to study the possible role of duodenal-content reflux esophagitis in a model of esophageal carcinogenesis with 2,6-dimethylnitrosomorpholine (2,6-DMNM) in which squamous cell carcinoma was the histological type usually induced.

Materials and methods

The induction of adenocarcinomas of the esophagus was accomplished in Sprague-Dawley rats under the combined influence of chronic esophagitis plus the carcinogenic effect of 2,6-DMNM. Chronic reflux esophagitis was produced by means of an esophagojejunostomy. This procedure, which diverts the biliary and pancreatic juice into the esophagus, significantly increased the number of animals with esophageal carcinomas (co-carcinogenic effect) after the chronic subcutaneous administration of 2,6-DMNM.

Results

Most strikingly, this model resulted for the first time in the induction of a significant number of carcinomas with glandular differentiation. It is of interest that reflux esophagitis of long duration (20-30 weeks), without administration of carcinogen, induced the development of foci of glandular metaplasia in the esophagus of rats. This finding suggests that glandular metaplasia may represent a morphological substrate from which the adenocarcinomas originate, because only squamous cell carcinomas were observed when 2,6-DMNM was given to rats that did not have esophagojejunostomy.

Discussion

In a subsequent study which aimed to determine which fraction of the duodenal-content reflux, pancreatic or biliary, contributed to the development of esophageal adenocarcinomas, it was found that adenocarcinomas developed only in those 2,6-DMNM-treated rats exposed to reflux of pancreatic secretions, either alone or in combination with bile. Adenocarcinomas were not observed in the group of carcinogen-treated rats exposed to bile reflux alone. Recent observations have demonstrated that duodenal-content reflux of longer duration (40-50 weeks) *per* se in the absence of exogenous carcinogens may induce the development of esophageal carcinomas, especially adenosquamous carcinomas. It was also common to observe the appearance of multiple foci of glandular metaplasia in the squamous epithelium. All these findings support the following hypothesis for the development of carcinomas arising in rat esophagus: pancreatic reflux injures the squamous epithelium, and unknown factors contained in this secretion promote a double differentiation capability in the proliferating stem cells of the basal layer of the squamous epithelium. In some cases foci of glandular metaplasia may also arise. Exogenous or endogenous carcinogens present in the biliopancreatic secretion, acting upon these stem cells, then induce the development of adenocarcinomas or adenosquamous carcinomas.

All these findings support the role of duodenal-content secretions on the process of mucus differentiation in the squamous epithelium. These observations may help to understand some aspects of the pathogenesis of Barrett's esophagus. In addition, the effect of biliopancreatic secretions on the sequence reflux esophagitis-mucus differentiation-adenocarcinoma/adenosquamous carcinoma in the rat model support the role of duodenal-content secretions in the process of malignant transformation in Barrett's esophagus as has recently been suggested.

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Biological investigations of environmental and occupational compounds using an alternative *in vitro* concept

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Assessment of cytotoxicity of inhalable substances such as gaseous or particulate compounds and complex mixtures have traditionally involved animal experiments. Difficulties in the calculation of human risk from animal data and the high number of relevant substances raise the question of effective alternative test systems to analyze the biological effects of airborne matter. *In vitro* systems offer the unique possibility to analyze the cellular reactions dependent on substance, concentration and time and to compare the data of several substances in the same system. The use of human cells, in particular, reduces difficulties in interpreting and extrapolating animal data to the human situation.

New cultivation and exposure techniques in the field of *in vitro* toxicology also enhance the efficiency of such cellular studies, as demonstrated by two experimental setups which allow direct exposure of cells from the respiratory tract at the air/liquid interface. The basic feature in both cases is the cultivation of the cells on porous transwell membranes, which are permeable for the culture medium.

The first system, called "CULTEX", is based on an intermittent medium supply of the cells. We have developed this alternative cultivation system, which provides a flexible and reproducible experimental protocol, to cultivate and expose cells to airborne material at the air/liquid interface, The method as well as the culture chamber are already patented. The medium will be pumped into four special modular culture units, each housing three transwells, through the transwell membrane to support the cells. At certain time intervals, the medium is be removed completely and the cells can be maintained and exposed at the air/liquid interface until the next medium supply without loss of viability for up to 72 h. Both the wells and the individual modules are connected via a network of glass tubes and hoses. Starting from a central medium supply, the medium is directed by a peristaltic pump via distribution nozzles to the relevant

modules and the transwells inside. The fill level in the transwells is regulated by an infrared sensor connected to an outer tube of the whole inlet pipe for controlling the medium level in the culture containers. The sensor acts as a photoelectric beam system. Depending on the light refraction, there is a change in the current through the phototransistor in the photoelectric beam. Each module also has a second drainage nozzle, which allows separate removal of the medium via the drainage tube, thus ensuring continual medium analysis, e.g., for cell secretory products, such as metabolites, during an experiment.

The constant temperature of the module is ensured by a regulated flow of temperature-controllable water through the modules of the whole cultivation chamber.

For exposure of the cells, special equipment has been constructed consisting of an aerosol generation system, and a second part for the dilution and distribution of the smoke. The design of the *in vitro* exposure unit allows simultaneous treatment of the cells with three aerosol concentrations, using one module as control unit for exposure to clean air. The technical details and physical characteristics of the system are presented in pilot studies with sidestream smoke.

The second system called "MIPEX" (mobile *in vitro* exposure system) offers the possibility to treat cultivated cells under indoor or outdoor conditions using a transportable exposure unit for inhalable particulate and/or gaseous compounds. The transwells with the adherently growing cells are placed in a compendium plate directly located under a corresponding plate with nozzles. The compendium plate can be rotated by motor, thus guaranteeing a homogenous impaction of the cells with particulate material. This experimental setup is located in a plastic chamber, which can be controlled with regard to temperature and atmospheric humidity. The nozzles are connected to three flues, which, depending on the adjusted air flow, allows the impaction of particle fractions of different sizes. It is therefore possible to expose the cells to particles which, for example, are normally deposited in the thoracic or alveolar regions. The cells can be treated for a maximum of 20 min. Afterwards they can be analyzed for cellular reactions or replaced in medium for further experiments. In a series of studies, the functional stability and reproducibility of this mobile *in vitro* exposure system was shown with human cells.

The application of new cultivation and exposure techniques will, in future, offer new testing strategies for comparable toxicological evaluation of soluble and inert substances, as well as complex mixtures and specially designed aerosols.