EFFECT OF CHRONIC ADMINISTRATION OF A SYNTHETIC AROMATIC RETINOID
(Ro 10-9359) ON THE DEVELOPMENT OF LUNG SQUAMOUS METAPLASIA AND
EPIDERMOID CANCER IN RATS

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INTRODUCTION

Since the first studies of Saffioti et al. (1) suggesting the preventive effect of high doses of vitamin A on squamous metaplasias of trachea and bronchis, several authors have used synthetic analogs of vitamin A, like retinoic acid, in chemoprevention and chemotherapy of tumors. Successful results were reported with skin (2) bladder (3) and mammary carcinogenesis (4). However for cancers of the respiratory tract the findings of Saffioti have not been confirmed in mice (5) hamsters (6) or rats (7). In our laboratory we have developed a model of lung carcinogenesis by using the combined effect of inhalation of radioactive particles and intratracheal instillation of benzo- pyrene. This model was used to examine the preventive and curative effect of a synthetic aromatic retinoid (Ro 10.9359) on the induction of development of metaplasia and epidermoid lung cancer. For this purpose, the compound was administered chronically at various times after carcinogen exposure.

METHODS

Animals

Pathogen free male Wistar rats, 8 weeks of age and weighing 200 to 220 g were obtained from Iffa Credo (L'arbresle, France).
Lung Carcinogenic Treatment

At day -15, animals were submitted to a nose only inhalation of plutonium dioxide ($^{239}$PuO$_2$) particles (median aerodynamic diameter, 2.06 μm, σ, 1.28) according to an already described procedure (8).

Initial lung burdens were determined by external chest counting 7 days after inhalation using a proportional counter (9). Lung burdens at death were determined in the same way. Less than 0.5% of the initial lung burden was translocated outside the lungs. Lung clearance was measured based on initial and final burdens (10). The data were fitted by a single exponential equation (half-time was 170 days) and the radiation dose delivered to lungs was calculated according to McClellan (11). At day 0, an intratracheal instillation of benzopyrene hematite (5 mg each) in 0.2 ml of saline solution was performed under halothane anesthesia, according to Saffioti et al. (1).

Retinoid Treatment

The aromatic retinoic acid analog Ro 10.9359, ethyl all-trans-9- (4-methoxy-2,3,6-trimethylphenyl)-3-7-dimethyl-2,4,6,8-nonatetraenoate (Hoffman La Roche), was presented as a hydrosuspendable galenical preparation. At different times after carcinogen exposure, depending upon the aim of experiment, i.e., metaplasia prevention, established metaplasia therapy or lung tumor therapy, rats received a chronic treatment with Ro 10.9359 given by stomach tube once a week. The doses used varied from 25 to 200 mg/kg per gastric administration.

Histopathology

The animals were autopsied after sacrifice at predetermined times in metaplasia prevention or therapy assays or after natural death in the lung cancer therapy assay. Organs presenting tumors or metastasis were studied histologically. In particular, lungs were removed in bloc and fully expanded by Bouin fixative and later embedded in paraffin. Lung tumors and lung metaplasia were diagnosed using large slices of whole organ sectioned along the longitudinal axis. The area of metaplasia was evaluated by a random observation of ten fields in different lobes using a grid ocular divided in 400 squares. For each field, the percentage of squares with metaplasia was checked, and results were expressed as the percent of positive squares for a mean number of 12,000 squares examined per lung. Lung tumors were diagnosed microscopically using also large slices of whole tissue and cancer staging was performed according to our previous studies (8).