Antitumorigenic Activity of 6-Methyl-1,3,8-trichloro-dibenzofuran and 8-Methyl-1,3,6-trichlorodibenzofuran in the 7,12-Dimethylbenz[a]anthracene-Induced Rat Mammary Tumor Model

A. McDougal and S. Safe
Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843-4466

1. Abstract

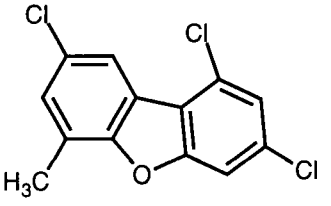
The effects of 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) and its analog 8-methyl-1,3,6-trichlorodibenzofuran (8-MCDF) as inhibitors of mammary tumor growth were determined in female virgin Sprague-Dawley rats treated with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). After initial development of mammary tumors (50 to 100 mm^3), rats were treated with 5, 10, or 25 mg/kg of the test compound in corn oil or corn oil alone for three successive weeks and sacrificed 7 days after the last treatment. A dose-dependent decrease in tumor growth was observed for both compounds. Hepatic microsomal ethoxyresorufin O-deethylase(EROD) activity was not significantly induced with any treatment.

2. Introduction

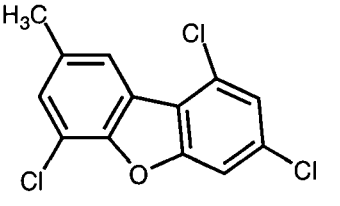
Kociba and co-workers first demonstrated that TCDD inhibits development of spontaneous uterine and mammary tumors in female Sprague-Dawley rats maintained for two years on a diet containing TCDD at 0.1, 0.01, and 0.001 μg/kg/day (1). Studies have shown that TCDD also inhibits tumor growth in mice (2) and female Sprague-Dawley rats treated with the complete mammary carcinogen DMBA (3). Subsequent studies on the female rat uterus and in MCF-7 human breast cancer cells have reported that TCDD inhibits 17β-estradiol (E2) induced proliferation and gene expression (4). MCDF was first investigated as a partial Ah receptor agonist which exhibited low toxicity but inhibited TCDD induced CYP1A1 gene expression and several other toxic responses (5,6). In contrast, MCDF appeared to be active as an antiestrogen in the female rat uterus (7,8) and in Ah-responsive human breast cancer cell lines (9). These studies have shown that MCDF and related analogs are relatively non-toxic, antagonize TCDD-induced toxic effects but themselves exhibit activity as antiestrogens both *in vivo* and *in vitro* models. MCDF is

approximately 300-700 times less potent than TCDD as an antiestrogen; and is at least 10,000-100,000 times less toxic than TCDD for traditional Ah-receptor mediated toxicities (8).

These results suggest that MCDF and related alkyl-PCDFs represent a new class of antiestrogens that act through the Ah-receptor and may have clinical potential for treatment of mammary tumors. This study reports antitumorigenic effects of MCDF and 8-MCDF in the DMBA-induced rat mammary tumor model.



MCDF



8-MCDF

3. Materials & Methods

Animal Treatment. Mammary tumors were induced in virgin 50 day old ± 16 hours Sprague Dawley rats obtained from Harlan (Houston, TX). They were treated (oral gavage) with a single dose of 20 mg DMBA per rat as previously described (3,10). After 30 to 60 days, carcinomas were detected by palpation in the ductal tubes of the mammary glands. After initial tumors reached a small size (50 to 100 mm^3), rats were dosed with MCDF or 8-MCDF in corn oil (1.6 ml/kg) once per week for 3 weeks by intraperitoneal injection. Control animals received corn oil alone. Tumor sizes were measured biweekly with calipers and volumes calculated using the formula (length/2) x (width/2) x (depth/2) x $(4/3\pi)$. The number of tumors detected in each rat was also recorded. One week after the third injection, rats were euthanized and tumors were removed, weighed, and sectioned. Animals were housed initially in groups of two, and isolated after formation of tumors to avoid cannibalism.

EROD Assay. Hepatic microsomal EROD activities were determined by fluorimetric procedures (530/590 excitation/emission) as previously described (11). This activity is a surrogate of potential AhR-mediated toxicity.

4. Results and Discussion

Figure 1 summarizes the results of two separate studies on the effects of MCDF and 8-MCDF in the DMBA-induced rat mammary tumor model. MCDF caused a dose-dependent decrease in both tumor mass and tumor volume and a significant inhibition of growth was observed at the lowest dose of both compounds used in this study (5 mg/kg). Previous studies with MCDF have shown that no toxic responses are observed in the dose range 5-25 mg/kg and the results confirm that liver weight changes and induction of CYP-1A1-dependent EROD activity were not observed any in the treatment groups. The effects of interchanging the 6-methyl and 8-chloro substituents on activity was also investigated by comparing the antitumorigenic activities of MCDF and 8-MCDF. The results showed that 8-MCDF was also a potent inhibitor of mammary tumor growth at all doses (5-25 mg/kg) used in this study. These results demonstrate that alkyl-PCDFs inhibit mammary tumor growth in the DMBA-induced rat mammary tumor model and studies on the antitumorigenic activities of other alkyl-PCDFs are ongoing. This research was supported by the National Cancer Institute (CA 64081).

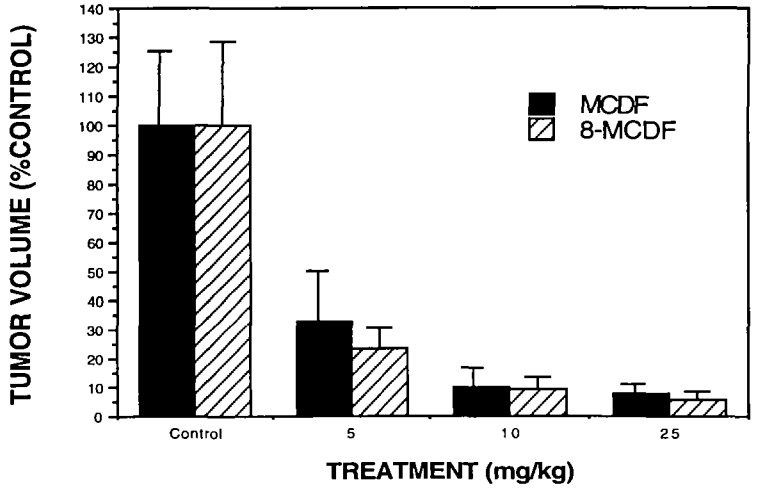


Figure 1. Effects of MCDF and 8-MCDF on mammary tumor growth. Two groups of 50-day old female Sprague-Dawley rats were treated orally with DMBA (20 mg/rat). After the initial tumor was detected (50-100 mm^3), rats were dosed with MCDF or 8-MCDF once per week for three weeks and euthanized one week after the third injection. Tumor volumes reported are those at the time of euthanasia.

5. References

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