Developmental Programming: Gestational Bisphenol-A Treatment Alters Trajectory of Fetal Ovarian Gene Expression. *Endocrinology*. 2013;154:1873–1884.
5. **Rubin BS.** Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. *J Steroid Biochem Mol Biol*. 2011; 127:27–34.
6. **Wetherill YB, Akingbemi BT, Kanno J, et al.** In vitro molecular mechanisms of bisphenol A action. *Reprod Toxicol*. 2007;24:178–198.
7. **Nagel SC, Hagelbarger JL, McDonnell DP.** Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. *Endocrinology*. 2001;142:4721–4728.
8. **Alonso-Magdalena P, Ropero AB, Soriano S, et al.** Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. *Mol Cell Endocrinol*. 2012;355:201–207.
9. **Teng C, Goodwin B, Shockley K, et al.** Bisphenol A affects androgen receptor function via multiple mechanisms. [published online April 4, 2013] *Chem Biol Interact*, doi:10.1016/j.cbi.2013.03.013.
10. **Meeker JD, Calafat AM, Hauser R.** Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic. *Environ Sci Technol*. 2010;44: 1458–1463.
11. **Kwintkiewicz J, Nishi Y, Yanase T, Giudice LC.** Peroxisome proliferator-activated receptor-γ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environ Health Perspect*. 2010;118:400–406.
12. **Sheng ZG, Tang Y, Liu YX, et al.** Low concentrations of bisphenol a suppress thyroid hormone receptor transcription through a nongenomic mechanism. *Toxicol Appl Pharmacol*. 2012;259:133–142.
13. **Zoeller RT, Bansal R, Parris C.** Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology*. 2005;146:607–612.
14. **Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G.** Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ Health Perspect*. 2010;118:1055–1070.
15. **Taylor JA, Vom Saal FS, Welshons WV, et al.** Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environ Health Perspect*. 2011;119:422–430.
16. **Mok-Lin E, Ehrlich S, Williams PL, et al.** Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. *Int J Androl*. 2010;33:385–393.
17. **Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K.** Exposure to bisphenol A is associated with recurrent miscarriage. *Hum Reprod*. 2005;20:2325–2329.
18. **Braun JM, Yolton K, Dietrich KN, et al.** Prenatal bisphenol A exposure and early childhood behavior. *Environ Health Perspect*. 2009;117:1945–1952.
19. **Miao M, Yuan W, He Y, et al.** In utero exposure to bisphenol-A and anogenital distance of male offspring. *Birth Defects Res A Clin Mol Teratol*. 2011;91:867–872.
20. **Tang WY, Morey LM, Cheung YY, Birch L, Prins GS, Ho SM.** Neonatal exposure to estradiol/bisphenol A alters promoter methylation and expression of Nsbp1 and Hpcal1 genes and transcriptional programs of Dnmt3a/b and Mbd2/4 in the rat prostate gland throughout life. *Endocrinology*. 2012;153:42–55.
21. **Walker DM, Gore AC.** Transgenerational neuroendocrine disruption of reproduction. *Nat Rev Endocrinol*. 2011;7:197–207.
22. **Hunt PA, Lawson C, Gieske M, et al.** Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. *Proc Natl Acad Sci USA*. 2012;109:17525–17530.
23. **Rivera OE, Varayoud J, Rodríguez HA, Muñoz-de-Toro M, Luque EH.** Neonatal exposure to bisphenol A or diethylstilbestrol alters the ovarian follicular dynamics in the lamb. *Reprod Toxicol*. 2011;32: 304–312.
24. **Adewale HB, Jefferson WN, Newbold RR, Patisaul HB.** Neonatal bisphenol-a exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons. *Biol Reprod*. 2009;81:690–699.
25. **Lee SG, Kim JY, Chung JY, et al.** Bisphenol A exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17β-estradiol synthesis via downregulation of aromatase in rat ovary. *Environ Health Perspect*. 2013
26. **Zhang J, Ji X, Zhou D, et al.** miR-143 is critical for the formation of primordial follicles in mice. *Front Biosci*. 2013;18:588–597.
27. **Yao G, Yin M, Lian J, et al.** MicroRNA-224 is involved in transforming growth factor-β-mediated mouse granulosa cell proliferation and granulosa cell function by targeting Smad4. *Mol Endocrinol*. 2010;24:540–551.
28. **Yin M, Lü M, Yao G, et al.** Transactivation of microRNA-383 by steroidogenic factor-1 promotes estradiol release from mouse ovarian granulosa cells by targeting RBMS1. *Mol Endocrinol*. 2012;26: 1129–1143.
29. **Xu S, Linher-Melville K, Yang BB, Wu D, Li J.** Micro-RNA378 (miR-378) regulates ovarian estradiol production by targeting aromatase. *Endocrinology*. 2011;152:3941–3951.
30. **Donadeu FX, Schauer SN, Sontakke SD.** Involvement of miRNAs in ovarian follicular and luteal development. *J Endocrinol*. 2012;215: 323–334.
31. **Soto AM, Vandenberg LN, Maffini MV, Sonnenschein C.** Does breast cancer start in the womb? *Basic Clin Pharmacol Toxicol*. 2008;102:125–133.
32. **Strohsnitter WC, Noller KL, Titus-Ernstoff L, et al.** Breast cancer incidence in women prenatally exposed to maternal cigarette smoke. *Epidemiology*. 2005;16:342–345.
33. **Troisi R, Hatch EE, Titus-Ernstoff L, et al.** Cancer risk in women prenatally exposed to diethylstilbestrol. *Int J Cancer*. 2007;121: 356–360.
34. **Missmer SA, Hankinson SE, Spiegelman D, Barbieri RL, Michels KB, Hunter DJ.** In utero exposures and the incidence of endometriosis. *Fertil Steril*. 2004;82:1501–1508.
35. **Avissar-Whiting M, Veiga KR, Uhl KM, et al.** Bisphenol A exposure leads to specific microRNA alterations in placental cells. *Reprod Toxicol*. 2010;29:401–406.
36. **Choi JS, OH JH, Park HJ, et al.** miRNA regulation of cytotoxic effects in mouse Sertoli cells exposed to nonylphenol. *Reprod Biol Endocrinol*. 2011;9:126.
37. **Meunier L, Siddeek B, Vega A, et al.** Perinatal programming of adult rat germ cell death after exposure to xenoestrogens: role of microRNA miR-29 family in the down-regulation of DNA methyltransferases and Mcl-1. *Endocrinology*. 2012;153:1936–1947.