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Chemical Carcinogenesis of the Gastrointestinal Tract in Rodents: An Overview with Emphasis on NTP Carcinogenesis Bioassays

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Abstract

Cancers of the stomach and large intestine (LI) are the second and fourth leading causes of human cancer mortality. A review of the National Toxicology Program (NTP) database and the Carcinogenic Potency Database (CPDB) reveals that chemically induced neoplasms of the gastrointestinal tract (GIT) are relatively common. Within the GIT, epithelial tumors of the forestomach in mice and rats and LI of the rat are most common. Generally, there is a high species concordance for forestomach with at least 26 chemicals inducing tumors in both species. Glandular stomach tumors are rare, and the few reported are usually neuroendocrine tumors (carcinoids) originating from the enterochromaffin-like (ECL) cells. Of 290 carcinogenic agents identified by the NTP, 19 (7%) caused intestinal neoplasia, 14 in the rat and 5 in the mouse. Neoplasms occurred in both males and females, exclusively in the small intestine (SI) of the mouse and in the LI or both SI and LI in the rat. Enteric carcinogens (NTP) frequently induced neoplasms at other alimentary sites (oral cavity, esophagus, and stomach). In conclusion, the most common induced GIT tumors are squamous neoplasms of the forestomach, glandular neoplasms of the stomach are rare, and rats appear more prone to developing LI (colorectal) cancer compared to mice.

Keywords

cancer; gastrointestinal tract; forestomach; stomach; intestine; rat; mouse; rodent; carcinoid; papilloma; carcinoma; adenoma; adenocarcinoma; neuroendocrine tumor; colon; colorectal; carcinogens; genotoxic; non-genotoxic; molecular biology; review; p53; animal model; NTP

Introduction

Cancers of the stomach and large intestine are the second and fourth leading causes of human cancer mortality (Shibuya et al. 2002). Although there are numerous publications on short-term experimental animal models of chemical-induced gastrointestinal carcinogenesis, there is a paucity of literature on chemically induced carcinogenesis of the gastrointestinal tract (GIT) in rodents mimicking lifetime exposure to carcinogens. This overview focuses on

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the incidences and causes of epithelial gastric and intestinal chemical carcinogenesis in the two major rodent species: rat and mouse. Emphasis is given to the results obtained in chronic (two-year) bioassays listed in the National Toxicology Program (NTP) database and the Carcinogenic Potency Database (CPDB).

Gastric Carcinogenesis

In humans, gastric cancer is the fourth most common cancer and the second leading cause of cancer-related mortality worldwide (Crew and Neugut 2004). In contrast, spontaneous or chemically induced gastric adenocarcinomas similar to that observed in man are extremely rare in rodents, although several experimental animal models exist. Spontaneous and chemically induced gastric tumors in rodents are primarily squamous cell papillomas or squamous cell carcinomas of the forestomach; while chemically induced tumors of the glandular stomach, which are rare, are almost exclusively neuroendocrine tumors (carcinoids) of the enterochromaffin-like cells. The background incidence of gastric neoplasia and the number of agents associated with gastric neoplasia in rats and/or mice are listed in Tables 1 and 2.

Forestomach

Proliferative lesions of the forestomach include hyperplasia, hyperkeratosis, and parakeratosis; and the neoplasms consist of squamous cell papilloma and squamous cell carcinoma (Frantz et al. 1991) arising from and recapitulating the lining epithelium (Figure 1).

Forestomach tumors are common and account for the vast majority of rodent gastric neoplasms. They are the sixth most common induced neoplasm in NTP (<http://ntp.niehs.nih.gov/>) studies, being induced in 35 (12%) of 290 agents found to be rodent carcinogens. The top five most common sites of tumor induction in the rat or mouse in over 570 NTP studies are liver, lung, kidney, mammary gland, and hematopoietic system. According to one estimate, a total of 120 genotoxic and non-genotoxic substances have been identified as carcinogenic to the forestomach in rodents including rats, mice, and/or hamsters (Proctor et al. 2007). The pathogenesis and genetic alterations in rodent forestomach tumors induced by genotoxic and non-genotoxic carcinogens are somewhat different. Genotoxic carcinogens, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), *N*-methylnitrosourea (MNUR) and *N*-methyl-*N*-nitrosourea (MNU) are considered to interact with DNA in their target organ cells, for example in the forestomach, by alkylating DNA bases or forming DNA adducts, resulting in DNA damage and finally causing genetic alterations that are considered to be irreversible (Hirose et al. 1979, 1989; Ohgaki et al. 1992; Cui et al. 1995). Although morphologic features of forestomach tumors induced by genotoxic and non-genotoxic carcinogens are similar, marked differences exist in alterations of genetic pathways or protein expression. *H-ras* and *p53* gene mutations were observed at high and relatively low frequencies, respectively, in forestomach tumors induced by genotoxic carcinogens, while forestomach tumors due to the non-genotoxic carcinogens had no mutations of the *H-ras* and *p53* genes (Kaneko et al. 2002). Relative overexpression of cyclin D1 and p53 was detected in forestomach tumors induced by the genotoxic carcinogens, while their non-genotoxic counterparts had a tendency to show low expression of those genes (Kaneko et al. 2002).

The mode of action can be different for each forestomach carcinogen such as butylated hydroxyanisole (BHA), caffeic acid (CA), or ethyl acrylate (EA), which tend to act through non-genotoxic mechanisms. For non-genotoxic chemicals, chronic inflammation or local irritation of forestomach mucosa due to physical trauma, or chemical-induced irritation and/or ulceration associated with high-dose regimens, may play a role in continuous induction of

cell proliferation and the ultimate development of carcinomas (Ito, Hirose, and Takahashi 1993; Hirose, Takahashi, and Shirai 1995; Rodrigues et al. 1986). The vital role of sustained cell proliferation in the progression of hyperplasia to neoplasia has been well characterized for EA, a non-genotoxic forestomach carcinogen of rats and mice. Carcinogenic doses of EA administered by gavage for three, six, or twelve months caused a sustained increase in forestomach squamous epithelial hyperplasia for as long as exposure to EA continued; however, in a stop-study, hyperplasia regressed, and no neoplasms developed when animals received EA for three or six months and were allowed to recover. In contrast, rats that were treated for twelve months and allowed to recover developed squamous cell carcinomas and/or papillomas in the forestomach (Ghanayem et al. 1993, 1994). This work indicates that cell proliferation, sustained for a sufficient time (twelve months), results in the development of neoplasia despite cessation of chemical administration.

Route of exposure influences forestomach carcinogenesis. Currently (2009), 48 genotoxic and non-genotoxic chemicals have been listed as carcinogenic to the forestomach in rats and/or mice (<http://ntp.niehs.nih.gov/>) by the NTP. Among these, 43 agents were administered by the oral route (gavage, dosed feed, or dosed water), 4 were administered by inhalation, and 1 was administered topically. In addition to the forestomach, a vast majority (38 chemicals) also induced tumors at other sites. A schematic representation of the two main proposed mechanisms involved in forestomach carcinogenesis is illustrated in Figure 2.

Glandular Stomach

The NTP has conducted over 570 two-year rodent bioassay studies with only a few occurrences of neoplasms of the glandular stomach. An adenoma and two adenocarcinomas were observed in male rats administered C.I. Disperse Yellow 3 (NTP, Technical Report no. 222), while two high-dose female rats had carcinomas with clonitralid (NTP, Technical Report no. 091). Likewise, spontaneous gastric adenomas and adenocarcinomas occur very rarely in most strains of mice or rats. There were no glandular stomach tumors in F344 rats ($n = 2,800$ rats) in a recent (May 2009) compilation of control rat tumor data from the NTP, while only 3 out of 1,498 female B6C3F1 mice had glandular stomach tumors. Spontaneous carcinoids of the glandular stomach have been observed in cotton rats (*Sigmodon hispidus*) and the African rodent *Mastomys natalensis*. Carcinoids originate from the enterochromaffin-like neuroendocrine cells (Waldum et al. 1999; Cui et al. 2000; Kumazawa et al. 1989). In conventional laboratory rodents, the background incidence of carcinoids is generally very low, with eight cases of gastric carcinoids reported in NTP studies (Leininger et al. 1999). A recent publication (Tsukamoto, Mizoshita, and Tatematsu 2007) on animal models to study human stomach cancer has an excellent overview on gastric carcinomas.

Chemically induced tumors of the glandular stomach observed in lifetime carcinogenesis bioassays are primarily carcinoids of the enterochromaffin-like cells (ECL cells). Hyperplasia of ECL cells and neuroendocrine tumors are reported for both rats and mice after treatment with a variety of pharmaceuticals such as omeprazole (Greaves 1990; Ekman et al. 1985), ranitidine (Havu et al. 1990), ciprofibrate (Spencer et al. 1989), H₂-receptor antagonist SK&F 93479 (Betton et al. 1987, 1988), and cimetidine (Colin-Jones et al. 1985). The mechanism of induction of carcinoids by antisecretory drugs such as omeprazole has been referred to as the “*gastrin hypothesis*” and is outlined in Figure 3 (Larsson et al. 1988; Håkanson and Sundler 1990). The hypothesis may be outlined as follows: (1) Inhibition of gastric acid secretion leads to elevated antral pH and, secondarily, to release of gastrin from the antral gastrin cells into the blood stream. (2) Gastrin causes both general hypertrophy of the oxyntic mucosa and hyperplasia of the ECL cells in the oxyntic mucosa. These drug-

induced effects are reversible and can be prevented by antrectomy, which supports the hypothesis that hypergastrinemia is a causative event in oxyntic mucosal hypertrophy and ECL cell proliferation (Larsson et al. 1986). Gastric ECL hyperplasia and/or carcinoids can be difficult to visualize unless correct staining techniques such as Grimelius and Sevier-Munger silver stains are employed (Carlsson et al. 1990). Hypergastrinemia secondary to inhibition of gastric acid secretion by drugs such as omeprazole is generally associated with a trophic effect on the fundic mucosa resulting in increased stomach weight and increased mucosal thickness (hypertrophy) (White et al. 1998; Rohr and Tuch 1992; Creutzfeldt et al. 1986).

In addition to pharmaceuticals (e.g., omeprazole, ranitidine, and cimetidine), carcinoids were also observed in both rats and mice in an NTP two-year bioassay with methyleugenol (Figure 4), a non-genotoxic chemical (NTP 2000). The cellular target or the mechanism of action for methyleugenol is not known. Short-term (thirty- and ninety-day) toxicity studies conducted with methyleugenol revealed elevated gastric pH and serum gastrin in rats and mice (NTP 2000), similar to those observed with antisecretory pharmaceuticals, suggesting “the gastrin hypothesis” may also be applicable to this chemical (Table 4).

Intestinal Carcinogenesis

The study of experimental colon carcinogenesis in rodents has had a remarkably long history, dating back almost eighty years (Krebs 1928). Since tumors of the lower bowel are the fourth leading cause of human cancer mortality (Shibuya et al. 2002), there are numerous animal models to study experimental intestinal carcinogenesis. Excellent reviews (Tanaka 2009; Rosenberg, Giardina, and Tanaka 2009; Corpet and Pierre 2005; Taketo and Edelman 2009) on animal models of intestinal carcinogenesis have been published recently. Published literature on chemically induced intestinal carcinogenesis mimicking lifetime exposure to potential carcinogens is generally very sparse. Proliferative lesions of the intestine include hyperplasia, reactive hyperplasia, focal atypical hyperplasia, and squamous metaplasia; and neoplasms include adenoma and adenocarcinoma (Figure 5) (Whiteley et al. 1996). The objective of this section is to present an overview of intestinal neoplasia in two-year chronic bioassays.

The spontaneous incidence of tumors of the intestines is generally low (Table 1) in rodents, ranging from 0.1% to 0.3% for the F344/N rat and 0.3% to 2.4% in the male B6C3F1 mouse in NTP studies. A review of the NTP database and the carcinogenic potency project database (CPDB) database (<http://potency.berkeley.edu/rev>) reveals a significantly higher number of intestinal carcinogens in rats rather than mice (Table 2). In NTP studies, 7% of rodent carcinogens target at least the intestinal tract compared to 12% for forestomach and 57% for liver (primarily mouse liver—data not shown). Furthermore, the numbers of chemicals that are carcinogenic to the large intestine are slightly greater than those that are carcinogenic in the small intestine. Of the 19 agents identified to cause intestinal cancer in NTP studies, none were enteric carcinogens in both the mouse and rat. More enteric carcinogens occurred in the rat (14) than the mouse (5); and of those 14, 8 (57%) caused neoplasms in both the small and large intestine (Table 3). Eighteen of the 19 intestinal carcinogens were by oral route of administration (gavage, feed, or drinking water); and many of the agents, such as 2,2-bis(Bromomethyl)-1,3-propanediol, 2,3-Dibromo-1-propanol, and sodium dichromate dihydrate (VI) induced neoplasms at multiple sites of the alimentary tract including oral cavity, esophagus, and/or stomach.

Chemical Structure and Intestinal Carcinogenesis: Nitrotoluenes and Trihalomethanes (THM)

The nitrotoluenes, *o*-nitrotoluene, and *p*-nitrotoluenes are structurally related, non-genotoxic chemicals that have been extensively studied by the NTP (Dunnick 1993; Dunnick, Elwell, and Bucher 1994; Dunnick et al. 2003). There was equivocal or no evidence of carcinogenic activity in rats and/or mice administered *p*-nitrotoluene in feed for two years (NTP 2002; Dunnick et al. 2003). In contrast to *p*-nitrotoluene, *o*-nitrotoluene administered in the feed for up to two years caused clear evidence for cancer at multiple sites in both rats and mice including large intestinal (cecal) tumors in mice (Sills et al. 2004). The cecal tumor response observed in B6C3F1 mice with *o*-nitrotoluene had both morphologic and molecular characteristics of human colon cancer. Morphologically, the tumors formed glands and were lined by tall columnar cytokeratin 20 positive epithelial cells (data not shown). Furthermore, these tumors had beta-catenin and p53 protein expression (Figure 6) as well as cyclin D1 expression with mutation in the *Catnb*, *p53*, and *K-ras* genes (data not shown). The alterations in cancer genes and proteins found in the mouse large intestinal tumors following *o*-nitrotoluene exposure are also hallmarks of human colon cancer (Sills et al. 2004).

The halomethanes make up one class of the approximately 600 drinking-water disinfection by-products (DBPs) that have been identified. Within the halomethane class are the trihalomethanes (THM) (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane). Although all four structurally related THMs are carcinogenic in rodents, intestinal carcinogenesis is observed only in rats administered bromoform (tribromomethane) or bromodichloromethane by gavage (NTP 1989; George et al. 2002), both of which are genotoxic chemicals. Chemical structures of nitrotoluenes and THM are illustrated in Figure 7.

The profound difference with regard to the carcinogenicity results observed between structurally similar chemicals illustrates the complexity in predicting carcinogenic potential by structure-activity relationship. In the case of nitrotoluenes, the position for substitution on the aromatic ring is important for predicting chemical carcinogenic response in rodents, although not necessarily the target site for chemically induced cancer (Dunnick et al. 2003). The nitrotoluenes (*o* and *p*) are both non-genotoxic chemicals.

In conclusion, B6C3F1 mice have a greater incidence of spontaneous neoplasms of the forestomach (up to 1.9%) and small intestine (up to 2.4%) compared to F344/N rats. The most common induced tumors of the rodent GIT are squamous neoplasms of the forestomach in mice, followed by intestinal carcinoma of the colon or rectum in rats. Rats appear more prone to developing intestinal (primarily colorectal) cancer compared to mice, and neuroendocrine tumors (carcinoids) are the most common type of glandular stomach neoplasm.

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Abbreviations

CPDB	Carcinogenic Potency Database
NTP	National Toxicology Program
GIT	gastrointestinal tract
ECL	enterochromaffin-like cells
MNNG	<i>N</i> -methyl- <i>N</i> -nitro- <i>N</i> -nitroguanidine
MNU	<i>N</i> -methyl- <i>N</i> -nitrosourea
SI	small intestine
GI	gastrointestinal
MNUR	<i>N</i> -methylnitrosourethane
BHA	butylated hydroxyanisole
CA	caffeic acid
EA	ethyl acrylate
DBPs	drinking-water disinfection by-products
THM	Trihalomethanes

Figure 1. Squamous cell papilloma (top panel) and squamous cell carcinoma in rats with invasion into submucosa and muscularis (bottom panel). H&E.

Figure 2. Schematic representation outlining the two main proposed pathways of forestomach carcinogenesis in rodents. *Salient features of forestomach tumors induced by genotoxic (MNNG) and non-genotoxic (BHA) chemicals are outlined (Kaneko et al. 2002; Kagawa et al. 1993).

Figure 3. Schematic representation of the “gastrin hypothesis”: a proposed mechanism for induction of gastric carcinoids in rodents by gastric acid blockade. Adapted from Håkanson and Sundler (1990).

Figure 4. Glandular stomach neuroendocrine tumors (carcinoids) observed in the NTP carcinogenicity study with methyleugenol in rats. Malignant neuroendocrine tumor (A, B, C). Three panels on the right (D, E, F) are serial sections of a malignant tumor stained with H&E, Sevier-Munger, and NSE respectively.

Figure 5.
Large intestinal adenoma (top panel) and adenocarcinoma (bottom panel) observed in the bromodichloromethane NTP carcinogenicity study in rats. H&E.

Figure 6. Cecal carcinoma observed in the *o*-nitrotoluene NTP carcinogenicity study in mice (A & B). Tumors were immunohistochemically stained for p53 (C) and beta catenin (D) (Sills, et al., 2004).

Figure 7.

Chemical structures of nitrotoluenes and Trihalomethanes (THM) to illustrate that structurally similar chemicals are not site specific carcinogens. *o*-nitrotoluene, bromodichloromethane, and tribromomethane (left panel) are intestinal carcinogens; whereas *p*-nitrotoluene, chlorodibromomethane, and chloroform are not intestinal carcinogens.

Table 1

Incidence of spontaneous gastric and intestinal neoplasms in control F344/N rats and B6C3F1 mice from recent NTP chronic (two-year) bioassay studies (in percentages).^a

Site and diagnosis	Male rat (1,398) ^b	Female rat (1,350)	Male mouse (1,449)	Female mouse (1,498)
Forestomach				
Squamous cell papilloma	0.2	0.3	1.4	1.7
Squamous cell carcinoma	0	0.1	0.5	0.1
Combined	0.2	0.4	1.8	1.9
Glandular stomach				
Adenoma	0	0	0	0.1
Adenocarcinoma	0	0	0	0.1
Neuroendocrine tumor	0	0	0	0
Combined	0	0	0	0.2
Small intestine				
Adenoma	0	0	0.9	0.5
Adenocarcinoma	0.1	0	1.6	0.3
Combined	0.1	0	2.4	0.8
Large intestine				
Adenoma	0.1	0.2	0	0
Adenocarcinoma	0.1	0.1	0.3	0
Combined	0.2 ^c	0.3 ^c	0.3 ^d	0

^a NTP Historical Controls Report (May 2009). There were 49 to 50 animals per group for 27 to 30 studies regardless of the route of administration reported in the past 5 years. The rat strain is F344/N and mouse strain B6C3F1.

^b Number of animals (*n*).

^c All were in colon or rectum.

^d All 4 were in the cecum.

Table 2Percentage of rodent carcinogens that target the gastrointestinal tract in NTP studies^a and CPDB.^b

Source of data	Target organ	Number and percentage of rodent carcinogens
NTP ^a	Stomach	36/290 ^c (12%)
	Intestines	19/290 (7%)
CPDB ^b (Rat)	Stomach	88/564 ^d (15%)
	Small intestine	29/564 (5%)
	Large intestine	32/564 (6%)
CPDB ^b (Mouse)	Stomach	69/442 (16%)
	Small intestine	6/442 (1.4%)
	Large intestine	3/442 (1%)

^a Out of over 570 chronic studies conducted by the NTP (<http://ntp.niehs.nih.gov/>).

^b Data obtained from the Carcinogenic Potency Database (CPDB). The numbers of chemicals that are listed as carcinogenic to the intestines were obtained from a comprehensive table listing frequency of target organs for 564 carcinogens in rats and 442 carcinogens in mice. (<http://potency.berkeley.edu/>). Many chemicals induce tumors at more than one site, and these are counted at each relevant target site.

^c Rodent carcinogens were those classified as some or clear evidence of carcinogenic activity or positive in NTP studies.

^d Rodent carcinogens were identified by CPDB criteria.

Table 3

Number of chemicals associated with intestinal neoplasia^a in NTP studies.

Site	Rats (<i>n</i> = 14 ^b)	Mice (<i>n</i> = 5 ^c)
Large intestines	14	1
Small intestines	8	4

^a Adenoma or adenocarcinoma.

^b 1-amino-2,4-dibromoanthraquinone; asbestos, chrysotile; 2,2-bis(bromomethyl)-1,3-propanediol; bromodichloromethane; C.I. acid red 114; C.I. direct blue 15; 2,3-dibromo-1-propanol; 3,3'-dimethoxybenzidine dihydrochloride; 3,3'-dimethylbenzidine dihydrochloride; glycidol; *o*-nitroanisole; phenazopyridine hydrochloride; 4,4'-thiodianiline; tribromomethane.

^c Captan; indium phosphide; methylene blue trihydrate; *o*-nitrotoluene; sodium dichromate dihydrate.

Table 4

Comparison of serum gastrin, gastric pH, and gastric neuroendocrine cell hyperplasia and neoplasia in female F344 rats administered Methylugenol.^a

Methylugenol dose (mg/kg/day)	14-week study ^b (female F344/N rats)			2-year study ^c (female F344/N rats)	
	Serum gastrin: 30 days (n = 10)	Serum gastrin: 90 days (n = 10)	Glandular stomach pH: 30 days (n = 10)	Glandular stomach pH: 90 days (n = 10)	Glandular stomach: Benign or malignant neuroendocrine tumor (n = 50)
0	62 ± 7	41 ± 3	2.2 ± 0.1	2.2 ± 0.3	0
37	25 ± 4 ^d	46 ± 3	2.4 ± 0.3	1.8 ± 0.1	1
75	22 ± 2 ^d	60 ± 6	1.7 ± 0.1	2.2 ± 0.2	25
150	57 ± 14	88 ± 11 ^d	1.9 ± 0.2	2.0 ± 0.2	34
300	127 ± 23 ^d	409 ± 68 ^c	1.6 ± 0.2 ^c	3.1 ± 0.5 ^c	41

A simplified table to illustrate the interrelationship between serum gastrin, gastric pH, and neuroendocrine cell hyperplasia or neoplasia of the glandular stomach.

^aNational Toxicology Program—Technical Report 391 (2000).

^bThe 30/90 day investigative study was conducted only in female F344 and male B6C3F1 mice. Data are presented as mean ± standard error.

^cThe 2-year carcinogenesis bioassay was conducted in both genders of F344 rats and B6C3F1 mice. Neuroendocrine tumors were observed in rats of both genders and male mice.

^dSignificantly different from controls.