Anti-Androgenic Potential of Endosulfan and its Microbial Transformation Products

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Abstract

The focus of our study has been to determine the potential of endosulfan and its microbial transformation products to cause reproductive disturbances through androgen-receptor mediated activity. Endosulfan is one of the last hexachlorocyclopentadiene insecticides remaining in widespread use throughout the world. It is of particular interest because of its persistance in the soil. Endosulfan (α and β isomers) and its characterized microbial transformation products (endosulfan sulfate, endosulfate alchol, endosulfan ether and endosulfan lactone) were tested for androgen-receptor binding affinity in a cell-free *in vitro* binding assay using cytosolic prostate tissue extract from mature rats and [^{3}H] methyltrienolone, a synthetic androgen. Among the compounds tested, endosulfan lactone caused the greatest reduction in binding of androgen to the androgen-receptor. Endosulfan α and endosulfan sulfate caused slight reduction in binding at high concentrations (1 mM), while the other compounds showed no activity in this assay. Preliminary experiments testing binary combinations of these compounds suggest additive effects but no strong synergistic activity. The biological relevance of our findings will be further studied using *in vitro* cell proliferation studies with the LNCaP androgen responsive cell line. Growth assays will be used to determine whether these compounds and mixtures induce or inhibit cell proliferation.

Introduction

Recent studies provide evidence that certain environmental contaminants cause a disruption in the endocrine system, resulting in altered reproductive function in humans and wildlife. These endocrine disruptors include certain pesticides, industrial chemicals and heavy metals. Xenobiotic-induced endocrine disruption can cause adverse health effects in fetuses, children and adults. Potential outcomes include alterations in sexual differentiation, decreased fertility and increased risk of cancers of the reproductive organs.

Endosulfan, one of the last hexachlorocyclopentadiene insecticides remaining in widespread use, is of particular concern due to its persistance and toxicity. Consumption of endosulfan by rats was shown to produce testicular atrophy (Gupta et al., 1979) and to lower gonadotrophin and testosterone plasma levels (Singh et al., 1990). Endosulfan has been shown to possess estrogenic activity (Soto et al., 1995), but has not previously been tested for androgen binding affinity. Microorganisms transform endosulfan (α and β) into several partial degradation products, including endosulfan sulfate, endosulfan alcohol, endosulfan ether and endosulfan lactone (Martens, 1977; Miles and Moy, 1979; Dreher and Podradtziki, 1988), none of which have

been tested for endocrine disruption potential.

The need to investigate endocrine disruptive activity of both parent compounds and stable microbial degradation products has been shown in the literature (Bitman and Cecil, 1970; Kelce et al., 1994; Kelce et al., 1995). Microbial processes have the potential to mitigate the harmful effects of various environmental contaminants (Mousa et al., 1996), yet there is also potential for an increase in endocrine disrupting effects. (Bitman and Cecil, 1970). It was reported that combinations of chlorinated pesticides with estrogenic activity act synergistically, with the potential to cause an effect at much lower concentrations in mixtures of two compounds (Arnold et al., 1996). Since the most commonly encountered mixtures of such chemicals in the environment are parent compounds and their transformation products, we are interested in studying the endocrine disruptive activity of endosulfan and its microbial transformation products alone and in combination.

Experimental Methods

The androgen receptor binding affinity of endosulfan and its microbial transformation products was determined using cytosolic tissue extract from prostates of mature rats (Kelce et al., 1994). This cell-free competitive binding assay used [^{3}H]methyltrienolone (R1881), a synthetic androgen. Microbial transformation products tested included endosulfan sulfate, endosulfan alcohol, endosulfan ether and endosulfan lactone.

Male Sprague Dawley rats of 120 to 150 days of age were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Rats were castrated at Harlan Sprague Dawley prior to air shipping. Castration was necessary because cytosolic androgen receptors in the prostate tissue are below detection limits in intact males (Kelce et al., 1994). The rats were housed in the MSU animal care facility upon arrival. Prostate tissue was harvested 24 hours after castration and immediately immersed in ice-cold TEDG buffer (5 ml/g tissue). The tissue was homogenized with a Polytron tissue homogenizer and the extract was centrifuged at 30,000 x g for 30 min. Unoccupied cytosolic androgen receptors remained in the supernatant, which was used in the binding assays. The TEDG buffer (pH 7.4) consisted of 10 mM Tris, 1.5 mM EDTA, 10% glycerol (v/v), and 1 mM each of dithiothreitol, phenylmethylsufonyl fluoride, and sodium molybdate.

Each total binding tube contained 300 µl of cytosolic prostate extract, 3 nM [^{3}H]R1881 and a specific concentration of endosulfan, a microbial transformation product or hydroxyflutamide (a known inhibitor). Binary combination tubes contained two test compounds. Parallel tubes containing a 500-fold molar excess of radioinert R1881 were used to determine nonspecific binding. Tubes were incubated for 20 hr at 4°C (Kelce et al., 1994). When the incubation was complete, bound ligand was isolated using hydroxylapatite extraction and measured by scintillation counting. Specific binding was determined by subtracting nonspecific binding from total binding in each set of parallel tubes.

To assess the biological significance of our findings, we will further assess endosulfan and its microbial transformation products' endocrine disruption potential using *in vitro* cell proliferation studies. The androgen responsive human prostatic cancer cell line, LNCaP, will be used to assess the androgenic activity of endosulfan and its microbial transformation products (Hall et al., 1994; Ko et al., 1994). Growth assays will be used to determine whether these comounds induce or inhibit cell proliferation. Cell proliferation in the presence of dihydroxytestosterone will be counteracted by compounds acting as androgen antagonists due to competitive inhibition of androgen receptor binding sites.

Results and Discussion

Endosulfan lactone competitively reduced binding of androgen to the androgen-receptor, with an inhibitor concentration necessary for 50% inhibition (IC_{50}) of 450 μM. Endosulfan α and endosulfan sulfate showed slight inhibition of binding at high concentrations (1 mM), while endosulfan β, endosulfan ether and endosulfan alcohol showed no activity in this assay. Hydroxyflutamide, a known inhibitor, had an IC_{50} of 1.7 μM in our laboratory. Preliminary experiments testing binary combinations of these compounds suggest additive effects but no strong synergistic activity. The biological relevance of our findings will be further studied by using *in vitro* cell proliferation studies with the LNCaP androgen responsive cell line. Growth assays will be used to determine the androgenic properties of the test compounds and mixtures as indicated by their ability to induce or inhibit cell proliferation.

Acknowledgements

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