Transplacental Induction of Pancreas Tumors in Hamsters by Ethanol and the Tobacco-specific Nitrosamine 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

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ABSTRACT

Epidemiological studies suggest that smoking during pregnancy and passive exposure of children to cigarette smoke may increase the cancer risk in children and young adults. We have previously shown that the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is an active transplacental carcinogen in Syrian golden hamsters when administered by s.c. injections to pregnant females. The majority of tumors in the offspring developed in the respiratory tract.

Since in smoking women the respiratory tract is the portal of entry of tobacco-related carcinogens, including NNK, we have investigated the transplacental effects of NNK given by intratracheal instillation to pregnant hamsters. The modulating effect of ethanol on the transplacental carcinogenicity of NNK in this system was also investigated because smoking and consumption of alcoholic beverages are observed in pregnant women. Our data show that exposure to NNK via the maternal respiratory tract causes a similar tumor incidence in the offspring as the s.c. route of administration. Ethanol greatly enhanced the carcinogenic response to NNK, and up to 60% of the offspring exposed in utero to ethanol and NNK developed tumors of the exocrine pancreas.

INTRODUCTION

Epidemiological studies suggest that smoking during pregnancy (1), as well as passive exposure of children to cigarette smoke (2), may increase the risk of cancer in children and young adults. The nicotine-derived nitrosamine NNK(1) is one of the most potent tobacco-specific carcinogens and thus is an excellent model compound for studies of the potential carcinogenic effects of cigarette smoke (3). NNK is an active transplacental carcinogen in mice (4) and Syrian golden hamsters (5). In the hamster model, up to 73% of the offspring develop tumors when hamster females are given s.c. injections on one of the last 3 days of pregnancy with NNK (5), and this effect is dose dependent (6). With the s.c. route of administration used in our previous studies (5, 6), the respiratory tract (nasal cavity, lungs, trachea, larynx) is the main target site for NNK-induced tumor development. Small ductular adenomas of the pancreas were also occasionally found but at an incidence of below 10% (5, 6).

In an effort to further our understanding of the transplacental effects of NNK, we have conducted a bioassay in female hamsters instilled intratracheally with NNK on day 15 of pregnancy. This route of administration makes the respiratory tract the portal of entry of the carcinogen, a situation which more closely resembles human exposure to tobacco-specific nitrosamines than the s.c. route.

Consumption of tobacco products and alcoholic beverages is observed among pregnant women, and it is well established that chronic consumption of alcoholic beverages enhances the cancer risk of smokers (7–9). Accordingly, we tested the modulating effects of ethanol on the transplacental carcinogenicity of NNK in this system. Our data show that exposure to NNK via the maternal respiratory tract causes an overall tumor incidence similar to that of the identical dose administered s.c. Ethanol greatly enhances the carcinogenic response to NNK, and animals exposed in utero to ethanol and NNK developed a high incidence of pancreas tumors.

MATERIALS AND METHODS

Outbred, Sendai virus-free, male and female Syrian golden hamsters were purchased from Charles River (Wilmington, DE) to establish a breeding colony at the University of Tennessee. The animals were handmated under observation by an experienced technician as previously described (5). The pregnant females were randomly assigned to 4 groups (Table 1). Two groups were given ethanol (10% v/v) in the drinking water from day 5 through the last day (day 16) of pregnancy. The females in one of these groups as well as an additional group not exposed to ethanol were intratracheally instilled with NNK solved in distilled water (50 mg/kg) under light anesthesia with Metofane (Pitman-Moore, Inc., Mundelein, IL) on day 15 of gestation. NNK was synthesized as previously described (10) and was >98% pure by high-performance liquid chromatography. The control group received identical intratracheal instillations with the vehicle of NNK (distilled water).

The offspring were weaned at the age of 4 weeks, and males and females were caged separately. The animals were observed until significant weight loss and/or clinical symptoms of disease or discomfort occurred. They were killed with an overdose (270 mg/kg) of pentobarbital (Butler Co., Columbus, OH) and necropsied, and all organs were processed for evaluation by histopathology as previously described (5). The control animals were killed at the time (45 weeks of age) when the last NNK-treated hamsters were euthanized. Due to budgetary restraints, only one histology slide per organ was prepared. For the same reason, the females which had been treated during pregnancy had to be killed when their offspring were weaned.

The incidences of all tumors combined and for specific organ sites were analyzed for statistically significant differences of all treatment groups from the control group as well as differences between the NNK and ethanol-pre-treated groups by the paired t test.

RESULTS

The numbers of male and female offspring per group and overall tumor incidences are listed in Table 1. Although the number of pregnant females used for each group was identical, the number of offspring born and surviving the weaning period varied considerably. The number of offspring per litter (including the control group) was smaller in all groups than in the control group of our previously studied experiment in which injections to pregnant females were by the s.c. route (mean value, 16.5 offspring/litter) (5). We believe that this is a reflection of high perinatal mortality caused by the more stressful manipulations (anesthesia, intratracheal instillation) used in the present study. Survival times among the treatment groups ranged from 39 to 45 weeks with no significant differences between groups. The group transplacentally exposed to ethanol and NNK demonstrated the highest overall tumor incidence in both sexes (50%) and females (77%) as compared with the group treated with NNK alone (males, 33.3%; females, 40%), ethanol alone (males, 5.5%; females, 4.3%), or the vehicle controls (no animals with tumors). In both NNK-treated groups, the females demonstrated a higher overall tumor incidence than the males.

The organ distribution of tumors in the various groups is listed in Table 2. Contrary to our earlier study with s.c. injections of NNK (5),...
none of the animals in this experiment developed tumors in the lungs, trachea, or larynx.

In the group treated with NNK alone, the main target organ of male offspring was the nasal cavity (22%, adenocarcinomas of the olfactory region), while in female offspring the adrenal glands (33%, pheochromocytomas) were the main site of tumor development. A significant number of females in this group developed lymphomas involving spleen, liver, intestines, and lungs, as well as colon polyps. Additionally, one female demonstrated focal ductular hyperplasias in the pancreas (Fig. 1).

The offspring of females treated with ethanol and NNK demonstrated a pronounced response in the pancreas which has no precedence to date in transplacental studies and which was more prominent in females than in males (Table 2; Fig. 1). Thirteen of the 17 females (76.5%) in this group had diffuse pancreatic ductular hyperplasia (Fig. 2) and acinar cell hyperplasias which were often accompanied by chronic pancreatitis and focal acinar cell atrophy. Ten of these females (59%) had ductular adenocarcinomas (Fig. 3), with occasional foci of acinar cell differentiation. These tumors were predominantly located in the body of the splenic lobe of the pancreas, measured between 3 and 20 mm in diameter, and invaded surrounding pancreas tissue. The same types of pancreatic lesions were found in the males but at a significantly \( P < 0.01 \) lower incidence than in females (Table 2; Fig. 1). Four males and 5 females had pheochromocytomas of the adrenal glands, and 2 animals of either sex had nasal cavity tumors (adenocarcinomas of the olfactory region).

Of the group treated with ethanol alone, one male developed an adenocarcinoma of the pancreas, and one female demonstrated a lymphoma involving the spleen, liver, and intestines. Thirteen of the 23 females in this group demonstrated pancreatitis and diffuse acinar cell atrophy, which was accompanied in 9 cases by diffuse hyperplasia of ductular and acinar cells (Fig. 1). Identical pancreatic lesions were found in some of the males but at a significantly \( P < 0.01 \) lower incidence than in the females (Fig. 1).

The control offspring, the mothers of which had received intratraheal instillations of distilled water, had no tumors and demonstrated normal histopathology of all organs including the pancreas, with the exception of occasional kidney amyloidosis, a common aging disease of hamsters which also occurred in the other experimental groups.

As in our previously reported study on the transplacental carcinogenicity of s.c. injected NNK (5), a significant number (42%) of the offspring exposed in utero to NNK alone developed liver cirrhosis. Offspring of hamster females treated with ethanol alone had an even
higher incidence of this disease (60%), while the incidence was significantly ($P < 0.01$) lower in the group exposed to both ethanol and NNK (15%). None of the control animals had liver cirrhosis.

**DISCUSSION**

Our data show that the simultaneous treatment of pregnant hamsters with ethanol and NNK significantly enhances the carcinogenic response in their offspring as compared to animals exposed in utero to NNK alone. Moreover, the simultaneous in utero exposure to ethanol and NNK resulted in the induction of a high incidence of ductular adenocarcinomas in the pancreas. This is an important finding, in that this tumor type is among the most common cancers found in humans and has a rather dismal prognosis (10). The pancreas tumors in our transplacental model are morphologically similar to the ductal pancreas adenocarcinomas induced in adult hamsters by chronic treatment with $N$-nitrosobis(2-oxopropyl)-amine and structurally related compounds (11). An experiment to test the transplacental carcinogenicity of $N$-nitrosobis(2-oxopropyl)-amine on the pancreas (12) yielded tumors in this organ at a low incidence (less than 10%) similar to that of our group treated with NNK alone. Although smokers are at a higher risk than nonsmokers for developing pancreas cancer (13, 14), experiments with adult laboratory rodents using chemical carcinogens contained in cigarette smoke have resulted predominantly in the induction of respiratory tract tumors (15). A low but significant incidence (11.25%) of acinar and ductular adenocarcinomas of the pancreas was reported in adult F344 rats given NNK for 2 years in the drinking water (16), while no pancreas tumors were reported in adult hamsters (15, 17). Similarly, our previous studies on the transplacental carcinogenicity of NNK administered to pregnant hamsters s.c. resulted in the induction of small ductal adenomas of the pancreas in 10% of the offspring (5). Using the maternal respiratory tract as the portal of entry for NNK caused ductular hyperplasia in the pancreas of one female but no pancreas tumors. Hence it appears that NNK alone is a weak pancreas carcinogen in the hamster when administered via the transplacental route. As is evident from the high incidence of hyperplasias along with the induction of one pancreas tumor in the group treated transplacentally with ethanol alone, ethanol may have a weak tumor-initiating effect in this transplacental hamster system. The simultaneous exposure to both of these stimuli led to a multiplicative effect on pancreas carcinogenesis in the current experiment.

It is well established that the consumption of alcoholic beverages has a multiplicative effect on the risk for a variety of epithelial cancers in smokers (7–9, 13, 14). Experiments aimed at reproducing this effect have mostly used protocols in which rodents were treated with ethanol during many weeks of exposure to nitrosamines (16–22). The observed responses varied greatly and were difficult to interpret because the ethanol exposure included both the initiation and promotion phase of tumor induction. In our study, a potential tumor-promoting effect of ethanol is excluded because ethanol exposure is discontinued on the day of NNK instillation. The observed modulating effects of ethanol on transplacental NNK carcinogenesis thus have to be caused by changes in the tumor-initiating events. With respect to the decreased incidence of liver cirrhosis in the ethanol/NNK-treated group as opposed to the groups receiving NNK, it is possible that NNK was metabolized to a greater extent in the livers of fetuses exposed simultaneously to ethanol. This interpretation is supported by our recent studies, which have shown an increase in the rate of NNK metabolism in the lungs and livers of hamster fetuses exposed in utero to ethanol, while NNK metabolism in maternal organs remained unchanged (23).

Chronic consumption of alcoholic beverages in humans results in an increased release of catecholamines from the adrenal medulla (24) and is an etiological factor for the development of hypertension (24) and chronic pancreatitis (24–26). It is therefore of particular interest that in our experiment the offspring exposed in utero to ethanol had a high incidence of chronic pancreatitis and hyperplasias, an effect which was even greater in the animals exposed to both ethanol and NNK (Fig. 1). It is likely that these chronic inflammatory and proliferative responses to ethanol in the pancreas are important factors in the cascade of events leading to the development of pancreatic tumors in this system. Interestingly, one of the main target organs of NNK carcinogenesis was the adrenal medulla, with a significant number of animals developing pheochromocytomas. This effect was significantly enhanced in the group exposed simultaneously to ethanol and NNK and was especially prominent in females (Table 2). Since pheochromocytomas generally result in elevated levels of catecholamines which in turn play an important role in the regulation of pancreatic secretion (27), the neoplastic response in adrenals and pancreas may be causally linked. In this context, it is of particular interest that we have recently found a direct interaction of NNK with adrenergic receptors in the hamster lung (28) and in a human lung cancer cell line (29). Since this receptor category also mediates the biological effects of the catecholamines in extrapulmonary mammalian organs, including the pancreas, future studies will address the potential involvement of adrenergic receptor-mediated signal transduction pathways in the molecular events leading to the development of pancreas cancer in this exciting new model system.

**REFERENCES**

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