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Published in:
Toxicological Sciences

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

Flusilazole disrupts retinoid signalling in fetal rodent testes

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Flusilazole is an agricultural azole fungicide that works by inhibiting the fungal cytochrome P450 enzyme CYP51. Flusilazole can likely also inhibit mammalian CYP enzymes, a mechanism that has been suggested to underlie some adverse effects seen in mammals, including reproductive disorders. Here, a main modality is the inhibition of CYP enzymes of the steroidogenesis pathway leading to sex hormone imbalance and subsequent abnormal sexual differentiation. Another potential mode of action, which remains largely unexplored, is the inhibition of CYP26B1, which would result in ectopic expression of retinoic acid (RA) in the fetal testes, leading to testis dysmorphology and failure in masculinization of the fetus.

Using *ex vivo* rat and mouse testis cultures, we have investigated whether flusilazole can affect retinoid signalling during gonadal sex differentiation. Wild-type Wistar rats or transgenic mouse lines (RARE-LacZ, Oct4-GFP (CD1) and Stra8-eGFP (C57BL/6)) were used. Fetal rodent testes were collected on gestational day 14.5 (rat) or 12.5 (mouse), cultured in hanging drops, and exposed to vehicle control, flusilazole, or the positive control RA for 48 hrs. Testes were harvested for RT-qPCR analysis, histological examination, staining, or imaging.

In fetal testis, we found that flusilazole exposure, in the same way as exposure to the positive control RA, lead to ectopic RA signaling. RA response element (RARE) activation and induction of pre-meiotic marker STRA8 were observed and additional preliminary data suggests that flusilazole exposure compromises the pluripotent germ cell pool. A panel of genes related to the retinoid system showed altered expression patterns after flusilazole exposure similar to those observed after RA exposure, and patterns were consistent between rat and mouse models.

In conclusion, flusilazole can disrupt retinoid signaling during gonadal sex differentiation with possible consequences for reproductive development and function.