TOXICOLOGICAL HIGHLIGHT

Organophosphate Antagonism of the Androgen Receptor

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Attention to xenobiotic actions on hormone-sensitive tissues as significant risks to humans and wildlife increased markedly during the 1990s. Maternal transfer of persistent organic pollutants during early development and perturbation of steroid hormone receptor-mediated tissue differentiation was proposed as having special environmental relevance (Collborn et al., 1993). Interactions of persistent organochlorines with estrogen receptors were initially identified. For example, Eroschenko and Wilson (1975) reported estrogenic activity of the insecticide chlordane (Kepone®) in Japanese quail oviduct. The list of xenoestrogens and antiestrogens expanded over the 1980s and 1990s (reviewed by Safe, 2000). In addition to persistent organochlorines, the high production industrial compounds bisphenol A and nonylphenol were found to bind estrogen receptors and activate estrogen-dependent genes in laboratory tests. Emphasis broadened to other steroid hormone receptors. Kelce et al. (1995) demonstrated that p,p'-DDE, a metabolite and the prominent environmental residue of technical DDT, was an androgen receptor antagonist. In light of increased public health concern a strategy for screening compounds for activity as endocrine disrupters was legislatively mandated effective August 1999. The Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) recommended a battery of tests (1998).

The article highlighted here identifies the organophosphate insecticide fenitrothion as an androgen receptor antagonist. In vitro and in vivo evidence was provided. Importantly, the in vivo assessment of antiandrogenic activity was studied in parallel with multiple measures of neurotoxicity. This provided valuable context for comparing fenitrothion potency as an antiandrogen and acetylcholinesterase inhibitor.

Transient transfection of HepG2 cells with the human androgen receptor and a luciferase reporter gene provided an in vitro system suitable for differentiating agonism and antagonism. Transformed yeast that contained human steroid receptors and an inducible β-galactosidase reporter were not consistently predictive of agonist versus antagonist activity (Gaido et al., 1997; O’Connor et al., 1999). Fenitrothion competitively inhibited dihydrotestosterone-dependent human androgen receptor activation of the luciferase reporter in HepG2 cells. The potency of fenitrothion was about 8-fold higher than that of p,p'-DDE and about 50% of flutamide, a pharmaceutical antiandrogen.

The Hershberger assay was the in vivo test for fenitrothion antiandrogenic activity. Weight of accessory sex glands was determined in castrated male Sprague-Dawley rats treated with testosterone propionate and 0, 15 or 30 mg fenitrothion/kg/day for 7 days. Both doses of fenitrothion significantly reduced weights of seminal vesicles, ventral prostate, and levator ani plus bulbocavernosus muscles. The higher dose slightly decreased terminal body weight. The positive control for antiandrogenicity, 50 mg flutamide/kg/day, much more markedly inhibited testosterone propionate-stimulated seminal vesicle and ventral prostate weight gain. Use of the Hershberger assay eliminated the ability to detect fenitrothion action at the anterior pituitary through inhibition of gonadotrophin release. In Long-Evans rats p,p'-DDE action as an androgen receptor antagonist appeared more potent centrally than at the male accessory sex glands (O’Conner et al., 1999). Nonetheless, the Hershberger assay clearly demonstrated peripheral action of fenitrothion as an antiandrogen.

Fenitrothion neurotoxicity was assessed in the Hershberger assay through measurements of motor activity as well as brain region and whole blood acetylcholinesterase activity. Both doses of fenitrothion reduced motor activity up to about 50% of total movements in controls. Whole blood acetylcholinesterase activity was significantly inhibited only by 30 mg fenitrothion/kg/day (60% of control). Brain region-specific changes were marked, however. Acetylcholinesterase activities of the striatal, hippocampal, and frontal cortex were inhibited by 15 mg fenitrothion/kg/day (12–22% of control) and 30 mg fenitrothion/kg/day (6–14% of control). Excess salivation and muscle tremors were also noted. These data for neurotoxicity clearly indicated the fenitrothion treated rats were in cholinergic stress. Use of castrated rats provided with exogenous androgen eliminated normal feedback loops of the hypothalamic-pitu-
itary-gonadal axis. Still, as recognized by the authors, the potential role of cholinergic stress in antiandrogenic activity of fenitrothion remained uncertain.

Safe (2000) critically reviewed the epidemiological and experimental evidence for endocrine disruption as a public health risk. The case for increased disease or decreased reproductive performance in humans environmentally exposed to persistent organochlorines was weak. The likelihood that wildlife populations suffered from such contamination was much greater. Since the 1970s uses of persistent organochlorine insecticides have been banned or greatly restricted in the United States. Environmental releases of industrially generated persistent organochlorines have also been greatly reduced over the past 20 to 30 years. The screening battery for endocrine disruptor potential now required was aimed at avoiding a new generation of problem chemicals (EDSTAC, 1998).

Organophosphate insecticides are widely applied in agricultural and residential settings. Tamura and colleagues recognized the structural similarity of the organophosphate fenitrothion and two known antiandrogens, the drug flutamide and the herbicide linuron. The highlighted study supports further study of organophosphates as endocrine disruptors; their preliminary work demonstrated antiandrogenic activity of other organophosphates, including methyl parathion, in their transiently transfected HepG2 cell system. Attention to organophosphate potency as a reproductive tract teratogen after in utero exposure was recognized as a priority for application to risk assessment. Incorporation of sex steroid receptors with reporter systems for their activation into the human HepG2 cell line appeared superior to similarly transfected yeast as a screening system for endocrine disruptors. As suggested by EDSTAC (1998), the Hershberger assay was effective for in vivo validation of androgen receptor activation in peripheral tissues. It also allowed direct comparison of fenitrothion potency as an endocrine disruptor and neurotoxin, an important consideration for risk assessment.

REFERENCES


