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Review Article

Lipoprotein Lipase as a Candidate Target for Cancer Prevention/Therapy

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Epidemiological studies have shown that serum triglyceride (TG) levels are linked with risk of development of cancer, including colorectal and pancreatic cancers, and their precancerous lesions. Thus, it is assumed that serum TG plays an important role in carcinogenesis, and the key enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma TG, may therefore be involved. Dysregulation of LPL has been reported to contribute to many human diseases, such as atherosclerosis, chylomicronaemia, obesity, and type 2 diabetes. Recently, it has been reported that *LPL* gene deficiency, such as due to chromosome 8p22 loss, *LPL* gene polymorphism, and epigenetic changes in its promoter region gene, increases cancer risk, especially in the prostate. In animal experiments, high serum TG levels seem to promote sporadic/carcinogen-induced genesis of colorectal and pancreatic cancers. Interestingly, tumor suppressive effects of LPL inducers, such as PPAR ligands, NO-1886, and indomethacin, have been demonstrated in animal models. Moreover, recent evidence that LPL plays important roles in inflammation and obesity implies that it is an appropriate general target for chemopreventive and chemotherapeutic agents.

1. Introduction

A high-calorie diet and low physical activity, part of the so-called "Westernization" of lifestyle, are associated with elevated incidences of the breast, colon, liver, pancreas, and prostate cancers. Moreover, they are also linked with the risk of obesity, type 2 diabetes, and dyslipidemia. The World Cancer Research Fund and American Institute for Cancer Research have evaluated causal relationships between body fat and cancer and provided strong evidence for roles in such as colorectum and pancreas cancers [1]. In Japan, overweight and obesity (body mass index ≥25) are reported to be associated with cancers of specific organs, such as the colorectum (male), postmenopausal breast (female), and the liver in individuals positive for hepatitis C virus infection [2–4].

Greater body fatness is a major risk factor for the metabolic syndrome, which presents as a combination of symptoms, such as dyslipidemia (elevated triglyceride (TG) levels or low high-density lipoprotein (HDL) cholesterol), elevated blood pressure, and elevated fasting glucose levels. Hypertriglyceridemia is associated with the risk of colon cancer in Japanese men (HR = 1.71) and being overweight with the risk of breast cancer (HR = 1.75) [5]. In addition, most epidemiological studies, including our own, have consistently showed that serum TG levels are associated with the risk of colorectal adenoma, a precursor lesion of colorectal cancer [6–11]. Thus, it is assumed that serum TG could play an important role in carcinogenesis and that the key enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma TG, may also be involved. In this paper, we focus on the roles of LPL in cancer development and further discussed possible approaches to cancer prevention/therapy.

2. Function, Structure, and Gene Regulation of LPL

2.1. Functions and Structure of LPL. LPL plays an important role in lipid metabolism as an enzyme responsible for hydrolysis of the TG component in circulating chylomicrons and very-low-density lipoprotein (VLDL) via binding with apolipoprotein C2 [12, 13]. Thus, lowering or deficiency of LPL expression is associated with hyperlipidemia [14, 15]. The LPL enzyme itself is composed of two structurally

distinct regions. The amino-terminal domain is responsible for catalysis with a catalytic center formed by three amino acids (Ser^{132}, Asp^{156}, and His^{241}). The carboxy-terminal domain of LPL is required for its binding to the lipoprotein substrate [3, 16–18].

2.2. LPL Gene Expression and Its Regulation. The human *LPL* gene is located on chromosome 8p22 and composed of 10 exons [19]. LPL is ubiquitously expressed in the whole body, but especially in the adipose tissue and the skeletal muscle [20, 21] and is regulated by hormonal and inflammatory stimuli, such as insulin [22, 23], glucocorticoid [24, 25], adrenaline [26], tumor necrosis factor (TNF)-α [27, 28], transforming growth factor (TGF)-β [29], and interleukin (IL)-1β [27].

The expression of LPL is controlled transcriptionally and posttranscriptionally. Basal promoter activity has been shown to be regulated by Oct-1 and the NF-Y binding motifs [30, 31], and the 5′-CCTCCCCC-3′ motif, which interacts with Sp1 and Sp3 [32]. Induction of *LPL* gene transcription is mediated by the peroxisome proliferator response element (PPRE) and the responsible element which binds to sterol regulatory element-binding protein (SREBP) [33, 34]. The effect of insulin on *LPL* expression is an example of posttranscriptional control, the hormone being suggested to increase *LPL* mRNA levels via mRNA stabilization [23, 35].

3. Relationship between LPL and Cancer: Human Studies

3.1. Loss of LPL and Resultant Common Disease. LPL has been reported to play key roles in many human diseases, such as atherosclerosis, obesity, type 2 diabetes, chylomicronaemia, Alzheimer's disease, and cachexia [15]. Especially, *LPL* gene deficiency is the cause of type I hyperlipoproteinemia (familial hyperchylomicronemia) [36]. Homozygous deficiency of *LPL* in humans is rare, but heterozygous deficiency is observed in around 3% of people with various ethnic backgrounds [37, 38]. Although these individuals have elevated serum levels of TG and decreased HDL cholesterol [39], it is not clear whether they are at increased risk of atherosclerosis, ischemic heart disease, type 2 diabetes, and cancer. There is a report that the *LPL* S447X mutation is associated with a higher risk of pancreatic calcification and steatorrhea in hyperlipidemic pancreatitis [40]. Since LPL provides fatty acids to the tissues and fatty acids evoke insulin resistance, *LPL* gene deficiency could affect glucose metabolism. However, whether heterozygous *LPL* deficiency reduces plasma glucose levels or not is still controversial. One paper described reduction of plasma glucose levels, but two others observed no effects as compared with LPL intact humans [41–43]. On the other hand, it has been reported that patients with poorly controlled diabetes frequently have dyslipidemia due to defects in LPL enzyme activity [44].

3.2. Effects of Chromosome 8p22 Loss and LPL Gene Polymorphisms on Cancer Risk. Alteration in genomic DNA, such as point mutations and deletions/amplifications or epigenetic changes such as CpG island hypermethylation and histone modification, can induce abnormal gene expression, which in the case of tumor suppressor genes or oncogenes could eventually lead to carcinogenesis. The human *LPL* gene has been mapped to chromosome 8p22 and previous studies on loss of heterozygosity (LOH) in colorectal tumors suggested that a putative tumor suppressor gene may lie within the short arm of chromosome 8, that is, 8p22-p21.3. Loss of 8p23.1-22 is also reported to be an important stage in initiation or promotion of hepatocellular carcinoma development and may also be the most frequent chromosomal alteration in prostate cancer [45]. It has been found that deletion of *LPL* is observed in 68% (52/76) of localized prostate cancers by FISH analysis [46]. It has further been reported that chromosomal region 8p23.1-8p21.1 may harbor one or more important prostate-cancer-susceptible loci based on linkage analyses in 159 hereditary prostate cancer families [47, 48]. To date, several new candidate cancer-susceptible genes have been cloned to 8p22, such as *deleted in breast cancer 2* (*DBC2*), *leucine zipper tumor suppressor 1* (*LZTS1*), *deleted in liver cancer 1* (*DLC1*), and *mitochondrial tumor suppressor 1* (*MTUS1*) [49–52]. Thus, cancer-susceptible genes mapped close to the *LPL* gene could be affected by *LPL* gene deletion, and exert combined effects in promoting carcinogenesis.

Moreover, an *LPL* Ser447stop polymorphism has been shown to be associated with prostate cancer risk [53] and the *LPL* gene is commonly methylated in prostate tumors [54]. *LPL* promoter CpG island methylation has been revealed in 45% of LPL-deleted tumors and in 22% of LPL-retaining tumors [54]. Biallelic inactivation of *LPL* by chromosomal deletion and promoter methylation may thus contribute to prostate tumorigenesis, but information is lacking regarding pancreatic cancer.

4. Relationship between LPL and Cancer: Animal Studies

4.1. Dyslipidemia Observed in Cancer-High-Susceptibility Animal Models. Elevated serum TG has been shown to promote carcinogen-induced colon carcinogenesis, and rats with hypertriglyceridemia such as the Zucker obese and Nagase analbuminemic strains and F344 rats fed a high-fat diet are all known to be more sensitive to carcinogen treatments than rats with normal serum lipid levels [55–57].

In the case of mice, the Apc^{1309} (C57BL/6J$^{Apc/Apc\Delta 1309}$) [58] and Min (C57BL/6-$Apc^{Min/+}$) animal models of human familial adenomatous polyposis (FAP) feature development of large numbers of intestinal polyps and hypertriglyceridemia [59, 60]. Although no significant differences between Apc^{1309} mice and wild-type mice were observed at 6 weeks of age, the average serum TG value in the former at 12 weeks was obviously increased almost 10-fold (~600 mg/dL) over that at 6 weeks. Similar increase of TG levels (~400 mg/dL) was observed in Min mice at 15 weeks compared to 8 weeks of age (Table 1). Along with TG elevation, mRNA levels of *LPL* in the liver and small intestine of Apc^{1309} and Min mice were suppressed. Of note, other lipogenic genes, such as *FAS* and *stearoyl-CoA*

Table 1: Summary of animal models with dyslipidemia and cancer high susceptibility.

Animal	Strain	Age (week-old)	Serum TG (mg/dL)	Treatment	Tumor	Reference
Mouse	Apc^{1309} (C57BL/6J$^{Apc/Apc\Delta 1309}$)	12	~600	—	Intestinal adenoma	[59]
	Min (C57BL/6-$Apc^{Min/+}$)	15	~400	—	Intestinal adenoma	[59, 60]
	KK-A^y	19	481	AOM	Colon cancer	[61]
	ICR	20	159	AOM + DSS	Colon cancer	[62]
Syrian golden hamster	—	6	300	BOP	Pancreatic cancer	[63]

Table 2: Summary of tumor suppressive effects of LPL inducers in animal models.

Agent	Dose	Animal model	Value to the untreated control group	Reference
Pioglitazone	200 ppm	Apc^{1309}	67%	[59]
	1600 ppm	Min	9%	[60]
	800 ppm	BOP-treated hamster	40%	[63]
NO-1886	800 ppm	Min	42%	[65]
Indomethacin	10 ppm	Min	25%	[66]

desaturase-1, β-oxidation genes like *acyl-CoA oxidase* and *carnitine palmitoyl transferase 1*, and gluconeogenesis genes, exemplified by *phosphoenolpyruvate carboxykinase*, demonstrated no variation from wild-type mouse expression.

Obese KK-A^y mice were found to be highly susceptible to azoxymethane- (AOM-) induced colorectal aberrant crypt foci (ACF) and colorectal carcinoma development compared to lean C57BL/6J mice [61]. Surprisingly, colorectal carcinomas developed within a very short-term period, 19 weeks, after AOM injection. The number of total ACF in KK-A^y mice was around 70/mouse and almost 8 times higher than that in lean C57BL/6J mice. The incidences of adenomas and adenocarcinoma were 84% and 88%, respectively, in KK-A^y mice, far higher than the 8% and 4% in C57BL/6J values. KK-A^y mice exhibit abdominal obesity, hypertriglyceridemia, and hyperinsulinemia at the time of ACF and tumor development. At 13 weeks of age, the average serum levels of TG, total cholesterol, and free fatty acids of KK-A^y mice undergoing AOM treatment were 484.1 mg/dL, 101.6 mg/dL, and 1,796 mEq/L, respectively (Table 1). It is interesting that hepatic *LPL* mRNA levels were also suppressed in KK-A^y mice compared with C57BL/6J mice. Moreover, serum proinflammatory adipocytokines, such as IL-6, leptin, and plasminogen activator inhibitor-1 (Pai-1), were elevated. Importantly, expression of proinflammatory adipocytokine mRNAs such as for IL-6, leptin, monocyte chemotactic protein (MCP)-1, Pai-1 and TNF-α was significantly increased in the visceral fat tissue; in contrast, that for adiponectin was decreased.

Tanaka et al. have developed a novel colitis-related colorectal carcinogenesis model, using AOM plus dextran sodium sulfate (DSS), a colitis-inducing agent [64]. In this model (AOM + 2% DSS in ICR mice), numerous colorectal adenocarcinomas occur within a short-term period and the serum TG levels demonstrate increase to about 134, 175 and 159 mg/dL at 5, 10, and 20 weeks, respectively [62] (Table 1).

Injection of *N*-nitrosobis(2-oxopropyl)amine (BOP) into Syrian golden hamsters is known to induce pancreatic ductal adenocarcinomas, with a histology very similar to typical human pancreatic ductal adenocarcinomas. Moreover, associated genetic mutations, that is, K-*ras* point mutations and *p16* aberrant methylation/homozygous deletions, are found in common in both hamster and human lesions. Interestingly, Syrian golden hamsters exhibit a hypertriglyceridemic state, almost 300 mg/dL at 6 weeks of age, even when not fed a high-fat diet [63] (Table 1). Also, in the case of this animal model, a low activity of LPL could be one of the causes of hypertriglyceridemia, activity of this enzyme in the liver being only 20% and 30%, respectively, of the values in C57BL mice and F344 rats.

5. Tumor Suppressive Effects of LPL Inducers

Pioglitazone, {(±)-5-[4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl]thiazolidine-2,4-dione monohydrochloride}, is a potent peroxisome proliferator-activated receptor (PPAR)γ ligand with a weak binding affinity for PPARα. In the promoter region of the *LPL* gene, there exists a PPRE, and pioglitazone treatment successfully induced LPL expression in the liver and intestinal epithelial cells in *Apc*-deficient mice. The total numbers of polyps in the groups treated with 100 and 200 ppm pioglitazone in the Apc^{1309} were reduced to 67% of the value in the untreated control group [59] (Table 2). With another *Apc*-deficient model, Min mice given 100–1600 ppm pioglitazone for 14 weeks showed decrease of intestinal polyps to 63–9% of the control number [60] (Table 2 and Figure 1).

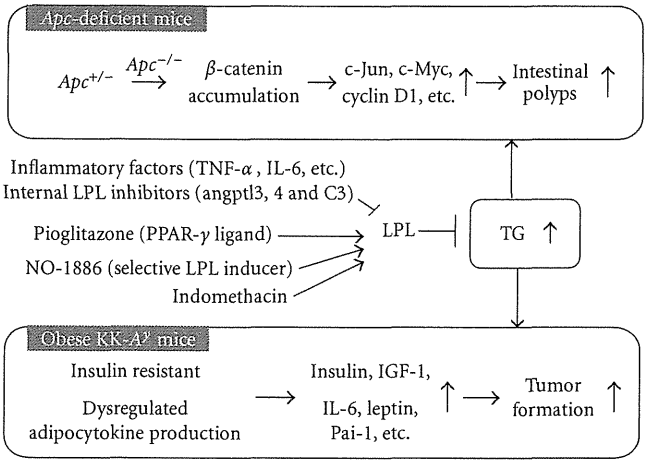

FIGURE 1: Involvement of triglycerides in animal intestinal carcinogenesis models. Angptl-3,4: angiopoietin-like protein-3,4; IGF-1: insulin like growth factor-1; IL-6: interleukine-6; LPL: lipoprotein lipase; Pai-1: plasminogen activator inhibitor-1; PPAR: peroxisome proliferator-activated receptor; TG: triglyceride; TNF-α: tumor necrosis factor-α.

Pioglitazone possesses other functions rather than just simply inducing LPL, such as causing cell growth arrest and apoptosis. Thus, data regarding LPL selective inducers are necessary for determining the relationship between hypertriglyceridemia and intestinal carcinogenesis. NO-1886, 4-[(4-bromo-2-cyanophenyl)carbamoyl] benzylphosphonate, chemically synthesized at Otsuka Pharmaceutical Factory [67] is one useful tool for clarifying this issue. Using a reporter gene assay, NO-1886 demonstrated no PPAR agonistic activity, unlike bezafibrate and pioglitazone [68].

Administration of 400 and 800 ppm NO-1886 also significantly decreased the total number of intestinal polyps to 48% and 42% of the untreated control value, respectively, in Min mice, along with causing marked increase in *LPL* mRNA levels in the liver and the small intestine. Moreover, treatment with NO-1886 also significantly decreased the numbers of colon polyps [65] (Table 2, Figure 1).

In the case of BOP-treated hamsters, pioglitazone has been demonstrated to improve hyperlipidemia and suppress ductal adenocarcinoma development. The incidences of ductal adenocarcinoma in the BOP plus 800 ppm pioglitazone and BOP alone groups were 38% and 80%, and the multiplicities were 0.55 and 1.37, respectively [63] (Table 2). Expression levels of hepatic LPL mRNA were elevated by treatment with 800 ppm pioglitazone. Moreover, quantitative real-time RT-PCR assays demonstrated almost 1.7-fold higher mRNA levels of LPL than that of pioglitazone-nontreated hamsters.

Indomethacin is a conventional nonsteroidal anti-inflammatory drug which has long been clinically employed to improve inflammation. It has demonstrated potent chemopreventive activity against intestinal tumor development in animal models, and a clinical trial in FAP patients also showed reduction in intestinal polyp development [69, 70]. We earlier reported that indomethacin suppresses intestinal polyp formation in Min mice together with ameliorating the hyperlipidemic state by regulating LPL, other lipid metabolic factors and inflammatory pathways [66]. Reduction of serum TG levels was 90% in Min mice with 10 ppm indomethacin treatment and higher than that with 400 ppm pioglitazone (83%) observed in our other previous study [59, 60]. The PPARγ agonistic activity of indomethacin is reported to be 50 times weaker than that of troglitazone, a well-established PPARγ agonist [71]. These results indicate that functions other than agonistic activity of indomethacin are responsible for its strong lipid-lowering effects (Figure 1).

6. Involvement of LPL in Inflammation, Obesity, and Others

6.1. LPL and Inflammation and Apoptosis. In addition to the lipid modifying function of LPL, two different mechanisms might be involved in *LPL* influence on carcinogenesis. The first involves anti-inflammatory action of LPL. It has been reported that LPL suppresses TNF-α- and interferon (IFN)-γ-evoked inflammation-related gene expression in endothelial cells through inactivation of transcription factor nuclear factor kappa B (NF-κB) [72]. Conversely, TNF-α, IFN-γ, IL-1β, IL-6, and leukemia inhibitory factor (LIF) decrease LPL activity.

It is well known that cyclooxygenase-2 (COX-2) is markedly elevated in human colon cancers, in AOM-treated rats, and in intestinal polyps of *Apc*-deficient mice. COX-2 is in fact thought to play important roles in both cancer cell proliferation and angiogenesis. Experiments conducted to clarify the mechanisms of NO-1886 effects on colon carcinogenesis revealed that the expression levels of mRNA for COX-2, in DLD-1 human colon cancer cells, were reduced under conditions of TGFα stimulation. On the other hand, there was no obvious change in the mRNA levels for COX-1 and inducible nitric oxide synthase (iNOS). The results obtained by RT-PCR analysis were also confirmed by

β-gal reporter gene assay in DLD-1 cells [65]. Consistent with the *in vitro* data, administration of 400 and 800 ppm NO-1886 reduced COX-2 mRNA levels in normal parts of small intestine of Min mice at 20 weeks of age [65]. In addition, NO-1886 ameliorates and induces regression of experimental steatohepatitis through increasing LPL activation and suppression of proinflammatory agents, such as TNF-α, IL-6, and COX-2 [73]. Recently, mice lacking *angiopoietin-like protein family 4* (*Angptl4*), which is the inhibitor of LPL, showed a severe and lethal phenotype characterized by fibrinopurulent peritonitis, ascites, intestinal fibrosis, and cachexia in response to a saturated fat diet [74].

The second mechanism is modification of the apoptosis pathway by LPL activation. Phosphatase type 2Cβ activation by unsaturated fatty acids has been demonstrated to induce apoptosis [75]. Unlike ester bodies of fatty acids, free fatty acids have cytotoxic effects *in vitro* and the products produced by hydrolysis of plasma TG may be implicated in such an apoptotic effect.

6.2. LPL and Obesity. Given the importance of LPL for lipid metabolism, its activity would be expected to be intimately involved in obesity effects and development of the metabolic syndrome. A large number of studies in rodents and humans have revealed that obesity results in increased LPL activity in adipose tissue [15, 35, 76–78]. Interestingly, LPL is regulated in opposite directions in adipose tissue and muscle. Feeding increases adipose LPL activity with a corresponding decrease in muscle LPL activity [35, 79]. Exercise stimulates LPL activity in the muscle and leads to increase fatty acid oxidation [80]. In an animal study, NO-1886 suppressed high-fat diet-induced fat accumulation in rats due to the increase of muscle LPL activity [81].

7. Conclusion

Targeting LPL activity or expression levels for development of reagents against cancer seems particularly challenging, because LPL is expressed ubiquitously and plays essential roles in maintaining homeostasis in the body. Data from LPL homozygous knockout mice, which die within one day of birth, underline its importance. However, appropriate suppression of serum TG levels could be achieved by using drugs, even if the number of selective inducers of LPL is limited. Thus, it might be important to develop selective LPL inducers or search for agents focusing on the aspect of "drug repositioning" to obtain the tools for investigating correlation between LPL and cancer. It should be borne in mind that LPL is inhibited by intrinsic factors, such as angptl3, angptl4, and C3 (Figure 1). These could clearly be candidate target molecules for development of LPL inducers. Considering that LPL activity has impact on obesity and metabolic syndrome, its targeting may also affect the regulation of adipocytokines, which may also be involved in carcinogenesis. Further investigations are warranted to clarify the importance of LPL and to accumulate evidence as to the worthiness as a target for cancer chemopreventive and chemotherapeutic agents.

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Induction of glandular stomach cancers in *Helicobacter pylori*-infected Mongolian Gerbils by 1-nitrosoindole-3-acetonitrile

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Helicobacter pylori (_H. pylori_) infection and high intake of various traditional salt-preserved foods are regarded as risk factors for human gastric cancer. We previously reported that Chinese cabbage contains indole compounds, such as indole-3-acetonitrile, a mutagen precursor. 1-Nitrosoindole-3-acetonitrile (NIAN), formed by the treatment of indole-3-acetonitrile with nitrite under acidic conditions, shows direct-acting mutagenicity. In the present study, NIAN administration by gavage to Mongolian gerbils (MGs) at the dose of 100 mg/kg two times a week resulted in three adduct spots (1.6 adducts/10^8 nucleotides in total), detected in DNA samples from the glandular stomach by ^{32}P-postlabeling methods. Treatment with six consecutive doses of 100 mg/kg of NIAN, two times a week for 3 weeks, induced well—and moderately—differentiated glandular stomach adenocarcinomas in the MGs at the incidence of 31% under _H. pylori_ infection at 54–104 weeks. Such lesions were not induced in MGs given broth alone, broth + NIAN or infection with _H. pylori_ alone. Thus, endogenous carcinogens formed from nitrosation of indole compounds could be critical risk factors for human gastric cancer development under the influence of _H. pylori_ infection.

Gastric cancer is the second most frequent cause of cancer death worldwide.[1] Although gastric cancer has become a relatively rare cancer in North America and most Northern and Western European countries, it remains common in East Asia, Eastern Europe, Russia, and selected areas of Central and South America.[2] *Helicobacter pylori* (*H. pylori*) is a well-established major risk factor for gastric cancer,[3–5] and the prevalence of *H. pylori* infection in East Asia countries, including Japan and Korea is reported to be relatively high.[6,7] In addition, the risk of gastric cancer is increased with a high intake of various traditional salt-preserved foods.[3] In fact, pickled vegetable consumption is reported to increase gastric cancer risk in Japan and Korea.[8–10] In Korea, kimchi, commonly prepared with Chinese cabbage or radish, is a traditional and popular food, which contains high levels of nitrate (median 1550 mg/kg).[11] Furthermore, Chinese cabbage is well known as a pickled vegetable commonly consumed in Japan. Moreover, ingestion of nitrate, mainly from food, is suggested to correlate with mortality from gastric cancer.[12–14] Ingested nitrate is mainly converted to nitrite by bacteria in the oral cavity after secretion into saliva.[15] Carcinogenic *N*-nitroso compounds can be formed from nitrite and secondary amines under acidic conditions. Furthermore, direct-acting *N*-nitroso compounds, such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)[16] and *N*-methyl-*N*-nitrosourea (MNU),[17] are known to induce cancer in the glandular stomach of experimental animals. Thus, it is suggested that *N*-nitroso compounds that are formed in the stomach under acidic conditions could be positively associated with the risk of gastric cancer. Nitric oxide, formed by nitric oxide synthase, is also reported to contribute to production of *N*-nitroso compounds.[18]

We have previously reported that treatments of various foodstuffs with nitrite under acidic conditions produce direct-acting mutagens towards *Salmonella* tester strains.[19,20] Among those foodstuffs, Chinese cabbage is shown to contain three indole compounds, indole-3-acetonitrile, 4-methoxyindole-3-acetonitrile and 4-methoxyindole-3-aldehyde as mutagen precursors. 1-Nitrosoindole-3-acetonitrile (NIAN), an *N*-nitroso-substituted compound formed by treatment of indole-3-

Key words: gastric cancer, *Helicobacter pylori*, Mongolian gerbil 1-nitrosoindole-3-acetonitrile, indole-3-acetonitrile

Abbreviations: DMSO: dimethyl sulfoxide; H&E: hematoxylin and eosin; *H. pylori*: *Helicobacter pylori*; MG: Mongolian gerbil; MNNG: *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MNU: *N*-methyl-*N*-nitrosourea; NIAN: 1-nitrosoindole-3-acetonitrile.

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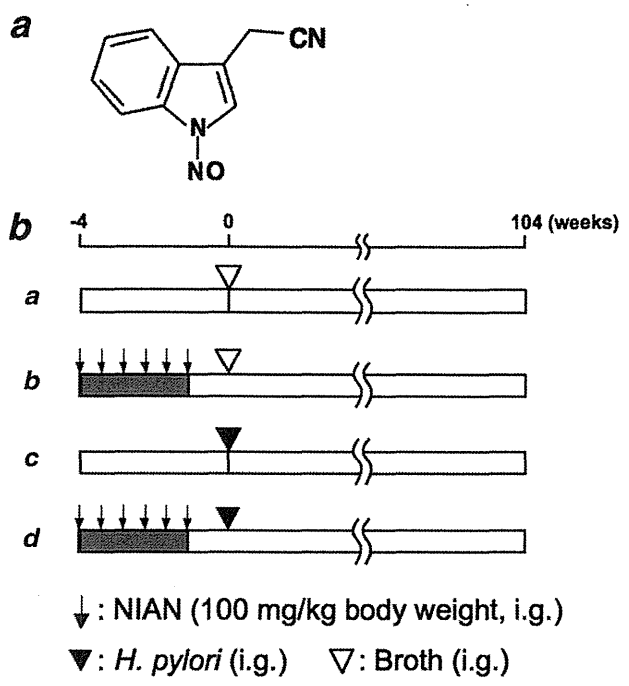

Figure 1. Chemical structure of NIAN and experimental protocol for the carcinogenicity study. (*a*) Chemical structure of NIAN. (*b*) Male 6-week-old MGs were orally administered NIAN (100 mg/kg) in 50% DMSO (groups B and D) or 50% DMSO alone (groups A and C) two times a week for 3 weeks. One week after the final administration, the animals were inoculated with *H. pylori* (ATCC 43504) (groups C and D) or sterilized broth (groups A and B).

acetonitrile with nitrite under acidic conditions, is a direct-acting mutagen in *S. typhimurium* and Chinese hamster lung cells,[20–22] and it is confirmed to form DNA adducts and to induce DNA single-strand scission in the rat glandular stomach.[23,24] Therefore, NIAN could play some role in gastric cancer development, as in the case of the well-known direct-acting mutagens, MNNG and MNU, in animal experiments.[16,17,25]

The Mongolian gerbil (MG) is reported to be susceptible to colonization by *H. pylori*, and *H. pylori* infection greatly enhances MNNG or MNU-induced gastric carcinogenesis in MGs.[26,27] Therefore, the MG is considered to be a useful animal model for evaluating the gastric cancer risk of direct-acting *N*-nitroso compounds, with or without *H. pylori* infection.

Chinese cabbage, containing nitrate and indole compounds, is commonly consumed in East Asian countries, including Japan, Korea and China, in which gastric cancer mortality is very high. In the present study, DNA adducts were detected with NIAN treatment in the glandular stomach of MGs, and the carcinogenicity of NIAN for gastric cancer *in vivo* was examined. The results clearly demonstrated that gastric cancer developed with a combination of NIAN administration and *H. pylori* infection in MGs. Possible involvement of indole compounds and nitrate derived from various foodstuffs, including Chinese cabbage, in gastric cancer development in humans is discussed.

Material and Methods

Materials

Indole-3-acetonitrile was purchased from Tokyo Food Techno (Tokyo, Japan), sodium nitrite from Wako Pure Chemical Industries (Osaka, Japan) and ammonium sulfamate from Kanto Chemical (Tokyo, Japan). Brucella broth was obtained from Becton Dickinson (Cockeysville, MD) and horse serum from Nippon Bio-Supply (Tokyo, Japan).

Preparation of NIAN

The chemical structure of NIAN is shown in Figure 1*a*. Indole-3-acetonitrile in 27 mM citrate-phosphate buffer (pH 3.0) was treated with 50 mM sodium nitrite for 1 hr at room temperature in the dark, as previously reported.[21] Nitrosation was stopped by addition of ammonium sulfamate at a final concentration of 50 mM. The reaction solution was filtered and the residue was washed with deionized water, then with *n*-hexane. The residual paste was dried and stored at −80°C until use. The preparation was >93% pure as judged by its UV absorbance on HPLC.

Bacterial culture

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was cultured in brucella broth supplemented with 10% heat-inactivated horse serum for 24 hr at 37°C under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2), as previously described.[28]

Animal treatment

Specific pathogen-free male, 6-week-old MGs (MGS/Sea, Kyudo, Fukuoka, Japan) were housed in a biohazard room, air-conditioned at 24°C ± 2°C and 55% humidity, on a 12 hr light–dark cycle and were allowed free access to commercial diet (CE-2; CLEA Japan, Tokyo, Japan) and water.

To analyze the formation of DNA adducts in the glandular stomach of MGs by NIAN treatment, NIAN was dissolved in 50% dimethyl sulfoxide (DMSO), and administered to three MGs by gavage of 0.5 ml solution, two times a week at a level of 100 mg/kg body weight. Two further MGs served as a control group receiving the solvent alone (0.5 ml). At 8 hr after administration of NIAN, both groups of animals were sacrificed under ether anesthesia, and their stomachs were resected and stored at −80°C until use. DNA was extracted by a standard procedure with enzymatic digestion of protein and RNA followed by extraction with phenol and chloroform/isoamyl alcohol (24:1, v/v).

The protocol for long-term gastric carcinogenicity in MGs treated with NIAN + *H. pylori* infection is illustrated in Figure 1*b*. The animals were randomly divided into four groups (groups A–D). Groups A and C were given 50% DMSO without NIAN (0.5 ml) whereas groups B and D were orally administrated NIAN (0.5 ml, 100 mg/kg body weight) dissolved in 50% DMSO by gavage, two times a week for 3 weeks. At one week after the last administration, the

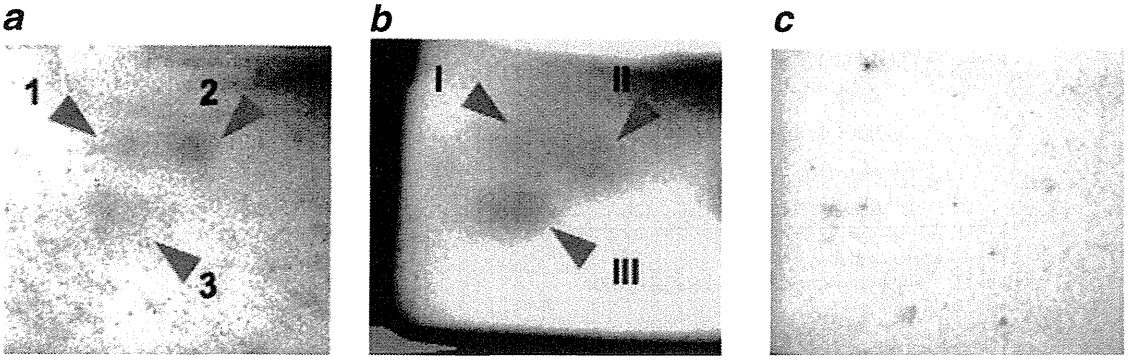

Figure 2. Autoradiograms of NIAN-DNA adducts in glandular stomach of MGs or calf thymus DNA treated with NIAN. Adducts were analyzed by ^{32}P-postlabeling method, as described in the Material and Methods. DNA samples were isolated from glandular stomach of MGs (*a*) or calf thymus DNA (*b*) after treatment with NIAN. DNA samples were also prepared from glandular stomach of MGs without NIAN treatment (*c*). Arrowheads indicate adducts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

animals of groups C and D were given an intragastric inoculation of *H. pylori* broth culture (0.5 ml, 0.9×10^8 CFU/animal) whereas animals of groups A and B were given sterilized broth alone (0.5 ml).[28]

During the experiments, animals which became moribund or emaciated (<80 g body weight) were sacrificed. At 104 weeks after *H. pylori* infection, all surviving animals were sacrificed under ether anesthesia. At performance of necropsy, all tissues were carefully checked macroscopically and the stomachs and major organs were removed and assessed for macroscopic lesion development. Effective numbers of animals were defined as those surviving until week 54 of the study, when gastric tumors were observed for the first time. In addition, in the *H. pylori*-infected groups, the animals developing gastritis observed on histological examination were regarded as effective. The percentages of gastritis-bearing animals by the single inoculation of *H. pylori* were 62% for group C and 76% for group D, being similar to those previously reported.[27] All animal experiments were performed according to the "Guidelines for Animal Experiments in the National Cancer Center" and were approved by the Institutional Ethics Review Committee for Animal Experimentation in the National Cancer Center.

Detection of DNA adducts by ^{32}P-postlabeling method

Calf thymus DNA (0.5 mg, Sigma, St. Louis, MO) treated with NIAN (3 mg) for 12 hr under neutral conditions was used for authentic NIAN-DNA adducts.[23] DNA samples from the glandular stomach of MGs and calf thymus DNA samples were digested with micrococcal nuclease and phosphodiesterase II, and subjected to ^{32}P-postlabeling analysis using the same procedure as described previously[23] except with solvent systems for two-dimensional development. The solvent system consisted of buffer A (4.0 M lithium formate, 7.7 M urea, pH 3.5) from bottom to top, and buffer B (0.90 M lithium chloride, 0.45 M Tris-HCl, 7.7 M urea, pH 8.0) from left to right, followed by 1.7 M sodium phosphate buffer, pH 6.0, from left to right, with 3.5 cm filter paper. Adducts were detected with a Bio-Image Analyzer (BAS 3000; Fuji Photo Film, Tokyo, Japan) after exposing the TLC sheets to Fuji imaging plates. Relative adduct labeling was determined by the methods of Reddy *et al.*,[29] and values were calculated as averages using data from three assays.

Histological examination

All excised stomachs were opened along the greater curvature and washed twice with saline, then fixed in 10% neutral-buffered formalin. The fixed stomachs were sliced along the longitudinal axis into 9–12 strips of equal width, and routinely processed to sections stained with hematoxylin and eosin (H&E). The degree of chronic active gastritis was graded according to criteria modified from the Updated Sydney System,[30] by scoring the infiltration of neutrophils and mononuclear cells. Other organs, in which macroscopic lesions were observed, were also fixed in 10% neutral-buffered formalin and routinely processed to sections stained with H&E for histological examination.

Statistical analysis

The significance of differences in quantitative data for gastric inflammation, gastric adenocarcinoma and tumors of other organs was analyzed by Fisher's exact test. Data for stomach wet weight and inflammation score were examined using Tukey's multiple comparison test. Significance was concluded at $p < 0.05$.

Results

DNA adduct formation by NIAN administration in the glandular stomach of MGs

To confirm the formation of NIAN-DNA adducts in the glandular stomach of MGs, NIAN was injected two times a week at a dose of 100 mg/kg by gavage, and then analyzed by ^{32}P-postlabeling method. Three adduct spots were observed in DNA samples derived from NIAN-treated animals (Fig. 2*a*). The adduct levels were 0.3 for adduct 1, 1.1 for adduct 2, 0.2 for adduct 3 and 1.6 adducts/10^8 nucleotides

Table 1. *H. pylori* infection induced-gastritis in MGs

Group	Treatment	Effective No.	Stomach wet weight (g)	Inflammation score
A	Broth	15	0.647 ± 0.097	0
B	NIAN + Broth	22	0.631 ± 0.094	0
C	*H. pylori*	18	1.432 ± 0.445*	2.22 ± 0.43*
D	NIAN + *H. pylori*	26	1.483 ± 0.445*	2.38 ± 0.64*

*$p < 0.01$ versus group A and B.
Values for results are expressed as averages ± SD.

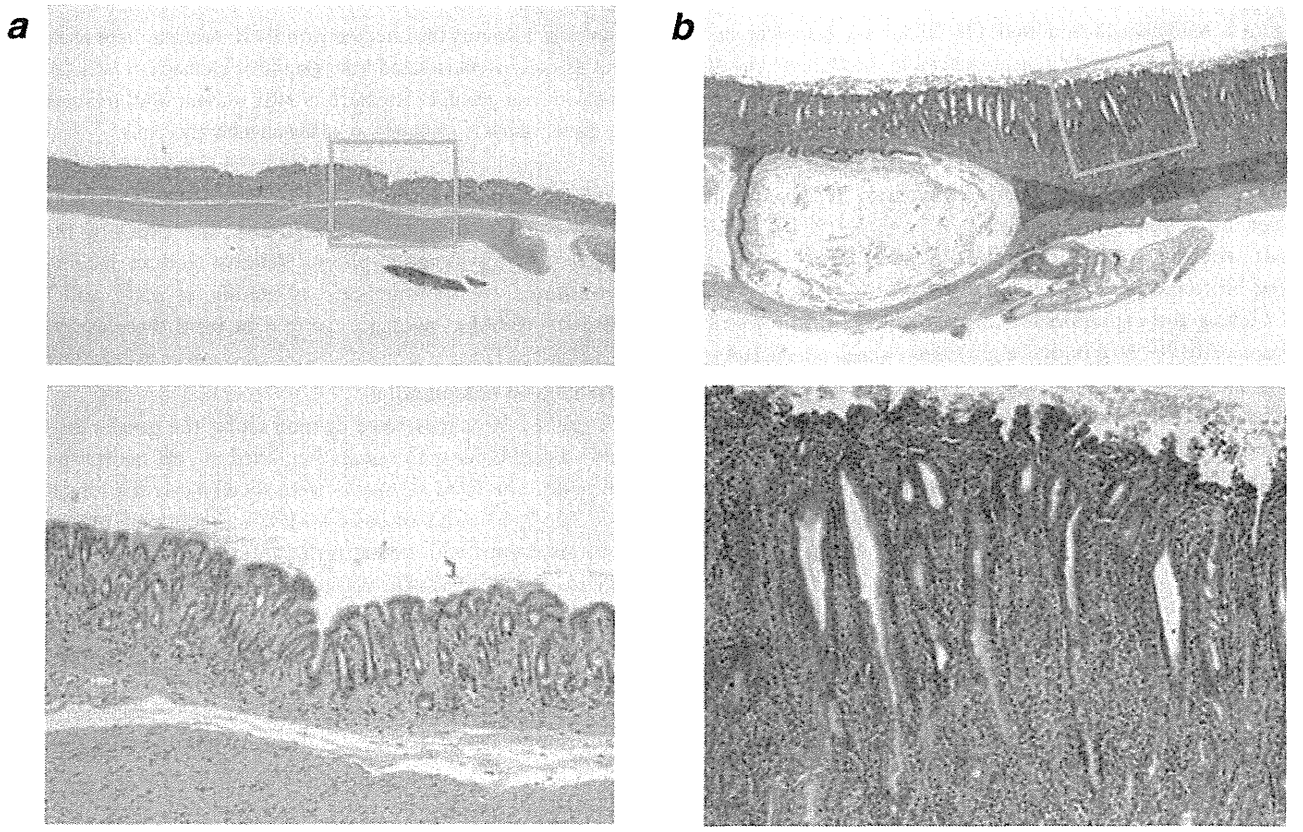

Figure 3. Macroscopic and microscopic views of gastritis in MGs infected or uninfected with *H. pylori*. (*a*) Normal gastric mucosa in group A. (*b*) Severe infiltration of many inflammatory cells with development of heterophilic proliferative glands in group C; H&E staining, ×40. Yellow boxes are shown at greater magnification below, ×200.

in total. This TLC pattern was similar to that in the *in vitro* reaction of calf thymus DNA with NIAN (total adduct level of 4.8 adducts/10^7 nucleotides, Fig. 2*b*). In the case of DNA samples derived from control animals, no adduct spots were seen on the TLC sheets (Fig. 2*c*).

Macroscopical and microscopical observation of *H. pylori*-induced gastritis in MGs

MGs were sacrificed until 104 weeks after *H. pylori* infection, and gastric disorders were analyzed. Stomach wet weights and gastric inflammation scores are shown in Table 1. Macroscopically, edematous thickening with hemorrhagic spots was apparent in the gastric mucosa in *H. pylori*-infected MGs (groups C and D), but not in animals uninfected with *H. pylori* (groups A and B). The stomach wet weight, reflecting edematous thickening, in animals infected with *H. pylori* (groups C and D) was significantly increased compared with that of animals not infected with *H. pylori* (groups A and B) ($p < 0.01$). No significant differences of stomach wet weight were detected between groups A and B and also between groups C and D.

Microscopically, gastritis, featuring infiltration of many inflammatory cells, and hyperplastic change of glandular epithelium, and erosion were observed in the pyloric regions of

Table 2. Incidence of glandular stomach adenocarcinoma in MGs

Group	Treatment	Effective No.	No. of animals with glandular stomach adenocarcinoma (%)		
			Total	Well dif.	Moderately dif.
A	Broth	15	0 (0)	0 (0)	0 (0)
B	NIAN + Broth	22	0 (0)	0 (0)	0 (0)
C	*H. pylori*	18	0 (0)	0 (0)	0 (0)
D	NIAN + *H. pylori*	26	8 (31)*	7 (27)	1 (4)

Well dif., well differentiated adenocarcinoma; Moderately dif., moderately differentiated adenocarcinoma.
*$p < 0.05$ versus group A and C and $p < 0.01$ versus group B.

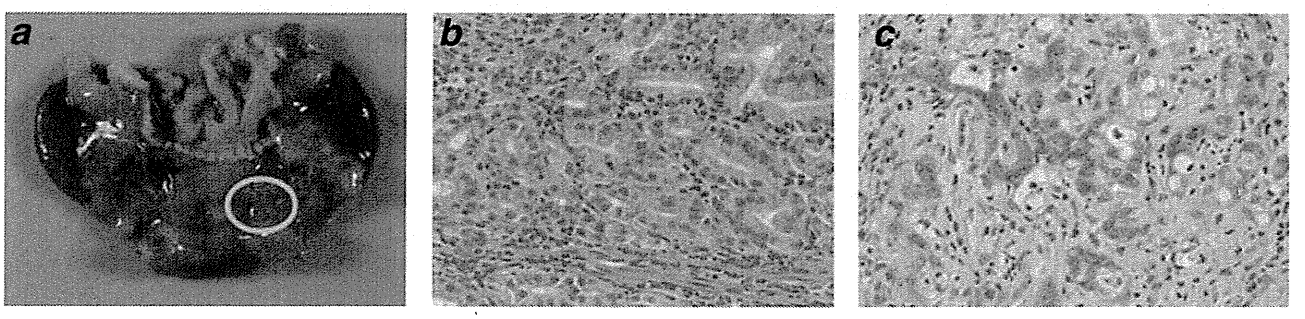

Figure 4. Histological findings of gastric adenocarcinoma in the animals treated with both NIAN and *H. pylori*. (*a*) Typical macrograph of a stomach. The yellow circle shows the suspected lesion of gastric cancer. (*b*) Well differentiated adenocarcinoma. (*c*) Moderately differentiated adenocarcinoma. (*b* and *c*) H&E staining, ×400.

the animals infected with *H. pylori* (groups C and D) (Fig. 3). Heterotopic proliferative glands, whose development is related to severe gastritis in *H. pylori*-infected MGs, were sometimes observed in *H. pylori*-infected groups (groups C and D). No gastritis was found in animals not infected with *H. pylori* (groups A and B). The gastric inflammation score in *H. pylori*-infected animals was significantly increased compared with that of animals uninfected with *H. pylori* ($p < 0.01$). There were no significant differences of gastric inflammation score between groups C and D.

Development of glandular stomach adenocarcinomas in MGs treated with both NIAN and *H. pylori*

The observed incidences of glandular stomach adenocarcinomas are shown in Table 2. Glandular stomach adenocarcinomas, histologically featuring tubular structures with cellular atypia infiltrating into the muscle layer, were found in eight animals treated with both NIAN and *H. pylori* (8/26 = 31%) at 54–104 weeks. All adenocarcinomas were observed in the pyloric mucosa and located in the lesser curvature of the stomach, where macroscopically severe edematous thickening was also seen (Fig. 4*a*). The observed adenocarcinomas in seven animals were of well differentiated (Fig. 4*b*), and a moderately differentiated lesion was observed in one animal (Fig. 4*c*). In the animals treated with broth alone, broth + NIAN and *H. pylori* alone (groups A, B and C), no glandular stomach adenocarcinomas were observed. The incidence of glandular stomach adenocarcinomas in group D was significantly higher than that in groups A, B and C ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively).

Irrespective of NIAN treatment and *H. pylori* infection, skin tumors, which histologically were well to poor differentiated squamous cell carcinomas, sebaceous carcinomas and melanomas, were found in one animal (1/15 = 7%) in group A, three animals (3/22 = 14%) in group B, two animals (2/18 = 11%) in group C and five animals (5/26 = 19%) in group D. A hemangioma was also observed in a kidney of one animal in group D (1/26 = 4%). No significant differences were apparent in these tumor incidences among groups A–D.

Discussion

In the present study, NIAN was found to induce glandular stomach adenocarcinomas in MGs in combination with *H. pylori* infection. NIAN-DNA adducts were also detected in the glandular stomach of MGs after treatment with NIAN, although clarification of their chemical structure(s) has yet to be performed. DNA adducts observed in the glandular stomachs of NIAN-treated MGs probably contain an indole-3-acetonitrile moiety. However, it is further likely that NIAN would act as an NO donor under aqueous conditions, thereby causing DNA modifications.[31–33] In fact, Lucas *et al.* demonstrated that NIAN can efficiently transfer nitroso groups to nucleophilic targets in purine nucleotides, causing *N*-nitrosation, deamination and the formation of a novel guanine analog, oxanine.[33]

Glandular stomach adenocarcinomas induced by NIAN treatment plus *H. pylori* infection were located in the pyloric region, similar to MNNG or MNU treatment plus *H. pylori* infection-induced glandular stomach adenocarcinomas in MGs.[26,27] Meanwhile, no glandular stomach cancers were observed in the groups of *H. pylori*-infected MGs without NIAN treatment, which is consistent with previous studies,[26,27] nor in the group treated with only NIAN. These findings indicated that *H. pylori* is a strong promoter of gastric carcinogenesis. Histological examination revealed that the tumors developed by NIAN + *H. pylori* were of well or moderately differentiated adenocarcinomas. Well or poorly differentiated adenocarcinomas and signet ring cell carcinomas were observed in *H. pylori*-infected MGs treated with MNNG or MNU.[26,27] Further studies are required to clarify the histological variety of stomach adenocarcinomas induced by NIAN, MNNG or MNU, since the type of cancer might depend on the genotoxic action of chemical carcinogens, rather than the effects of *H. pylori* infection.[27] In addition, tumors were observed in skin and kidney, which were suspected to spontaneously develop. The MGs have been reported to develop spontaneous skin tumors such as sebaceous and squamous cell carcinoma.[34]

Epidemiological studies have indicated that nitrate intake increases gastric cancer risk, and major sources are vegetables including Chinese cabbage, spinach and parsley.[14] Indole-3-acetonitrile, a precursor of NIAN, is distributed widely in cruciferous vegetables including Chinese cabbage and sprouts.[35] Furthermore, fava beans (*Vicia faba*), which are commonly consumed in Colombia, give rise to a potent mutagen in the presence of nitrite under acidic conditions.[36] The nitrosatable precursor of the mutagen in fava beans and the major product of nitrosation are reported to be an indole compound, 4-chloro-6-methoxyindole and an *N*-nitroso compound, 4-chloro-2-hydroxy-N^1-nitroso-indolin-3-one oxime, respectively.[37] Other indole compounds are also reported to produce direct-acting mutagens after nitrite treatment under acidic conditions.[38,39] In general, conversion of indole derivatives to nitrosated forms *in vitro* is known to be rapid and efficient at physiologically feasible nitrite concentrations with the low pH of the human stomach.[37] Thus, it is conceivable that nitrosation of indole compounds such as indole-3-acetonitrile probably occurs in human stomach. On the other hand, nitric oxide is suggested to be produced by activated macrophages in inflamed organs with *H. pylori* infection.[18] Therefore, nitrosation of indole compounds could be mediated by both acid catalysis and inflammatory responses in the human stomach.[18,20,37–40] On the basis of the conversion rate of NIAN from indole-3-acetonitrile under physiological conditions, the dose of NIAN used in the present study appears about 500–1000 fold the expected human exposure to NIAN *via* fresh or pickled Chinese cabbage. However, humans continually consume various kinds of foods containing indole compounds and nitrate during ordinary life. Thus, it is probable that the total amount of nitroso-indole compounds would be much closer to the dose of NIAN used in the present study. Moreover, it has been reported that low doses of chemical carcinogens, such as MNNG and MNU, could induce glandular stomach cancers in rodents under inflammation conditions including NaCl treatment and *H. pylori* infection, but hardly induce glandular stomach cancer without NaCl treatment and *H. pylori* infection. Therefore, the continuous intake of indole compounds and nitrate may play an important role for gastric carcinogenesis in East Asian countries still with a high salt consumption and *H. pylori* infection rate.

Gastric cancer is tending to decline in most countries.[41–43] One of the explanations for this tendency is the reduced prevalence of *H. pylori* infection.[42] Changes in dietary habits, mainly being lower salt consumption, could be also related to reduced gastric cancer incidence. However, the gastric cancer prevalence in East Asian countries, such as Japan and Korea, is still high.[2] At present, we have not succeeded in detecting NIAN in human bodies nor the exposure levels of the precursor, indole compounds for humans. Thus, it is necessary to estimate the human exposure levels to nitroso-indole compounds including NIAN, and to study further animal experiments and epidemiological analyses for clarification of contribution of nitroso-indole compounds under *H. pylori* infection in humans gastric carcinogenesis.

In conclusion, the present study demonstrated that NIAN can induce gastric cancer in *H. pylori*-infected MGs. It is noteworthy that nitrosatable precursors widely exist in foods. Thus, it is suggested that *N*-nitroso indole compounds including NIAN might contribute to the frequent development of gastric cancer in East Asian countries such as Japan and Korea in which the prevalence of *H. pylori* infection is relatively high. Further studies of interaction with other dietary elements appear warranted to promote the prevention of human gastric cancer.

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Review Article

Prevention and Intervention Trials for Colorectal Cancer

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There have been a number of candidates for chemopreventive agents from synthetic drugs and natural compounds suggested to prevent colorectal cancer. However, they have shown modest efficacy in humans. The reason for this could be partly explained by the use of inappropriate models *in vitro* and *in vivo,* and the limitation of chemoprevention trials. In Japan, there are no cancer chemopreventive medicines, and few cancer chemoprevention trials to date. In contrast, an increase in the prevalence of colorectal cancer in Japan has forced us to develop more efficient chemopreventive strategies. It is now a good time to review in detail the current status and future prospects for chemoprevention of colorectal cancer with respect to the future development of chemopreventive medicines, particularly using synthetic drugs and natural compounds in Asian populations. The role and mode of action of available synthetic drugs, mainly aspirin and metformin, are reviewed. In addition, the possible impact of natural compounds with anti-inflammatory/immunosuppressive properties, such as ω3 polyunsaturated fatty acid and lactoferrin, are also reviewed.

Key words: chemoprevention – aspirin – metformin – ω6-PUFAs – lactoferrin

INTRODUCTION

The prevalence of colorectal cancer (CRC) is increasing in Asia, including Japan, believed to be caused by changing dietary habits and lifestyle, interacting with genetic characteristics. Many Asian countries have experienced a 2- to 4-fold increase in CRC incidence over the past few decades (1). Fortunately, a natural history of sporadic CRC, evolution from normal mucosa to developing overt cancer, spans on average 10–20 years, thereby allowing us an opportunity for effective prevention and intervention. CRC can be prevented by lifestyle modification, i.e. taking regular physical activity, abstaining from smoking and taking healthy nutrition. Moreover, there are population screening methods for the early detection of CRC and adenomatous polyps, precursor lesions for CRC, such as using fecal occult blood testing and endoscopy. However, the efficacy of such screening and surveillance strategies for patient uptake is often suboptimal, limiting real effectiveness. Thus, there is a clear imperative to consider alternative preventative strategies, such as using cancer chemopreventive agents.

In contrast to 'chemotherapy', the term 'chemoprevention' was first introduced by Sporn (2). Chemoprevention is now defined as the use of specific agents, including natural and chemical compounds, to suppress, delay or reverse carcinogenesis, and thereby to prevent the development of cancers (3). As the user of cancer chemopreventive agents is not a cancer patient, the ideal cancer chemopreventive agent needs to meet several criteria: (i) it should have a convenient dosing schedule; (ii) it should be easily administered; (iii) it should be low cost; and most importantly; (iv) it should have very low side effects.

Subjects adopted for cancer chemopreventive trials are general populations or those who are in cancer high-risk

groups. Generally speaking, it is considered that patients in cancer high-risk groups, that is well-defined and representative for common CRC, are suitable subjects. Two conditions fall under this consideration, i.e. familial adenomatous polyposis (FAP) and Lynch syndrome. FAP is a rare autosomal dominant inherited disorder due to *APC* gene mutation, characterized by the occurrence of many polyps in the colorectum and other parts of the intestine. It has been reported that half of the patient population develops adenocarcinoma from intestinal polyps by the age of 40 years (4). Lynch syndrome is also known as a hereditary non-polyposis colon cancer, carrying a breakdown in DNA mismatch repair gene. Of note, there are other polyposis syndromes, but they are very rare and lack clear relevance to the general population, and hence not suitable as trial subjects. For instance, these are cases of juvenile polyposis, in which the responsible gene is *SMAD4*, Peutz–Jeghers syndrome, in which it is *STK11*, and Cowden syndrome, in which it is *PTEN*.

Based on reports of chemopreventive activity in the literature and/or efficacy data from *in vitro* models, animal models and human trials, the most promising drugs are aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). The effectiveness of these may be attributed to their potent inhibition of cyclooxygenase (COX) enzymes. In this review, important aspects of the current status and future prospects for chemoprevention of CRC, particularly using synthetic drugs (aspirin and metformin) and natural compounds (ω3 polyunsaturated fatty acid and lactoferrin), involving data of Asian populations, are summarized. Meanwhile, recent clinical trials have been requested to be registered in public trial registries (www.clinicaltrials.gov, www.actr.org.au, www.ISRCTN.org, www.umin.ac.jp/ctr/index/htm or www.trialregister.nl) so that anyone can obtain updated trial records in the database after the publication of this review.

CHEMICAL COMPOUNDS

Aspirin

A large number of epidemiological and experimental studies have indicated that NSAIDs reduce the risk of CRC. As COX-2 expression and prostaglandin (PG) E_2 synthesis is elevated in CRC, PGE_2 is more likely to enhance colorectal carcinogenesis than other prostanoids. The COXs/PGH synthases have two enzymes, COX-1 and COX-2, which catalyze both oxidative and reductive reactions in the PG synthesis pathway. The constitutive enzyme COX-1 is detectable, but has low expression in normal human colorectal tissue, whereas for the inducible enzyme COX-2, its expression is elevated under conditions of inflammation and cancer.

Aspirin is a conventional NSAID, and irreversibly inhibits COX-1 and COX-2, through selective acetylation of a specific serine residue of Ser^{529} and Ser^{516}, respectively (5). Low-dose aspirin (70–100 mg/day) is widely used for cardiovascular disease prevention. Aspirin has a short half-life (~20 min) and it preferentially inhibits platelet COX-1 in the presystemic circulation when administered at low doses once a day. Moreover, aspirin is a medicine that has been in use for a long time and its adverse events are well-defined; it meets all the criteria of chemopreventive agents, and thus has become the most widely studied pharmacological agent for the prevention of CRC.

Observational Studies

Kune et al. (6) first reported in humans that there is an inverse association between use of aspirin and the risk of CRC, in a study conducted in Australia. They investigated the relationship between risk of CRC and several chronic illnesses in 715 CRC cases and 727 age/sex-matched controls, and it was found that those who had used aspirin-containing medications in the past were less likely to develop CRC (relative risk, RR = 0.53, 95% confidence interval, CI 0.40–0.71). Moreover, a prospective cohort study (7) was conducted in the USA with 47 900 middle-aged male health professionals, who responded to a mailed questionnaire in 1986. The questionnaires aimed to assess the use of aspirin and other variables, including the occurrence of cancer in 1986, 1988 and 1990; 251 new patients were diagnosed with CRC during the study period. Regular users of aspirin (≥2/week) in 1986 had a lower risk of total CRC (RR = 0.68, CI 0.52–0.92). A meta-analysis of the case–control studies, on available data on the association between aspirin use and CRC by 2007 that included 20 815 cases of CRC, revealed that there was significantly lower use of aspirin or NSAIDs in cases than in control studies (8). These results support the contention that regular use of aspirin decreases the risk of CRC.

Randomized Controlled Trials

Observational studies can powerfully identify causal associations, but much has been learned from intervention trials. Reports of the randomized controlled trials of aspirin, such as APACC (9), AFPPS (10), CALGB (11) and ukCAP (12), revealed that aspirin (75–325 mg/day for 3 years) reduces the risk of any recurrent colorectal adenoma by 17% and advanced adenoma by 28% (13) (Table 1). Moreover, meta-analysis of aspirin on the long-term risk of death due to CRC in randomized trials of aspirin vs control revealed that the use of aspirin for around 5 years reduces the incidence of and mortality due to CRC by 30–40% after 20 years of follow-up (14). In the case of trials in cancer high-risk patients, such as FAP (CAPP1 or J-FAPP study) and Lynch syndrome (CAPP2 study) patients, they also present evidence of the effectiveness of aspirin as shown below (Table 1).

CAPP1

The CAPP1 was a double-blind, randomized trial in FAP patients with four arms: aspirin for 600 mg/day plus matched