Lycopene Supplementation Prevents Smoke-Induced Changes in p53, p53 Phosphorylation, Cell Proliferation, and Apoptosis in the Gastric Mucosa of Ferrets

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ABSTRACT  Cigarette smoking increases the risk for gastric cancer. Higher intakes or blood levels of lycopene are associated with a decreased risk of gastric cancer. However, the biological mechanisms by which lycopene may protect against gastric carcinogenesis are poorly understood. We evaluated the effects of lycopene supplementation on smoke-induced changes in protein levels of p53, p53 target genes (p21\textsuperscript{Waf1/Cip1} and Bax-1), cell proliferation, and apoptosis in the gastric mucosa of ferrets. Ferrets were assigned to cigarette smoke exposure or to no exposure and to no, low-dose, or high-dose lycopene supplementation (2 × 3 factorial design) for 9 wk. Lycopene concentrations were significantly elevated in a dose-dependent manner in the gastric mucosa of ferrets supplemented with lycopene alone, but were markedly reduced in ferrets supplemented with lycopene and exposed to smoke. Although ferrets were given lycopene containing 95% all-trans isomers, cis isomers were the predominant forms in the gastric mucosa. Total p53 and phosphorylated p53 levels were greater in ferrets exposed to smoke alone than in all other groups. Levels were ~300 and 500% of the controls, respectively. However, smoke-elevated total p53 and phosphorylated p53 were markedly attenuated by both doses of lycopene. p21\textsuperscript{Waf1/Cip1}, Bax, and cleaved caspase 3 were substantially decreased, whereas cyclin D1 and proliferating cellular nuclear antigen (PCNA) were increased in ferrets exposed to smoke alone. Lycopene prevented smoke-induced changes in p21\textsuperscript{Waf1/Cip1}, Bax, cleaved caspase 3, cyclin D1, and PCNA in a dose-dependent fashion. These data indicate that lycopene may prevent smoke exposure–induced changes in p53, p53 phosphorylation, p53 target genes, cell proliferation, and apoptosis in the gastric mucosa of ferrets.

KEY WORDS:  • lycopene  • smoke  • cell proliferation  • apoptosis  • ferrets

Gastric cancer is the second leading cause of cancer deaths worldwide (1). Most studies have reported a positive association between cigarette smoking and the risk of gastric cancer (1). Metabolically activated carcinogens contained in tobacco smoke can directly affect the p53 tumor suppressor gene, which plays a pivotal role in the balance of cell proliferation and apoptosis, in the cellular responses to various stresses (2), and in suppressing gastric carcinogenesis (3). p53 is overexpressed in gastric cancer (4,5). Reactive oxygen intermediates generated from cigarette smoke interact with DNA and produce oxidative DNA damage with potentially mutagenic consequences (6). Upon DNA damage, p53 is activated, leading to the induction of p53 target genes, p21\textsuperscript{waf1/cip1} and Bax-1 (7,8). p21\textsuperscript{waf1/cip1}, a cyclin-dependent kinase (CDK)\textsuperscript{3} inhibitor, is a key component in the cell cycle arrest in G1. Bax is a pro-apoptotic member of the Bcl–2 family, p21\textsuperscript{waf1/cip1} and Bax-1 function as mediators to promote p53-dependent apoptosis (7–9). p53 phosphorylation, especially at serine 15, is an early cellular response to various genotoxic carcinogens and stresses that produce reactive oxygen species, and facilitates both accumulation and functional activation of p53 (10,11). Continued exposure to genotoxic carcinogens and reactive oxygen species can damage p53 and lead to its loss of function and mutations, resulting in the inactivation of the p53 stress-response pathway (7). Such changes would provide precancerous cells with a selective advantage for unregulated growth and the acquisition of genetic instability (7).

Higher consumption of tomato and tomato-based products (major sources of lycopene), and higher intakes or plasma levels of lycopene are associated with a reduced risk of gastric cancer in epidemiologic studies in various populations (12–14), suggesting a protective effect of lycopene against the development of gastric cancer. However, the potential biological mechanisms by which lycopene might inhibit gastric carcinogenesis are poorly understood. Previous experimental studies from the same research group reported that administration of lycopene...
LYCOPENE REDUCES EFFECTS OF SMOKE

INCREASES ANTIMUTATIONAL AND ANTIPROLIFERATIVE ACTIVITIES OF TUMOR SUPPRESSORS

MATERIALS AND METHODS

Animals, diet and study groups. Male adult ferrets (1.0–1.2 kg) from Marshall Farms consumed a semipurified ferret diet (Research Diets) that contained no lycopene, as determined by HPLC analysis in our laboratory, and water ad libitum. Before the experiment started, all ferrets were quarantined for at least 2 wk to evaluate their health. Male ferrets (n = 36) were randomly assigned to 6 groups (n = 6/group) for 9 wk as follows: 1) control; 2) smoke exposed (SM); 3) low-dose lycopene (LLyco; 1.1 mg/(kg·d)); 4) high-dose lycopene (HLyco; 4.3 mg/(kg·d)); 5) SM + LLyco; 6) SM + HLyco. During the 9-wk experimental period, ferret body weights were recorded weekly. All ferrets were terminally exsanguinated under deep isoflurane anesthesia after the 9-wk experimental period. Tissues and plasma were collected and stored at −80°C until analysis. All experimental procedures were approved by the Animal Care and Use Committee at the Human Nutrition Research Center on Aging and conducted under the supervision of the Animal Care and Use Committee.

Cigarette smoke exposure. The cigarette smoke-exposure procedure used in the present study was described elsewhere (18). Briefly, the ferrets were put in a chamber connected with a smoking device. Cigarette smoke was drawn out of the cigarettes (Type 1R4F, Tobacco and Health Research Institute, Cigarette Smoke Exposure Procedure) and passed into the chamber. During the first 2 wk of study, the number of cigarettes was increased progressively to a rate of 10 cigarettes/30 min twice in the morning and twice in the afternoon and then maintained for the rest of the 9-wk experimental period. In our previous study, we demonstrated that the concentration of urinary cotinine equivalents (≈0.1 mmol/L urine) of this amount of smoke-exposure to ferrets is similar to that in humans smoking 2 packages of cigarettes/d (18).

Lycopene supplementation. Because the total absorption of β-carotene by the ferret is ≈80% less than that by humans (22,23) and lycopene is similar in chemical structure to β-carotene, the calculation for lycopene dosage was based upon the assumption that ferrets absorb lycopene 80% less efficiently than humans. Lycopene 10% dry powder (Lycovit®, 10%, BASF) was dissolved in 0.5 mL corn oil and given orally to ferrets (not gavage) at either a low or high dose every morning for 9 wk. This lycopene product contained 95% all-trans isomers in the HPLC analysis. The ferrets that were not supplemented with lycopene were given corn oil only (0.5 mL/kg·d). Ferrets in the HLyco group were supplemented with 1.1 mg/kg·d, which corresponds to ≈15 mg of lycopene/d intake in a 70-kg person. This dose of lycopene is higher than the average intake of lycopene (9.4 ± 0.3 mg/d) in men and women in the United States (24). Ferrets in the HLyco group were supplemented with 4.3 mg/(kg·d), which corresponds to ≈60 mg of lycopene/d intake in a 70-kg person and is attainable in a diet enriched with tomato products or supplements. The dose of lycopene administered in a human Phase II randomized clinical trial of lycopene for the prevention of prostate cancer progression was 15 mg 2 times/d (25,26). This dose of lycopene was within the range of lycopene doses used in the present study.

RESULTS

Lycopene supplementation in the gastric mucosa of ferrets after 9-wk treatment. Lycopene supplementation at either the low or high dose for 9 wk significantly increased the concentrations of lycopene isomers in the gastric mucosa of the ferrets; the increases were dose dependent (Table 1). Because the semipurified ferret diet used in this study contained no lycopene, we did not detect lycopene in the gastric mucosa of the ferrets; the increases were dose dependent (Table 1). Because the semipurified ferret diet used in this study contained no lycopene, we did not detect lycopene in the gastric mucosa of the ferrets; the increases were dose dependent (Table 1).
Levels of total p53 and phosphorylated-p53 in the gastric mucosa of ferrets after 9 wk of treatment. Smoke exposure alone markedly increased both total p53 (~300% of controls; Fig. 1A) and phosphorylated p53 (~500% of controls; Fig. 1B) protein levels. Total p53 and phosphorylated p53 protein levels did not differ from the control group in ferrets supplemented with lycopene alone or when combined with smoke exposure.

Levels of Bax-1 and cleaved caspase 3 in the gastric mucosa of ferrets after 9-wk treatment. Compared with the control group, Bax-1 protein levels were downregulated 80% in the group exposed to smoke alone and 50% in the SM + LLyco group (Fig. 2A). Similarly, cleaved caspase 3 protein levels were 75% less than in controls in the SM alone group and 40% less in the SM + LLyco group (Fig. 2B). Bax-1 and cleaved caspase 3 protein levels in ferrets supplemented with LLyco alone or HLyco with or without exposure to smoke did not differ from those in the control group (Fig. 2A and B).

Levels of p21\textsuperscript{Waf1/Cip1}, cyclin D1, and PCNA in the gastric mucosa of ferrets after 9-wk treatment. Compared with the control group, p21\textsuperscript{Waf1/Cip1} protein levels were reduced 80% in the group exposed to smoke alone (Fig. 3A). In contrast, compared with the controls, protein levels of cyclin D1 were increased ~350% (Fig. 3B) and PCNA 400% (Fig. 3C) in the SM alone group. None of the protein levels differed among the other 5 groups.

DISCUSSION

Cigarette smoke exposure reduces circulating concentrations of lycopene in humans (28) and ferrets (18). In the present study, we also demonstrated that cigarette smoke exposure substantially lowered lycopene concentrations in the gastric mucosa of ferrets (Table 1). In addition, cis isomers of lycopene predominated in the gastric mucosa, although ferrets were given lycopene containing 95% all-trans isomers. This finding is consistent with previous studies, suggesting that isomerization of lycopene occurs in the gastrointestinal tract, particularly in the stomach (29), and cis isomers of lycopene are more bioavailable than all-trans lycopene (30).

Smoke carcinogens have an effect on the p53 tumor suppressor gene, a critical component in regulating the balance between cell proliferation and apoptosis and the responses to cellular stresses (2) as well as in suppressing gastric carcinogenesis (3). p53 overexpression was demonstrated in human gastric cancer (4,5). The elevated level of p53 was also shown in the gastric mucosa of rats exposed to cigarette smoke (31). Because malfunction of p53 may result in increased cell proliferation and decreased apoptosis and eventually tumor development and progression, elevated levels of p53 accumulation and phosphorylation may serve as markers for monitoring genotoxic effects and tumorigenesis.

However, no animal or human studies have examined the effects of lycopene on p53 and p53 phosphorylation in the gastric mucosa. In the present study, smoke exposure markedly decreased apoptosis and eventually tumor development and progression, elevated levels of p53 accumulation and phosphorylation may serve as markers for monitoring genotoxic effects and tumorigenesis.

![FIGURE 1](https://academic.oup.com/jn/article-abstract/136/1/106/4664095) Effects of lycopene supplementation and smoke exposure on the protein levels of total p53 (panel A) and phosphorylated-p53 (panel B) in the gastric mucosa of ferrets. Values are means ± SD, n = 6. Means without a common letter differ, P < 0.05. The upper portion of each panel is a representative Western blot. Groups are in the same order as in the histogram.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>13-Cis lycopene</th>
<th>9-Cis lycopene</th>
<th>Trans lycopene</th>
<th>5-Cis lycopene</th>
<th>Total lycopene</th>
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<tbody>
<tr>
<td>Control (sham exposure)</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SM</td>
<td>10.1 ± 2.1\textsuperscript{a}</td>
<td>3.1 ± 1.1\textsuperscript{a}</td>
<td>14.7 ± 3.9\textsuperscript{a}</td>
<td>132.7 ± 17.3\textsuperscript{a}</td>
<td>160.6 ± 19.7\textsuperscript{b}</td>
</tr>
<tr>
<td>LLyco</td>
<td>3.4 ± 1.0\textsuperscript{a}</td>
<td>1.2 ± 0.3\textsuperscript{a}</td>
<td>4.4 ± 1.5\textsuperscript{a}</td>
<td>39.6 ± 13.5\textsuperscript{a}</td>
<td>48.6 ± 15.1\textsuperscript{a}</td>
</tr>
<tr>
<td>SM + LLyco</td>
<td>93.2 ± 22.3\textsuperscript{d}</td>
<td>71.0 ± 7.1\textsuperscript{c}</td>
<td>53.5 ± 9.9\textsuperscript{b}</td>
<td>481.3 ± 89.3\textsuperscript{c}</td>
<td>699.0 ± 76.6\textsuperscript{d}</td>
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<tr>
<td>SM + HLyco</td>
<td>54.0 ± 10.5\textsuperscript{b}</td>
<td>20.4 ± 7.9\textsuperscript{b}</td>
<td>38.6 ± 11.6\textsuperscript{b}</td>
<td>347.1 ± 104.6\textsuperscript{b}</td>
<td>460.1 ± 107.9\textsuperscript{b}</td>
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</table>

\textsuperscript{1} Values are means ± SD, n = 6. Means in a column without a common letter differ, P < 0.05.

\textsuperscript{2} ND, not detected.
increased total p53 and phosphorylated p53 protein levels in the gastric mucosa of ferrets. More importantly, supplementation with either a low or a high dose of lycopene substantially reduced the excess increase in total p53 and phosphorylated p53 protein levels due to smoke exposure. These data provide evidence that lycopene supplementation prevents the changes in p53 and p53 phosphorylation induced by cigarette smoke exposure in the gastric mucosa of ferrets.

p53 controls cell cycle checkpoints in response to a variety of cellular stresses (9). The p21<sub>waf1/cip1</sub> CDK inhibitor is directly regulated by p53. Once activated, p21<sub>waf1/cip1</sub> protein binds to both the cyclin-CDK complex and PCNA, subsequently suppressing their activities and cell proliferation (9). Previous studies showed that in contrast to p53 expression, the expression of p21<sub>waf1/cip1</sub> is lower in gastric tumors than in the normal gastric mucosa (5). In addition, negative expression of p21<sub>waf1/cip1</sub> and overexpression of p53 are associated with aggressive behavior of gastric tumors and poor survival among patients with gastric cancer (5,32,33). There are few studies evaluating the relation of smoke exposure to p21<sub>waf1/cip1</sub> expression. In particular, there are no reports that evaluated smoke exposure in relation to p21<sub>waf1/cip1</sub> expression using gastric cell lines or gastric tissues. Components of smoke exposure were shown to stimulate gastric cell proliferation and promote gastric tumor growth in mice (34) and in human gastric cancer cells (35). Although lycopene supplementation inhibits proliferation of several types of cancer cells such as prostate, breast, endometrium, and lung (36,37), there are no available data for gastric cancer. In the present study, smoke exposure substantially decreased levels of
accompanied by increased levels of cyclin D1 and PCNA, cell proliferation indices, in the gastric mucosa of ferrets. Supplementation with either low or high doses of lycopene prevented the changes in p21\(^{Waf1/Cip1}\), cyclin D1, and PCNA caused by smoke exposure in a dose-dependent fashion. Findings from the present study provide the first evidence that the inhibitory effect of lycopene supplementation on smoke-induced cell proliferation is mediated in part by p21\(^{Waf1/Cip1}\), a p53 target gene.

Bax-1, a proapoptotic member of the Bcl-2 family of proteins that plays a key role in programmed cell death, is a downstream mediator of p53-dependent apoptosis (9), p53 upregulates the expression of Bax-1 gene through a p53 DNA-binding element within the promoter region of the Bax gene (9). The expression of Bax-1 was identified in normal tissues (38), adenomas (39), and tumor tissues (39,40) of the stomach. Studies showed that the expression level of Bax-1 (41,42) and caspase-3 (43,44), an apoptosis index, is higher in normal gastric tissues than in gastric carcinomas. In addition, most caspase-3 in gastric cancer cells is inactivated, suggesting that cancer cells may evade programmed cell death by inhibiting caspase 3 activation (43,45). An early study showed that short-term passive cigarette smoke exposure for up to 9 d upregulates apoptosis in the gastric mucosa of rats (31) and in human gastric epithelial cell lines (46). However, there is no report on how long-term cigarette smoke exposure influences apoptosis in the gastric mucosa. In the present investigation, exposing ferrets to smoke for 9 wk significantly decreased both cleaved caspase 3 and Bax-1 in the gastric mucosa, indicating that long-term smoke exposure downregulates p53-dependent apoptosis in the gastric mucosa. Lycopene or tomato increases apoptosis in several cancer cell lines including the prostate (47,48), leukemia (49), and colon (50). Limited data exist concerning the effect of lycopene on apoptosis in gastric cancer. Additionally, no data exist concerning the effect of lycopene on smoke-induced changes in apoptosis in the stomach. In the only previous study of gastric cancer using a chemical carcinogen [N-methyl-N'-nitro-N-nitroso-guadinine (MNNG)] in rats, lycopene supplementation inhibited MNNG-reduced expression of Bax and caspase 3 in the gastric tissues (17); it is unclear, however, whether these changes in apoptosis are related to p53 because the expression of p53 and other p53-associated molecules was not examined. In the present study, lycopene dose dependently prevented smoke-reduced protein levels of Bax-1 and cleaved caspase 3 in the gastric mucosa of ferrets, suggesting that lycopene can overcome the adverse changes in apoptosis caused by cigarette smoke exposure. We demonstrated that smoke-reduced apoptosis, reflected by decreased levels of Bax-1 and cleaved caspase 3, was associated with smoke-elevated cell proliferation, indicated by increased levels of cyclin D1 and PCNA and decreased p21\(^{Waf1/Cip1}\), as well as with levels of total p53 and phosphorylated p53 increased by smoke exposure. Furthermore, we showed that lycopene dose dependently reversed smoke-induced changes in p21\(^{Waf1/Cip1}\), Bax-1, cleaved caspase 3, cyclin D1, PCNA, and p53. These data suggest that lycopene protects against the development of gastric cancer associated with cigarette smoke exposure at least in part through its effect on p53-dependent apoptosis and cell proliferation.

Helicobacter pylori infection was shown to upregulate the expression of p53 in human gastric mucosa and cell lines (51,52). Previously, we observed that p53 protein was detected in the gastric mucosa of ferrets infected with Helicobacter mustelae (6 of 6 ferrets) but not in that of the specific pathogen-free ferrets (0 of 5 ferrets) (Wang XD and Fox JG, unpublished data). In the present study, we also detected total p53 and phosphorylated p53 in control ferrets and observed that total p53 and phosphorylated p53 levels were slightly lower in the groups given lycopene supplementation alone compared with the controls, suggesting that lycopene might also alleviate Helicobacter mustelae–related p53 alterations.

In conclusion, this study demonstrates that lycopene supplementation at either low or high dose may prevent smoke exposure–related changes in p53, p53 phosphorylation, p53 target genes (p21\(^{Waf1/Cip1}\) and Bax-1), cell proliferation, and apoptosis in the gastric mucosa of ferrets. Our study provides evidence that lycopene may protect against the development of gastric cancer by modulating p53-dependent cell cycle control and apoptosis.

**LITERATURE CITED**


