In vitro and in vivo studies on the toxicology of di-n-butyl phthalate (DBP)

Effects on reproductive, endocrine, and immune systems

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Abstract

Chemical pollution is an increasing societal problem and has a major impact on human and environmental health. One important source of chemical pollution is plastic, which contains many different compounds with often largely unknown hazards. Phthalates are one group of chemicals in plastic that has been associated with several adverse effects in humans, particularly reproductive system impairments. Studies have also suggested a link between exposure to phthalates and negative effects on the immune system. One of the most widely used phthalates is di-n-butyl phthalate (DBP), which is frequently detected in humans and the environment. DBP has been associated with decreased male fertility and reduced levels of testosterone. However, the mechanisms behind these anti-androgenic effects are not entirely understood, and most studies have focused only on developmental exposure.

This thesis aims to, for the first time, investigate persistent effects on the reproductive and immune systems of adult male mice after exposure to DBP. Adult male mice were orally exposed to DBP (0, 10 or 100 mg/kg/day) for 5 weeks. A persistent and significant decrease in testicular testosterone levels was shown together with an increase in the levels of several steroidogenic enzymes 1 week after the conclusion of exposure. The decrease in testosterone may be related to the demonstrated increase in oxidative stress, which may affect enzyme activity. Additional mechanistic studies were conducted in the human adrenal cell line H295R. The testosterone levels decreased also *in vitro*; however, the levels of several steroidogenic enzymes in the cells decreased, which is in contrast with the *in vivo* study. Several additional steroid hormones were affected *in vitro*, but not *in vivo*. The animal study further revealed significantly increased levels of the key testicular proteins DAZL, vimentin, SOX9, and SULT1E1.

Moreover, a persistent immunosuppressive effect was demonstrated in the DBP-exposed mice, supporting previous data indicating that endocrine disruptors can affect the immune system. DBP-induced leukopenia, reduced numbers of T helper cells, and increased levels of immunosuppressive cells were observed. In addition, the distribution of two main DBP metabolites to three proposed target tissues (liver, testes, and adipose tissue) was examined, and the presence of the metabolites was confirmed 24 h after the final dose. The glucuronidation pattern in the mice was shown to be more similar to that previously observed in humans than in rats.

In conclusion, the results in this thesis support that the testes and immune system are key targets for DBP-induced toxicity. DBP decreased the testosterone levels both *in vivo* and *in vitro*, but certain differences in the effects on steroidogenesis were observed between the experimental models. Further studies are required to determine the No Observed Adverse Effect Level (NOAEL) for the effects identified in the animal model and to understand the underlying mechanisms completely.

Keywords: *Dibutyl phthalate, Endocrine disrupting chemicals, Male reproductive health, Phthalates, Immunotoxicity, Toxicology, Steroidogenesis.*

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