

# Induction of oral cancer by 7,12-dimethylbenz[*a*]anthracene in rats with liver cirrhosis

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The effects of carbon tetrachloride-induced liver cirrhosis and xerostomia on oral carcinogenesis were studied in rats given the lipid-soluble carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA). The first carcinoma was detected after only 1 month of DMBA applications. After a further 8 months 85% of the animals had developed one or more squamous cell carcinomas. The latency period for DMBA-induced oral cancer in cirrhotic rats with xerostomia was markedly reduced in comparison with previous findings from xerostomic rats without liver cirrhosis. The results support earlier epidemiologic studies indicating a relationship between liver cirrhosis and oral cancer. □ *Experimental study; lipid-soluble carcinogen; oral carcinogenesis; squamous cell carcinoma; xerostomia*

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An association between alcohol-induced liver cirrhosis and oral cancer in man has been claimed in several epidemiologic investigations (1, 2). The mechanisms by which cirrhosis promotes the development of oral cancer are, however, incompletely understood.

To study the role of different pathogenetic factors in oral carcinogenesis, several experimental model systems have been elaborated (3). Using the rat palate model, Wallenius (4) showed that repeated intraoral applications of a 0.5% solution of the lipid-soluble carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) resulted in oral squamous cell carcinoma in 30% of rats when treated for 16 months. After complete inhibition of the salivary secretion, however, 100% of the animals developed carcinomas after 11 months (4, 5). When animals with either intact or inhibited salivary secretion were exposed to the water-soluble carcinogen 4-nitroquinoline *N*-oxide (4NQO), onset of oral cancer occurred even earlier (6, 7).

In similar experiments, Lekholm & Wallenius (8) observed that rats with liver cirrhosis showed a reduced latency period for development of oral cancer after treatment

with 4NQO. Subsequent biochemical analyses of clinically normal palatal epithelium of cirrhotic rats revealed an altered fatty acid composition of the lipids (9).

The present investigation, which is an extension of previous studies, deals with the effects of liver cirrhosis and xerostomia on the development of oral cancer in rats exposed to DMBA.

## Materials and methods

### *Animals*

Thirty-eight female albino rats of the Sprague–Dawley strain (Anticimex AB, Stockholm, Sweden), with a mean weight of 150 g, were used. The animals were fed standard pellets and water ad libitum.

### *Induction of liver cirrhosis*

Liver cirrhosis was induced by repeated injections of chemically pure carbon tetrachloride (CCl<sub>4</sub>) mixed with paraffin (9, 10). The injections were given intraperitoneally (20 µl CCl<sub>4</sub>/0.3 ml paraffin/100 g body weight/injection) twice a week during the

Table 1. Number and location of tumors in relation to time of DMBA exposure

	Months of DMBA application								
	1	2	3	4	5	6	7	8	9
No. of tumors in the palate	1	3	0	2	0	2	3	2	3
No. of tumors in the lower jaw	0	1	1	0	0	1	3	1	3
Total no. of tumors	1	4	1	2	0	3	6	3	6

first 6.5 months. Thereafter, and until the end of the experiment, the injections were given once a week, but with a double dose of CCl<sub>4</sub> mixed with the same amount of paraffin.

Out of a total of 38 rats, 5 died during the period of induction of liver cirrhosis. Of the remaining rats, 5 were used as controls, while the other 28 were selected for tumor induction.

#### Induction of oral cancer

The lipid-soluble carcinogen DMBA (Fluka AG, Chemische Fabrik, Buchs SG, Switzerland) in a 0.5% solution in acetone was used. The carcinogen, or the vehicle alone, was continuously applied three times a week to the hard palate of test and control rats, as previously described (4). To enable the carcinogen to penetrate the mucosa, the salivary secretion was inhibited by injecting all animals with methylscopolamine nitrate (1 mg/kg body weight) subcutaneously immediately before application of the carcinogen. The duration of the induced xerostomia was approximately 8 h, during which period all water bottles were withdrawn from the cages.

All rats showed increasing signs of poor physical condition throughout the carcinogen application period. Seventeen of the 28 animals died spontaneously. Seven of these could not be examined owing to severe autolysis and were therefore excluded from further analysis. The remaining animals were killed either when tumor growths were observed or when the rats showed evidence of poor physical condition.

#### Histopathology

The animals were autopsied for registration of gross tumor growth, metastases included, and biopsy specimens were taken from suspected areas. Specimens were always taken *en bloc* from the upper jaw, including the palate. Additional specimens were taken from the left and right side of the mandible, the tongue, and the right anterior lobe of the liver.

The tissue specimens were fixed in 4% neutral buffered formaldehyde, embedded in paraffin, sectioned at six different levels, and stained for routine histopathologic examination. The diagnosis of oral cancer was verified microscopically.

#### Results

The first carcinoma was histologically confirmed in the palate of a rat that had been painted with DMBA for 1 month. After a further 8 months 18 of the 21 rats had developed squamous cell carcinomas. The remaining three rats died within the first 3 months of carcinogen application, without any signs of malignancy. The mean time of carcinogen application necessary for cancer development was 5.6 months. The number and locations of the histologically verified tumors and their latency periods are given in Table 1.

The tumors were highly differentiated and characterized by an infiltrative growth of relatively uniform cells in sheets and strands (Fig. 1). Clear single-cell infiltration was often difficult to discern. Except for single

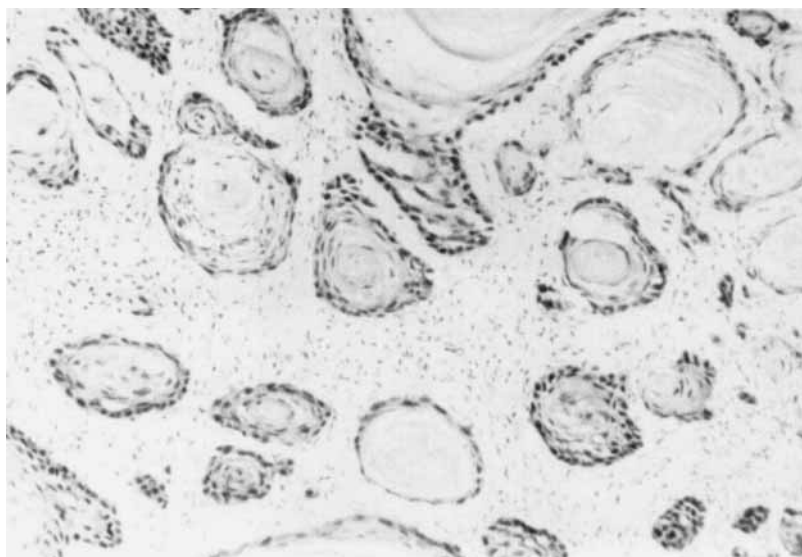


Fig. 1. Highly differentiated squamous cell carcinoma of the palate in a rat painted with DMBA for 7 months. (Hematoxylin and eosin; magnification,  $\times 175$ .)

cells or clusters of cells with dyskeratosis, areas of excessive keratinization were rare. Scattered mitotic figures were observed. The surrounding stroma showed, as a rule, a mild inflammatory reaction. Microscopically, areas of multifocal tumor growth were observed in about half the animals. Tumors developing after 8 months of applications were, in general, larger and grew more aggressively, with destruction and invasion of the underlying jaw bone. No metastases were observed in the regional lymph nodes, the lungs, or the liver in any of the animals.

Only mild inflammatory reactions and moderate hyperplasia were seen in the palatal mucosa of the control rats painted with acetone alone for 10 months. No signs of atypia or malignant transformation were observed.

All liver specimens from both test and control rats showed manifest liver cirrhosis (Fig. 2).

## Discussion

The present study shows that the latency period for development of oral cancer after continuous DMBA applications is reduced in rats with liver cirrhosis and xerostomia in

comparison with rats with xerostomia alone (4). In fact, the first carcinoma among the cirrhotic rats was detected after 1 month of DMBA applications. After 9 months 85% of the animals had developed one or more squamous cell carcinomas. The mean time of carcinogen application necessary for cancer development was 5.6 months. The corresponding figure for xerostomic rats without liver cirrhosis has in other studies been reported to be 11 months (4, 5). Our experimental design differed somewhat from previous studies. In the present experiment most animals died spontaneously or were killed owing to poor physical condition prior to clinical signs of tumor growth. The diagnosis oral cancer was therefore in most cases verified histologically.

A somewhat shortened latency period for development of oral cancer has previously also been demonstrated in cirrhotic rats exposed to the water-soluble carcinogen 4NQO (8). However, in that study the first carcinoma was not detected until after 4 months of carcinogen application, and the total time reduction for tumor development was only 15% compared with control rats (6, 8). The increased susceptibility of the oral mucosa to carcinogens in rats with liver cirrhosis is thus further emphasized by the

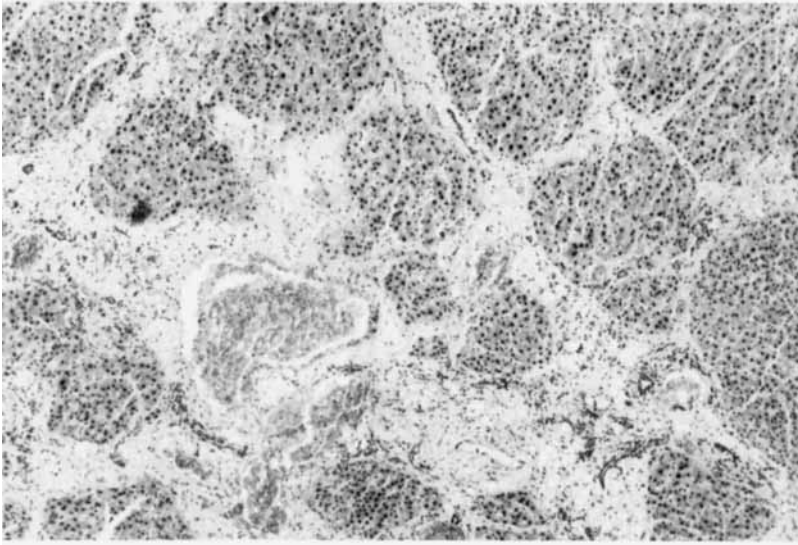


Fig. 2. Liver specimen showing manifest cirrhosis in a rat after injections of  $\text{CCl}_4$ . (Hematoxylin and eosin; magnification,  $\times 125$ .)

present results. The reason for the altered susceptibility is, however, incompletely known.

Earlier studies of cirrhotic rats have revealed an altered fatty acid composition of the phospholipids in oral epithelial cells, leading in part to changed physiologic conditions of the oral mucosa (9). These changes are most likely due to both local and general disturbances caused by the cirrhotic state. Furthermore, Lekholm et al. (11) have noted a focal loss of lipids, especially choline-containing lipids, in oral epithelial cells during DMBA-induced carcinogenesis in normal rats. The alterations appeared well before the development of invasive carcinomas. Such changes, in combination with cirrhosis-associated lipid disturbances, might also contribute to the rapid cancer development seen in this investigation.

The reason for inducing xerostomia in this experiment was to facilitate penetration of the lipid-soluble carcinogen into the mucosa, since the saliva normally constitutes an effective barrier against penetration of lipid-soluble carcinogens (4). The absence of a protective salivary layer on the mucosa can lead to mucosal alterations, including epi-

thelial atrophy, inflammation, fissuring, and ulceration (12). Such effects also have to be considered when discussing factors of importance for tumor development.

The etiology of oral cancer in man is very complex. Several concurrent factors, including tobacco, alcohol, iron deficiency, chronic candidosis, herpes simplex virus, and liver cirrhosis, are known to be of importance (1, 2, 12–15). Clinical studies of the significance of single factors are therefore difficult, and the role of, for example, liver cirrhosis as such in the development of oral cancer cannot be assessed by epidemiologic studies alone.

In this study we present evidence in support of a relationship between liver cirrhosis and oral cancer. Clinically, such a correlation has also been found in alcohol abusers (1, 2, 13). Additional studies are, however, needed to clarify the exact mechanisms by which cirrhosis promotes the development of oral cancer.

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