Chemoprevention of Cancer of the Upper Respiratory Tract of the Syrian Golden Hamster by Aerosol Administration of Difluoromethylornithine and 5-Fluorouracil

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Abstract
Research aimed at identifying effective chemopreventive compounds active against carcinogenesis of the upper respiratory tract (URT) has been largely unsuccessful. We are addressing this problem by efforts at agent identification and by using aerosol delivery. Two compounds, difluoromethylornithine (DFMO) and 5-fluorouracil (5-FU) were investigated. DFMO is an irreversible inhibitor of ornithine decarboxylase, an enzyme important in cell proliferation. It has been used widely by oral administration for chemoprevention. 5-FU is a pyrimidine analog used extensively as a chemotherapeutic agent. It is generally administered i.v. and can cause considerable toxicity. However, aerosol administration for therapy of lung cancer in humans has been reported to be without adverse effects (Tatsumura et al., Br J Cancer 1993;68:1146–9). The experimental model used herein entailed six intratracheal administrations of methylnitrosourea (MNU) to hamsters. Each of the test agents was started about 1 week after MNU and was continued for 29 weeks with DFMO. Infiltrating squamous cell carcinomas of the URT occurred in 92% of the controls and were reduced by 50% in animals receiving DFMO (P = 0.0001). The experiment with 5-FU was of shorter duration being terminated 20 weeks after MNU. Thirty percent of the controls had infiltrating carcinomas and were reduced by 60% in animals receiving 5-FU (P = 0.0274). Both compounds resulted in a significant increase in the percent of cancer-free animals. These two agents may have selected use in subjects at high risk of cancer of the URT. 

Introduction
Chemoprevention of carcinogenesis of the upper respiratory tract (URT; pharynx, larynx, and trachea) is poorly developed, and few agents show significant efficacy. The aim of the present studies is to identify new compounds that will have the capacity to prevent infiltrating squamous cell carcinoma in these tissues particularly when administered in the post-initiation period. Aerosol administration has been used because it has the positive advantages of delivering agents into direct contact with the tissues at risk. This produces a high concentration of these compounds in the target tissue as compared with the concentrations elsewhere (1–5). Two compounds were chosen for the present study. The first is difluoromethylornithine (DFMO), which is an irreversible inhibitor of ornithine decarboxylase. This enzyme converts ornithine to polyamines, which are important for cell proliferation (6, 7). The enzyme has an almost ubiquitous distribution in tissues of animals and humans. DFMO has been shown to have chemopreventive effects against carcinogenesis in a variety of animal tissues including tongue, skin, large bowel, urinary bladder, and breast (8, 9). A preliminary study of the effect of oral administration of DFMO on methylnitrosourea (MNU) induced carcinogenesis of the respiratory tract of the hamster has been reported (10). High doses were used that produced minimal efficacy. In the human, efficacy against progression of actinic keratoses to cancer of the skin has been obtained and also against biomarker endpoints in large bowel, prostate, cervix, and urinary bladder (6, 11–13).

The second compound chosen for study was 5-fluorouracil (5-FU). The major use of this compound has been as a cancer therapeutic agent. The compound has antiproliferative activity, which appears related to its capacity to inhibit thymidylate synthase (14, 15). 5-FU is generally administered i.v., and under these conditions, serious bone marrow and intestinal toxicity can occur. However, in a study published by Tatsumura et al. (4) in which 5-FU was given by aerosol for the treatment of lung cancer in humans, the authors reported that no adverse effects were seen. Initial studies in dogs and subsequent studies in humans showed high concentrations of 5-FU in trachea and bronchi and low concentrations in peripheral tissues and blood. Aerosol administration to a small group of patients with lung cancer showed an antitumor response in 60% of the participants. On the basis of the lack of toxicity, evidence of an antineoplastic effect in the respiratory tract, and the distribution studies, it was decided to determine whether 5-FU might have applicability in chemoprevention.

The animal model used in the studies to be presented was MNU-induced squamous cell carcinoma of the URT of the Syrian Golden Hamster. MNU was given by intratracheal intubation using a modification of a technique described previously (16). The aerosols were started about 1 week after the last dose of MNU. Both DFMO and 5-FU exerted significant chemopreventive effects on squamous cell carcinogenesis in this experimental model. The implications of these findings, particularly for subjects at high risk of developing infiltrating cancer in the respiratory tract, will be discussed.

Materials and Methods

Animal Procedures. Male Syrian Golden hamsters obtained from the Charles River Laboratories were used throughout. They were shipped at 7 weeks of age and held for 2 weeks before initiation of any experimental procedures. At that time, they were given the first of six intratracheal administrations of carcinogen. The first three were 1% MNU in 0.05 ml of saline. They were separated by 2-week intervals. Two weeks later, the first of three administrations of 0.5% MNU was started. These were separated by intervals of 1 week. In testing for chemoprevention by DFMO, four experimental groups were used. There were two control groups. One did not receive aerosol administrations and is designated as the absolute control. A second group was given the solvent, which was water, and this group was designated as the solvent control. A third group was exposed to an inhaled dose of DFMO of 23 mg/kg body weight and the fourth to an inhaled dose of 46 mg/kg body weight.

The hamsters were randomized by weight before the start of the aerosol administrations and were reweighed weekly. The aerosol exposures were started 10 days after the last dose of MNU. They were administered 5 days/week for 90 s each day. The experiment was terminated 30 weeks after the last dose of MNU. For testing 5-FU, the same general procedures were...
used. In this experiment, there were four groups, as follows: an absolute control group, a solvent control group, a group receiving 5-FU at 1.45 mg/kg body weight and a group receiving 5-FU at 2.08 mg/kg body weight. The aerosols were administered for 1 min three times per week starting 7 days after the last dose of MNU. This experiment differed from that with DFMO in that the aerosols were administered for 16 weeks, and the protocol was terminated 4 weeks later.

Animals were necropsied after termination of the protocol, or if prior sacrifice occurred because of weight loss of ≥10 g in a period of 1 week. The lung/trachea/larynx/pharynx were removed as a complete unit and fixed in zinc formalin. The liver, kidney, and spleen were also removed and fixed. After fixation, the individual lobes of the lungs were removed. All muscle and connective tissue were dissected from the block of tissue containing trachea, larynx, and pharynx. This block was then cut transversely into eight pieces each about 2–3 mm in width. All these blocks were decalcified, dehydrated, and embedded in Paraplast X-tra (Oxford Labware, Sherwood Medical, St. Louis, MO), with all eight pieces in a single block. Transverse step sections cut at 6 μm were taken every 200 μm until all tissue had been sectioned. The slides were stained with H&E and scored for pathology.

**Aerosol Procedures.** The aerosol system was designed to generate solid particles with a uniform size distribution and concentration. The aerosol was generated with an ultrasonic nebulizer (a modified Holmes Visible Mist; Holmes Product Corp., Milford, MA). The nebulizer allows for a variable airflow rate as well as a relatively high range of output rates. The precise engineering allows for control of the output aerosol concentration from the solution containing the agent. The large capacity nebulizer provides for a long duration of operation without refilling, and the baffle system minimizes reservoir evaporation. Most importantly, the modified nebulizer operates in the kilohertz regimen and yields particles of drug after drying that have an aerodynamic size of about 2.5 μm. This is ideal for deposition in theURT, the site at which the cancers occur. For carrying out the aerosol procedures in the hamsters, the apparatus is located in a high-velocity hood. All procedures relevant to the aerosol exposures are performed within the hood. The hamsters are exposed to the aerosols for 90 s each day in the DFMO study and 60 s in the 5-FU study. The animals are exposed individually by placing the nose of the animal in the nose cone. The procedures for determining the concentration of chemopreventive agent in the aerosols, the particle sizing, and the estimated dose delivered to the animals has been described previously (1, 2).

**Statistics.** It is hypothesized in this study that MNU-induced cancers are more likely to occur in the control groups than in the chemoprevention groups, and hamsters in the low-dose chemoprevention group are more likely to occur in the control groups than in the chemoprevention groups. To test this hypothesis, a statistical method based on ordered 2 × 2 contingency tables was used (17). A test result with a one-sided P < 0.05 is significant. The data from two control groups (absolute controls and solvent controls) are combined for the computation.

### Results

The results of the chemoprevention study with DFMO are shown in Table 1. It will be seen that the percentage of hamsters developing infiltrating carcinomas is significantly reduced in order going from the control group to the low-dose DFMO group to the high-dose DFMO group (P = 0.0001) and that DFMO significantly increased the number of animals free of all cancers (P = 0.0269; i.e., in situ cancer and infiltrating cancer). In the high-dose DFMO group, there is an increased incidence of in situ carcinoma compared with other groups. This finding, in conjunction with the lower incidence of infiltrating carcinomas, indicates a delay or possibly a block in the progression from in situ carcinoma to infiltrating cancer. The aerosol administration of DFMO did not alter weight gain as compared with the controls during the course of the experiment.

The results of the chemoprevention study with 5-FU are shown in Table 2. This experiment was terminated 20 weeks after the last dose of MNU in contrast to the 30-week interval used for DFMO, and the carcinogenic response was smaller. 5-FU significantly reduced the occurrence of infiltrating carcinoma (P = 0.0274) and also significantly increased the number of animals free of all cancers (P = 0.0163; i.e., in situ cancer and infiltrating cancer). Aerosol 5-FU did not retard weight gain as compared with the controls. In Table 3, the results of a 5-week toxicity study with aerosol 5-FU administered for 1 min three times per week are shown. 5-FU did not cause gross or microscopic pathology in the oral cavity, skin, small bowel, or large bowel nor was there a significant alteration of the white blood count.

### Discussion

In the present study, both DFMO and 5-FU prevented infiltrating squamous cell carcinoma of the URT by ≥50%. Both compounds also increased the percentage of animals free of cancer. DFMO is an antiproliferative agent, and there is a great deal of information pertaining to its pharmacology and toxicology in both animals and humans (6). The compound basically is well tolerated at concentrations at which it produces chemoprevention in most tissues (6, 11, 12). Dose-related reversible ototoxicity is its major adverse effect. Many individuals with precancerous lesions of the URT are at high risk of
developing infiltrating carcinoma and could benefit from an effective agent inhibiting this progression.

The potential for use of 5-FU differs from DFMO because of considerations pertaining to toxicity. 5-FU is a chemotherapeutic agent with modest activity as such when given for therapy of cancer of internal organs. Topically, it has been effective in preventing the progression of actinic keratoses of the skin to cancer in humans (18). It has also produced chemoprevention of benzo(a)pyrene-induced epidermal carcinogenesis in the mouse (19). However, in its use for chemotherapy of internal organs, 5-FU is usually given as an i.v. infusion that can result in serious side effects. The issue of the possible use of 5-FU as a chemopreventive agent for carcinogenesis in the respiratory tract has arisen as a result of the work of Tatsumura et al., in which the treatment of lung cancer with aerosol 5-FU was carried out without evident toxicity, a consideration of importance for use in chemoprevention. This feature is supported in our own studies. At the present time, these toxicity data are encouraging but not exhaustive. However, they do focus on a possible special use of 5-FU for a subset of individuals. These subjects present under conditions in which they are at very high risk of developing cancer in the respiratory tract but do not have evidence of cancer when they are first seen. They include individuals with extensive head and neck preneoplasia, individuals with multiple advanced bronchial dysplasias, and individuals who have had a resection of an early pulmonary carcinoma without residual cancer being demonstrable. In essence, these represent situations in which advanced preneoplasia is present and possibly early cancer as well. For such individuals, an agent such as 5-FU with both preventive and therapeutic capacities would be useful. Lower doses than those required for therapy of overt cancer may be effective and provide an acceptable risk benefit ratio for these “high-risk precancer/potential early cancer” subjects.

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References

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