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Relationship between the Time of Sustained Ethyl Acrylate Forestomach Hyperplasia and Carcinogenicity

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Chronic administration of ethyl acrylate (EA) by gavage at 100 or 200 mg/kg/day resulted in a significant dose-dependent increase in the incidence of forestomach (FS) squamous cell papillomas and carcinomas in both sexes of F344 rats and B6C3F$_1$ mice. Subsequent work in this laboratory was designed to investigate the relationship between EA-induced FS hyperplasia and carcinogenicity. Current studies have focused on determining the time required for sustained FS hyperplasia to produce neoplastic transformation. Results of these studies demonstrated that gavage administration of EA to male F344 rats at 200 mg/kg/day for 6 or 12 months caused a sustained increase in FS epithelial hyperplasia for as long as exposure to EA continued. However, FS hyperplasia regressed, and no neoplasms developed when animals receiving EA for 6 months were allowed to recover until they were sacrificed at 24 months of age. In contrast, rats treated for 12 months and allowed 9 months recovery developed FS squamous cell carcinomas (3/13) and papillomas (1/13) for a combined incidence of 4/13. No gross lesions were detected in the liver of any of the rats treated with EA or corn oil vehicle, confirming the tissue specificity in the relationship between EA-induced FS hyperplasia and carcinogenesis. In conclusion, the present work has demonstrated that FS hyperplasia is selectively sustained at the site of EA-induced carcinogenicity for as long as EA is administered and has also demonstrated a temporal relationship between FS mucosal hyperplasia and the development of FS neoplasia by EA. It is speculated that whereas a temporal relationship probably exists between FS hyperplasia and tumor development for many chemicals known to increase cell proliferation, this relationship may vary significantly from one chemical to another and with the target tissue and may be influenced by the mutagenicity of chemicals.

Introduction

Ethyl acrylate (EA), the ethyl ester of acrylic acid, is produced in large volumes for the manufacture of polymers and copolymers that are used to make latex paint, textiles, paper coatings, dirt release agents, and specialty plastics such as dental and medical devices. Acute oral gavage administration of EA to rats caused severe mucosal and submucosal edema accompanied by inflammatory infiltration (*1–3*). In prechronic studies, EA caused severe forestomach (FS) epithelial hyperplasia and hyperkeratosis (*4,5*). A recent stop-study in which 100 or 200 mg EA/kg was administered daily, 5 days/week, for 3 months showed that EA-treated rats exhibited severe epithelial hyperplasia of the FS. Forestomachs of EA-treated rats that were allowed a 19-month recovery showed further decline in the incidence and severity of mucosal cell hyperplasia. No treatment-related lesions were observed at any other site (*5*).

Chronic gavage administration of EA resulted in a significant dose-dependent increase in the incidence of squamous cell papillomas and carcinomas of the FS in both sexes of F344 rats and B6C3F$_1$ mice (*6*). No treatment-related neoplastic lesions were detected at any other sites. EA was negative in most mutagenicity assays with and without metabolic activation, as indicated by testing in several *Salmonella typhimurium* strains and in Drosophila (*7*). However, EA was positive in one micronucleus mutagenicity study in mice (*8*), a finding that was later disputed by Ashby et al. (*9*).

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Chemically-induced cell proliferation is thought to play a role in the development and progression of chemically induced neoplasia (*4,10–13*). Work in this laboratory has addressed the role of cell proliferation in EA-induced FS carcinogenesis. In particular, current studies have focused on determining the time required for sustained hyperplasia induced by EA to lead to neoplastic transformation in the rat FS and the tissue specificity of EA-induced cell proliferation.

Methods

Chemicals. Ethyl acrylate [99%, inhibited with 15-20 ppm of hydroquinone monomethyl ether (HQMME)] was purchased from Aldrich Chemical Company (Milwaukee, WI). Corn oil (Mazola brand) was obtained from Best Foods, CPC International Inc. (Englewood Cliffs, NJ).

Animals and Treatments. Male F344 rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were housed in polycarbonate cages and fed NIH 31 diet and water *ad libitum* in facilities with a relative humidity of 50 ± 10%, temperature of 21-22°C, and a 12-hr dark-light cycle. Rats were housed under these conditions for a minimum of 2 weeks before treatment. All animals were approximately 3 months old at the start of treatments. Rats were assigned numbers that were entered into a computer and were divided into four groups using a locally developed computer program that uses body weight as the basis for randomization. All animal husbandry and experimental operations were conducted under NIH guidelines.

Effect of EA Administration for 3, 6, or 12 Months and Recoveries. Two hundred milligrams of EA/kg in corn oil (5 mL/kg) was administered to rats daily, 5 days/week, for 6 or 12 months. Control rats received 5 mL corn oil/kg/day, 5 days/week, for 12 months. Five rats from each treatment group were sacrificed using CO_2 euthanasia at 24 hr after the last dose. The remaining rats in each treatment group were killed at 24 months of age.

At the time of sacrifice, animals were euthanized under CO_2, and organs and tissues were examined for gross lesions. Tissues were taken and preserved in 10% aqueous buffered neutral formalin. Stomachs were removed free of other tissues, opened across the lesser curvature, pinned to a piece of cardboard, and rinsed before examination. Stomachs were sliced along the long axis and immediately immersed in 10% aqueous buffered neutral formalin. Formalin-fixed stomachs were sliced into 3-4 sections, which extended perpendicularly from the junction of the FS and glandular stomach and covered the full length of the FS, embedded in paraffin, and stained with hematoxylin/eosin (H&E). Microscopic evaluation of coded slides was performed without prior knowledge of treatment.

Results

Data presented in this report demonstrated that EA administration to male F344 rats resulted in the development of FS hyperplasia, which was sustained for as long as EA administration continued. FS hyperplasia induced by continued administration of EA for 6 months was largely reversible after cessation of dosing (Table 1). No treatment-related neoplastic lesions were observed in the FS of rats exposed to EA for 6 months and sacrificed at 24 months of age (Table 1).

After 12 months of EA administration, forestomachs of all treated rats showed evidence of hyperplastic lesions, but there was no evidence of gross or microscopic tumors. However, when animals receiving EA for 12 months were sacrificed after 9 months of recovery, gross examination showed papillomas/carcinomas arising from the FS mucosa of some chemical-treated animals. Microscopic diagnosis in H&E-stained sections further confirmed the development of FS tumors in these EA-treated rats (Table 1). The incidence of FS squamous cell carcinomas and papillomas in EA-treated rats was 3/13 and 1/13, respectively, (combined incidence of 4/13).

No significant increase in the incidence of hyperplasia or neoplasia was seen in the FS of rats treated with the corn oil vehicle for 12 months and sacrificed at 24 months of age. Further, no neoplastic lesions were grossly observed in the liver or any other organ of rats (except the FS) treated with EA or corn oil vehicle.

Discussion

In a recent stop-study in which 100 or 200 mg EA/kg was administered daily, 5 days/week, for 3 months, rats exhibited epithelial hyperplasia of the FS as long as exposure to EA continued (*5*). Histopathologic eval-

Table 1. Incidence of neoplastic and non-neoplastic lesions in the forestomach of male F344 rats treated with ethyl acrylate (EA) for 6 or 12 months followed by recovery to 24 months of age.

	Treatment					
Forestomach Lesions	12 Months CO	6 Months EA	12 Months EA[a]	12 Months CO + 9 Months recovery[b]	6 Months EA + 15 Months recovery	12 Months EA + 9 Months recovery
Mucosal hyperplasia	0/5	5/5	5/5	0/16	0/18	8/13
Squamous cell Papillomas and Carcinomas	0/5	0/5	0/5	0/16	0/18	4/13

[a]Ethyl acrylate was dissolved in corn oil (CO) and administered by gavage at 200 mg/kg/day, 5 days/week for 12 months. Dose volume=5 mL/kg.
[b]Rats treated with 5 mL CO kg/day, 5 days/week for 12 months and allowed 9 months recovery.

uation of forestomachs of EA-treated rats that were allowed a 19-month recovery (with no exposure to EA) showed a significant decline in the incidence and severity of mucosal cell hyperplasia (*5*). No treatment-related neoplasms were observed in these rats. Considering the fact that EA is a FS carcinogen in a 2-year gavage administration study, current investigations have focused on determining the time required for sustained hyperplasia induced by EA to lead to neoplastic transformation in the rat FS.

The current studies confirmed earlier findings (*5*) that EA-induced hyperplasia in the rat FS is sustained as long as EA administration continues. These lesions were selective to the site of EA administration. FS hyperplasia was sustained for 6 months of EA administration and was largely reversible after cessation of chemical exposure, with no neoplasms occurring when these animals were sacrificed at 24 months of age. Hyperplastic lesions in the rat FS were also sustained for 12 months of EA administration. However when animals receiving EA for 12 months were sacrificed after 9 months of recovery, they had developed FS squamous cell carcinomas (3/13) and papillomas (1/13) at a combined incidence of 4/13. It is concluded that a temporal relationship exists between mucosal hyperplasia and the development of FS neoplasia. EA-induced hyperplasia in the skin and respiratory passages of rats receiving EA by dermal application (3 months) and inhalation (6 months), respectively, has been reported to reverse after cessation of exposure to EA, and no neoplastic lesions were detected (*14,15*). This may suggest that a temporal relationship between EA-induced hyperplasia and carcinogenicity may exist in other organs directly exposed to EA. The lack of a carcinogenic response in the two studies may be related, at least in part, to the relatively short time of exposure to EA and, hence, short period of sustained hyperplasia in the skin and the respiratory tract (*14,15*).

As observed in the bioassay (*6*), no significant increase in hyperplasia or neoplasia was detected in the present study in any other organ of the rats treated with EA or corn oil vehicle. This further confirms the tissue specificity in the relationship between EA-induced hyperplasia and carcinogenesis.

Based on the lack of a carcinogenic response after 3 or 6 months of sustained FS hyperplasia and the fact that EA is nonmutagenic, we believe that the primary mechanism of FS neoplasia after exposure to EA is a consequence of promotion of spontaneously initiated cells. Initiation may be caused by trace chemical contaminants and/or natural carcinogens in the diet by activation of oncogenes or by DNA replication errors introduced and fixed in the course of cell replication. Chemical contaminants of animal feed and naturally formed carcinogens such as *N*-nitrosamines were suggested to cause initiation of tumors by the FS carcinogen, butylated hydroxyanisole (*12*).

Finally, we speculated that, whereas a temporal relationship probably exists between cell proliferation and tumor development for most chemicals known to induce proliferation, this relationship may vary significantly from one chemical to another and with the target tissue. Since a mutagenic chemical that also induces increased cell proliferation should both initiate and promote its own carcinogenicity, the time of exposure necessary to induce tumor development should be significantly shorter than that observed for nonmutagenic carcinogens.

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