The effect of aflatoxin B_1 treatment on expression of transient receptor potential melastatin 8 in mouse ovary and testes

Kristina S. Boss,*[,1], Courtney M. Sutton*, Kathleen J. Austin*, Kristi M. Cammack[†], Rebecca R. Cockrum[‡], and Brenda M. Alexander*

*Animal Science Department, University of Wyoming, Laramie, WY 82071; [†]West River Ag Center, South Dakota State University, Rapid City, SD; and [‡]Virginia Polytechnic Institute and State University, Blacksburg, VA

Transl. Anim. Sci. 2019.3:1683–1685
doi: 10.1093/tas/txz057

INTRODUCTION

Aflatoxin is an *Aspergillus flavus* or *Aspegillus parasiticus* mold product commonly found in corn, oats, barley, wheat, and other livestock feeds. Of the four aflatoxin forms, aflatoxin B_1 (AFB1) is considered the most potent and exposure can lead to reproductive deficiencies in a variety of species, including humans, livestock, and mice (Uriah et al., 2001; Verma and Nair, 2002; Kanora and Maes, 2009). In male mice, AFB1 exposure is tied to histological changes in the testes, decreased sperm counts, and differences in sperm motility and litter size (Uriah et al., 2001; Verma and Nair, 2002). In female rats, AFB1 exposure has been shown to negatively impact uterine and ovarian size, as well as disturb cyclicity of the estrus cycle and reduce conception and litter sizes. AFB1 has also been linked to increased rates of fetal resorption (Supriya et al., 2016). AFB1 reduces steroidogenesis by competitively binding to the StAR protein in rats (Supriya et al., 2014). A reduction in circulatory testosterone may impact transient receptor potential melastatin 8 (TRPM8) expression. TRPM8 channels are expressed in the prostate, testis, sperm, vascular tissue, lung tissues, uterus, placenta, liver, skin, eye, bladder urothelium, heart, dorsal root ganglion sensory neurons, trigeminal ganglia sensory neurons, and taste papillae of higher animals (De Blas et al., 2009; Almaraz et al., 2014; Asuthkar et al., 2015a; Majhi et al., 2015). Recently, TRPM8 was found to act as an ionotropic testosterone receptor and may play a role in testosterone-induced behaviors including sexual drive, aggressiveness, fear conditioning, and other behavioral traits (Asuthkar et al., 2015a, 2015b). Due to TRPM8's role as a testosterone receptor and AFB1's influence on steroid hormone production and fertility, we hypothesized AFB1 exposure could influence the expression of TRM8 channels in reproductive tissues.

MATERIALS AND METHODS

Female mice were paired with proven male mice, four to a cage, and mated over the course of 1 week. At the end of the week, males were removed and females were fed aflatoxin 0.1 mg/kg BW (n = 8) in the form of oral drench using corn oil as vehicle for approximately 3 weeks before parturition. Control females (n = 7) were fed a placebo of corn oil. Fertile male mice were treated with either 50 µg/kg/day AFB1 (n = 4) using corn oil as a vehicle or corn oil alone (n = 3) for 45 days via intraperitoneal injection (Austin et al., 2012). Mice were weighed weekly and dosages of AFB1 and placebo were adjusted accordingly. Mice were killed by cervical dislocation and exsanguination. Gonads were excised, preserved in 4% paraformaldehyde, paraffin infused, and sectioned at 6 µm per standard immunohistochemistry procedures.

[1]Corresponding author: kboss1@uwyo.edu
Received April 4, 2019.
Accepted May 15, 2019.

Rabbit TRPM8 polyclonal antibody was purchased from Lifespan Biosciences, Inc. (Seattle, WA) and used 1:100 dilution. Anti-rabbit HRP conjugated secondary antibody (Jackson Labs, Bar Harbor, ME; 1:10,000) with positive staining detected using a DAB substrate kit from Vector Laboratories (Burlingame, CA). All slides were dehydrated through graded ethanol and equilibrated in xylene. Coverslips were mounted using Permount (Thermo Fisher, Waltham, MA). Positive staining appeared brown. Negative control staining was obtained in the absence of primary antibody.

Images of stained tissue were captured using Cell Sense Software with a consistent light setting at 200× magnification. Mean gray scale intensity was calculated for granulosa cells, theca cells, and seminiferous tubules using ImageJ software (NIH). All images were converted to gray scale. Minimum and maximum gray value, mean gray value, and limit to threshold were recorded for all measurements. Four measurements per cell type per image were recorded. An average mean gray value was calculated for each cell type per image. Smaller mean gray values indicated darker shades of gray suggesting darker TRPM8 staining and thus greater TRPM8 channel expression.

All statistical analysis were performed using GLM (Minitab 18). Average intensity per cell/tissue type was calculated for each animal. Intensity differences between granulosa and theca cells was determined. Treatment effects were determined for theca cells, granulosa cells, and seminiferous tubules. To determine if expression differed by follicle type, average intensity of granulosa cells within secondary and tertiary follicles were determined and analyzed for treatment, follicle type, and treatment by follicle type interactions using GLM analysis.

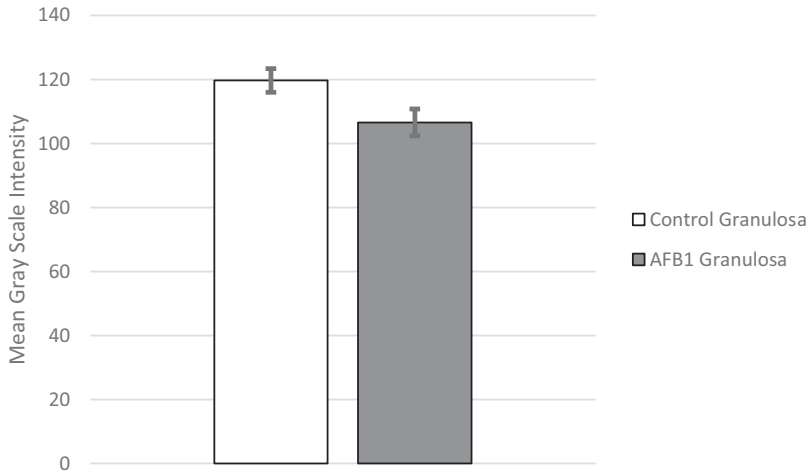

Figure 1. Mean gray scale intensity of granulosa cells from females treated with AFB1. Aflatoxin decreased gray scale intensity ($P = 0.04$) reflecting greater staining intensity and presumed increased expression of TRPM8 channels.

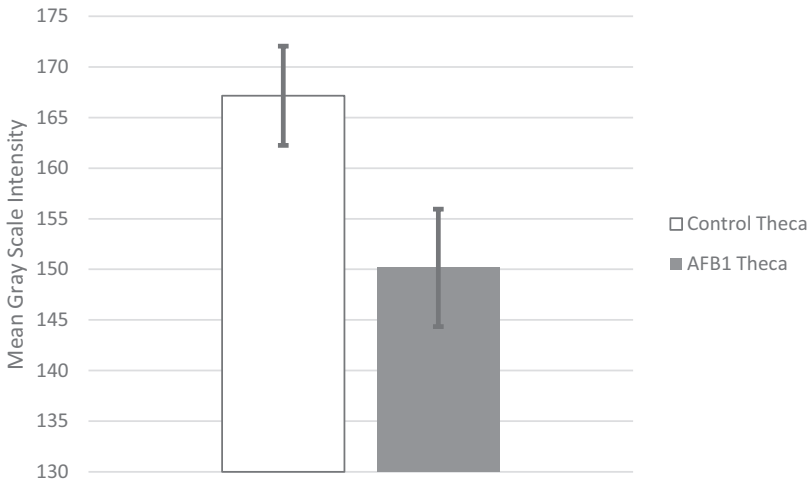

Figure 2. Mean gray scale intensity of theca cells from females treated with AFB1. Aflatoxin decreased gray scale intensity ($P = 0.05$) reflecting greater staining intensity and presumed increased expression of TRPM8 channels.

RESULTS AND DISCUSSION

Robust TRPM8 channel expression was detected in both the granulosa and theca cells of the ovary. Granulosa cells appear to have greater expression of TRPM8 channels when compared with theca cells as reflected by greater staining intensity and measured with decreased ($P < 0.001$) gray scale. Both cell types have steroidogenic capacity under the control of the gonadotropins which utilize calcium in their signaling pathway. TRPM8 is a putative testosterone receptor (Asuthkar et al., 2015b), and it is possible TRPM8 channels may influence this signaling pathway especially in granulosa cells which are responsive to testosterone. Since gray scale measurements of granulosa cells did not differ ($P = 0.5$) by follicle type, it is unlikely that expression of the TRPM8 channels differ as the follicle matures.

Reproductive effects of aflatoxins have been reported in domestic animals (Cortinovis et al., 2014) and murine models (Supriya et al., 2016). Aflatoxins are toxic to the gametes (Liu et al., 2015) and influence steroidogenesis (Adedara et al., 2014; Cortinovis et al., 2014). Treatment of female mice with AFB1 decreased gray scale intensity in granulosa ($P = 0.04$; Figure 1) and theca ($P = 0.05$; Figure 2) cells stained for expression of TRPM8 channels. Decreased gray scale reflects greater staining intensity and thus increased expression of the TRPM8 channels. Although treatment of AFB1 increased the expression of the TRPM8 channels, it is not clear how that would influence fertility. Because the TRPM8 channel is a testosterone receptor, it would be expected that it may influence cell response to testosterone which may have greater implications in the granulosa cells.

Seminiferous tubules from the mice testes stained positively for TRPM8 channels, but treatment with AFB1 did not influence ($P = 0.2$) staining intensity of TRPM8 channels. Thus, suggesting aflatoxin did not influence the expression of the TRM8 channels even though steroidogenesis (Adedara et al., 2014) may have been influenced.

This study indicates aflatoxins increased the expression of TRPM8 in granulosa and theca cells from the ovary and may be partially responsible for the reproductive consequences of aflatoxin exposure observed in females. Similar effects were not observed in the testes suggesting TRPM8 changes are not a factor in the decreased fertility of males exposed to aflatoxins.

LITERATURE CITED

Adedara, I. A., M. K. Nanjappa, E. O. Farombi, and B. T. Akingbemi. 2014. Aflatoxin B1 disrupts the androgen biosynthetic pathway in rat Leydig cells. Food Chem. Toxicol. 65:252–259. doi:10.1016/j.fct.2013.12.027

Almaraz, L., J. A. Manenschijn, E. Peña, and F. Viana. 2014. TRPM8. In: Mammalian transient receptor potential (TRP) cation channels: handbook of experimental pharmacology 222. Berlin/Heidelberg: Springer-Verlag; p. 547–579.

Asuthkar, S., L. Demirkhanyan, X. Sun, P. A. Elustondo, V. Krishnan, P. Baskaran, K. K. Velpula, B. Thyagarajan, E. V. Pavlov, and E. Zakharian. 2015a. The TRPM8 protein is a testosterone receptor: II. Functional evidence for an ionotropic effect of testosterone on TRPM8. J. Biol. Chem. 290:2670–2688. doi:10.1074/jbc.M114.610873

Asuthkar, S., P. A. Elustondo, L. Demirkhanyan, X. Sun, P. Baskaran, K. K. Velpula, B. Thyagarajan, E. V. Pavlov, and E. Zakharian. 2015b. The TRPM8 protein is a testosterone receptor: I. Biochemical evidence for direct TRPM8-testosterone interactions. J. Biol. Chem. 290:2659–2669. doi:10.1074/jbc.M114.610824

Austin, K. J., R. R. Cockrum, A. M. Jons, B. M. Alexander, and K. M. Cammack. 2012. Renin mRNA is upregulated in testes and testicular cells in response to treatment with aflatoxin B1. Theriogenology 77:331.e1–337.e1. doi:10.1016/j.theriogenology.2011.08.004

Cortinovis, C., F. Caloni, N. B. Schreiber, and L. J. Spicer. 2014. Effects of fumonisin B1 alone and combined with deoxynivalenol or zearalenone on porcine granulosa cell proliferation and steroid production. Theriogenology 81:1042–1049. doi:10.1016/j.theriogenology.2014.01.027

De Blas, G. A., A. Darszon, A. Y. Ocampo, C. J. Serrano, L. E. Castellano, E. O. Hernández-González, M. Chirinos, F. Larrea, C. Beltrán, and C. L. Treviño. 2009. TRPM8, a versatile channel in human sperm. PLoS One 4:e6095. doi:10.1371/journal.pone.0006095

Kanora, A., and D. Maes. 2009. The role of mycotoxins in pig reproduction: a review. Veterinari Medicina. 12:565–576.

Liu, J., Q. C. Wang, J. Han, B. Xiong, and S. C. Sun. 2015. Aflatoxin B1 is toxic to porcine oocyte maturation. Mutagenesis 30:527–535. doi:10.1093/mutage/gev015

Majhi, R. K., S. Saha, A. Kumar, A. Ghosh, N. Swain, L. Goswami, P. Mohapatra, A. Maity, V. K. Sahoo, A. Kumar, and C. Goswami. 2015. Expression of temperature-sensitive ion channel TRPM8 in sperm cells correlates with vertebrate evolution. PeerJ 3:e1310. doi:10.7717/peerj.1310

Supriya, C. H., B. Akhila, K. Pratap Reddy, B. P. Girish, and P. Sreenivasula Reddy. 2016. Effects of maternal exposure to aflatoxin B1 during pregnancy on fertility output of dams and developmental, behavioral and reproductive consequences in female offspring using a rat model. Toxicol. Mech. Methods 26:202–210. doi:10.3109/15376516.2016.1151967

Supriya, C., B. P. Girish, and P. S. Reddy. 2014. Aflatoxin B1-induced reproductive toxicity in male rats: possible mechanism of action. Int. J. Toxicol. 33:155–161. doi:10.1177/1091581814530764

Uriah, N., I. N. Ibeh, and F. Oluwafemi. 2001. A study on the impact of aflatoxin oh human reproduction. AFJ Reprod. Health. 5:106–110.

Venkatachalam, K., and C. Montell. 2007. TRP channels. Annu. Rev. Biochem. 76:387–417. doi:10.1146/annurev.biochem.75.103004.142819

Verma, R. J., and A. Nair. 2002. Effect of aflatoxins on testicular steroidogenesis and amelioration by vitamin E. Food Chem. Toxicol. 40:669–672.