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Aflatoxin B_1 disrupts the androgen biosynthetic pathway in rat Leydig cells

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ABSTRACT

The present study investigated if Aflatoxin B_1 (AFB_1), a potent and naturally occurring mycotoxin, interferes with the steroidogenic pathway in rat Leydig cells. Testicular Leydig cells are the predominant source of the male sex steroid hormone testosterone (T) that maintains the male phenotype and support fertility. Leydig cells, isolated from 35-day-old male Long-Evans rats (*Rattus norvegicus*), were incubated with AFB_1 at 0, 0.01, 0.1, 1, 10 μM followed by measurement of T secretion by radioimmunoassay and analysis of protein expression in western blots. Results demonstrated that AFB_1 suppressed testosterone secretion in a dose-dependent manner and inhibited expression of cholesterol transporter steroidogenic acute regulatory protein (StAR) and steroidogenic enzymes [(3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase enzyme (HSD17B3)]. Protein expression analysis showed that AFB_1 treatment increased ERK phosphorylation but suppressed p38 MAPK and JNK activation in Leydig cells. AFB_1-induced inhibition of Leydig cells was alleviated by co-treatment with the ERK inhibitor UO 126, implying that ERK mediates, at least in part, the inhibitory effects of AFB_1 in Leydig cells. The findings highlight potential extra-hepatic effects of aflatoxin exposure and indicate that exposure to AFB_1 has significant reproductive health implications for consumers of contaminated products even under conditions of low dietary toxin levels.

1. Introduction

Contamination of foodstuff and animal feeds by aflatoxin-producing molds is a public health concern (Adedara et al., 2010; Shuaib et al., 2010). Aflatoxin B_1 (AFB_1), a toxic secondary metabolite of *Aspergillus species*, occurs naturally, and is a major food contaminant in hot and humid regions of the world. This is concerning because large segments of the population in tropical and subtropical regions consume cereals, tree nuts, corn, oilseeds, peanuts, sorghum and dried fruits and are potentially exposed to aflatoxins (Strosnider et al., 2006). While mold contamination of food products is considered universal, aflatoxin concentrations in grain products varies widely across regions (Probst et al., 2007). Exposure to AFB_1 in a recent outbreak of aflatoxin poisoning in Africa was estimated at 50 mg/day (Probst et al., 2007). Acute aflatoxin exposures can result in rapid death and chronic exposures have been associated with hepatocellular carcinoma, immune-deficiency with increased susceptibility to infectious diseases and growth retardation in young individuals (Kensler et al., 2011). Although liver is the primary target organ, AFB_1 reportedly caused reproductive anomalies in mice, rats, pigs, sheep, and cattle (Agnes and Akbarsha, 2003; Wangikar et al., 2005). For example, AFB_1-treated mice exhibited histological changes in the testis, including germ cell loss (Agnes and Akbarsha, 2003; Austin et al., 2012; Prabu et al., 2013), whereas aflatoxicosis was linked to decreased sperm production and increased sperm abnormalities in male subjects (Uriah et al., 2001; Ibeh et al., 1994). However, it remains to be determined if adverse reproductive effects due to aflatoxicosis result from impairment of Leydig cell function and altered steroid hormone secretion. Leydig cells are the predominant source of testosterone (T), which supports sperm production and regulate the male phenotype (Hancock et al., 2009; Sherrill et al., 2010).

The pituitary gonadotropin luteinizing hormone (LH) is the primary regulator of Leydig cells. Androgen receptors (AR) and estrogen receptors alpha (ESR1) and beta (ESR2) are hormone transcription factors which play a significant role in the differentiation, development and growth of the male reproductive system (Yamashita, 2004; Sierens et al., 2005). These receptors are widely distributed in the male reproductive tract including testicular Leydig cells and are subject to regulation by androgen and estrogen (Akingbemi, 2005; Lucas et al., 2008; Nanjappa et al., 2012). In addition, Leydig cells have growth factor receptors, including insulin-like growth factor 1 (IGF1Rβ) and epidermal growth factor receptors (EGFR), which mediate estrogenic activity

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