Promises and Perils of Lycopene/Tomato Supplementation and Cancer Prevention

Can Smoke-Exposed Ferrets Be Utilized to Unravel the Mechanisms of Action of Lycopene?[1]

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EXPANDED ABSTRACT

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Beneficial effects of carotenoid-rich fruits and vegetables on lung cancer risk have been found in many epidemiological studies. The failure of the β-carotene intervention trials to show a benefit against lung carcinogenesis in smokers indicates that other carotenoids or phytonutrients may account for the protective effects of fruits and vegetables on lung cancer risk that has been reported from observational studies. Epidemiological studies provide supportive evidence that lycopene may have a chemopreventive effect against a broad range of epithelial cancers, including lung cancer (1–4). Although the exact mechanism by which lycopene functions has not been well defined, lycopene may protect against carcinogenesis by: *1*) functioning as a natural antioxidant, *2*) enhancing cellular gap junction communications, *3*) inducing phase II enzymes involved in activation of the antioxidant response element transcription system, *4*) suppressing insulin-like growth factor-1 (IGF-1)[3]-stimulated cell proliferation by inducing IGF binding protein, and 5) inhibiting neoplastic transformation of normal cells. It has also been proposed that certain actions of lycopene may be mediated by its oxidative metabolites (**Fig. 1**). However, a better understanding of lycopene metabolism and the mechanistic basis by which lycopene promotes chemoprevention must be elucidated under well-controlled experimental conditions. Particularly, the molecular properties of lycopene and its metabolites need more investigation, and knowledge of their metabolic pathway, dose effects, tissue specificity, and possible adverse effects with tobacco smoking and alcohol consumption must be addressed. This information is critically needed for future human studies involving lycopene for the prevention of cancer in the lung and at other tissue sites.

Unfortunately, few animal models are suitable for addressing the effectiveness of dietary lycopene as a chemopreventive agent against smoke-induced lung cancer, and this has limited progress toward understanding the mechanistic actions of dietary lycopene. One reason for this is that humans can absorb significant amounts of intact carotenoids (e.g., β-carotene and lycopene) and accumulate high concentrations of carotenoids in the peripheral tissues, whereas most animal species, except at high doses, absorb virtually no carotenoids intact. For example, Kim et al. (5) provided evidence that lycopene decreased the incidence of lung tumors in B6C3F1 mice. However, Guttenplan et al. (6) observed enhancement of benzo[*a*]pyrene-induced-mutagenesis by lycopene in the colon and lung of *LacZ* mice. It is difficult to interpret the effect of lycopene and understand the mechanism(s) of lycopene using these animal models, because either the lycopene levels in plasma and tissue were not described or the doses of carotenoids were very high.

Ferrets (*Mustela putorious furo*) offer an excellent model for mimicking the conditions of carotenoid intervention studies in humans because ferrets and humans are similar in terms of lycopene absorption, tissue distribution and concentrations, and metabolism. We conducted a study to evaluate the effects of lycopene supplementation at both a low dose and a high dose on blood and lung tissue lycopene levels in ferrets with or without 9 wk of cigarette smoke exposure (7). Ferrets in the low-dose lycopene group were supplemented with 1.1 mg/(kg · d) of lycopene, which is equivalent to an intake of 15 mg/d in humans. This dose of lycopene is slightly higher than the mean intake of lycopene (9.4 ± 0.3 mg/d) in U.S. men and women (8). Ferrets in the high-dose lycopene group were supplemented with 4.3 mg/(kg · d) of lycopene, which is equivalent to 60 mg/d in humans and is achievable in a diet enriched with tomato products or supplements. The results

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[3] Abbreviations used: IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor; IGFBP-3, insulin-like growth factor binding protein-3; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3′-kinase. PKB, protein kinase B.

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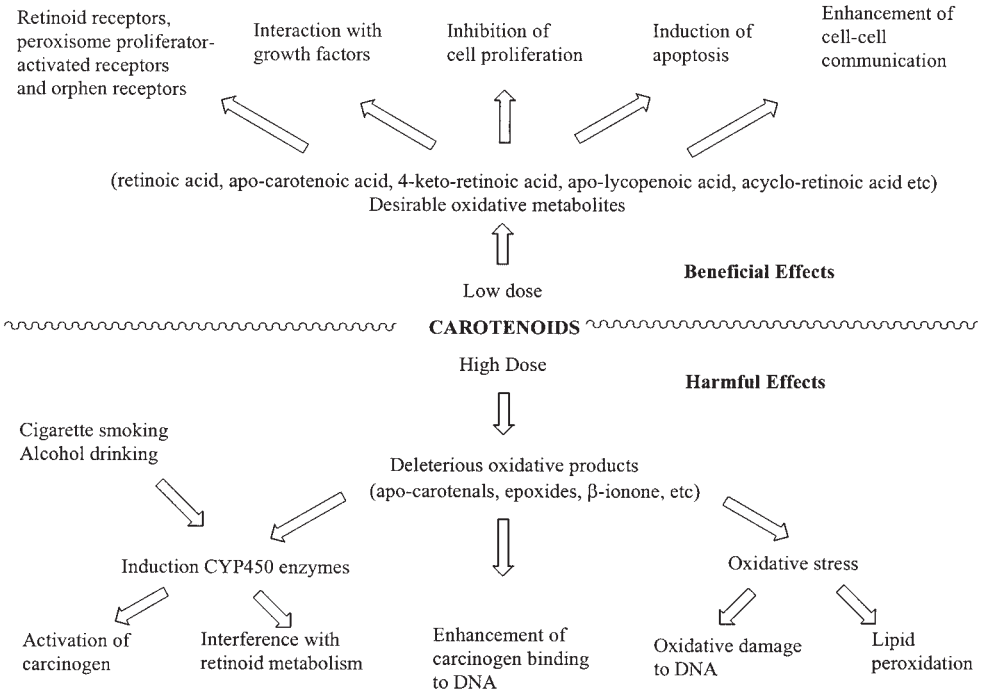

FIGURE 1 Simplified schematic illustration of the possible mechanism(s) of carotenoids and their oxidative metabolites on their beneficial and detrimental effects to human health (16).

show that lycopene supplementation at both a low dose and a high dose for 9 wk substantially increased the concentrations of lycopene in both plasma and lung tissue. The concentration of plasma lycopene (range, 226–373 nmol/L) in ferrets after lycopene supplementation was similar to the lycopene concentration (range, 290–350 nmol/L) reported in humans (9,10). Furthermore, the lycopene concentrations in the lungs of ferrets that were given a low dose of lycopene (equivalent to 15 mg/d in humans) reached 342 nmol/kg, which is within the range of lung lycopene concentration in normal humans (100 to 500 nmol/kg) (11). Lycopene concentrations in ferrets supplemented with a high dose of lycopene increased 3.4-fold in lung tissue and 1.6-fold in plasma compared with that in ferrets supplemented with a low dose of lycopene. The observation that a higher increase in lycopene concentrations occurred in lung tissue than in plasma after lycopene supplementation should be studied further in terms of possible harmful effects of lycopene supplementation. In addition, smoke exposure decreased plasma and lung lycopene concentrations in both the low-dose and the high-dose lycopene groups; the percentages of decrease in lycopene concentrations were ~40% in plasma for both the low-dose and the high-dose lycopene groups and 90% in lung tissue for both the low-dose and the high-dose lycopene groups. This is consistent with data from the National Health and Nutrition Examination Survey III, in which smokers had lower serum concentrations of lycopene compared with nonsmokers (12). These data clearly indicate a similarity between humans and ferrets with respect to lycopene absorption and accumulation and demonstrate that cigarette smoke exposure decreases lycopene levels in plasma and lung tissue. Notably, in a study of ferrets supplemented with 30 mg β-carotene/d, the concentration of β-carotene in lung tissue reached 26 μmol/kg, and this was associated with enhanced development of lung squamous metaplasia induced by cigarette smoke exposure (13). In ferrets supplemented with 60 mg lycopene/d, the concentration of lycopene in lung tissue was only 1.2 μmol/kg, which caused no harmful effects but rather prevented the development of lung squamous metaplasia and cell proliferation induced by smoke exposure (7). The different outcomes between the lycopene and β-carotene studies in ferrets may be due to the differences in the levels of carotenoids that accumulated in lung tissue.

To explain why cigarette smoke decreased lycopene levels in the lung, we analyzed metabolites of lycopene in lung tissue using HPLC and spectral analysis. Of the several metabolites of lycopene that were detected in the lung tissue of ferrets supplemented with lycopene, 1 was identified as apo-10′-lycopenol (14). We cloned a full-length carotene-9′,10′-monooxygenase that catalyzes the excentric cleavage of lycopene at the 9′,10′ double bond forming apo-10′-lycopenoids in ferrets (Hu et al., unpublished observations). The results show that the carotene-9′,10′-monooxygenase in ferrets encodes a protein of 540 amino acids and has 82.2% identity with human carotene-9′,10′-monooxygenase and 79.3% identity with mouse carotene-9′,10′-monooxygenase. Most recently, we showed that the apo-10′-lycopenoids mediate the chemopreventive activity of lycopene by inhibiting cell proliferation and modulating the activation of extracellular signal-regulated protein kinase in A549 lung cancer cells (15).

We demonstrated that the potential mechanisms for the harmful effects of high-dose β-carotene supplementation in smokers observed in human clinical trials may be associated with the production of undesirable oxidative metabolites of β-carotene in lung tissue (16). The formation of oxidative by-products from β-carotene can induce cytochrome P450 enzymes (e.g., CYP1A1 and 2A1) and interfere with retinoic acid metabolism (17) as well as downregulate retinoic acid receptor β (18). However, the excentric cleavage product from low-dose β-carotene could be converted into retinoic acid and provide protection (18–21). The biological activity of lycopene metabolites under high-dose and low-dose lycopene supplementation must be investigated. It is unclear whether there is an interaction between smoke and lycopene metabolism, as exists for β-carotene, and whether the function of lycopene is organ specific. That is, lycopene may protect against prostate cancer, but might also interact with tobacco smoke to produce undesirable degradative products of lycopene that could have unexpected adverse effects in other tissues (e.g., lung). Lyco-

pene and β-carotene protect against oxidative DNA damage (induced by xanthine/xanthine oxidase) in HT29 cells at relatively low concentrations (1–3 μmol/L), but lose this capacity at higher concentrations (4–10 μmol/L) (22). Lycopene and β-carotene at a high concentration (20 μmol/L) substantially enhanced levels of lipid peroxidation induced by a lipid-soluble radical generator [2,2′-azobis (2,4-dimethylvaleronitrile)] (23). These studies highlight the dose-response relations (both physiological and pharmacologic) of lycopene against lung tumorigenesis, and the interaction of cigarette smoke and lycopene metabolism should be examined carefully. Further characterization of metabolic pathways of lycopene, regulation of lycopene cleavage enzymes, and biological functions of the oxidative metabolites of lycopene would, most likely, elucidate the mechanisms of the beneficial (or harmful) effects of lycopene on cancer prevention.

The ferret model can be used for investigating the mechanism(s) of lycopene action. The IGFs are mitogens that play a pivotal role in regulating cell proliferation, differentiation, and apoptosis (24). Disruptions of normal IGF-1 system components lead to hyperproliferation and survival signals and have been implicated in the development of various tumor types. Several lines of evidence implicate IGF-1 and its receptor, IGF-1R, in lung cancer and other malignancies (24,25). The downstream pathway of the IGF-1R signaling involves the activation of both phosphatidylinositol 3′-kinase (PI3K)/Akt/protein kinase B (PKB) and Ras/Raf/mitogen-activated protein kinase (MAPK) pathways (**Fig. 2**). Epidemiological evidence indicates that increased levels of IGF-1, reduced levels of IGFBP-3, or an increased ratio of IGF-1 to IGFBP-3 in circulation are associated with an increased risk for the development of several common cancers, including that of the breast, prostate, colon, and lung (25). IGF-1–stimulated cell growth was reduced by physiological concentrations of lycopene in endometrial, mammary (MCF-7), and lung (NCI-H226) cancer cells (26,27). Lycopene treatment was also associated with an increase in membrane-associated IGFBPs (27). Two studies in humans have also shown that higher intakes of cooked tomatoes or lycopene are associated with lower circulating levels of IGF-1 and higher levels of IGFBP-3 (28,29). In a recent animal study, lycopene supplementation reduced local prostatic IGF-1 expression in the Dunning prostate cancer model (30). We hypothesize that cigarette smoke exposure may promote cell proliferation and neoplasia by affecting normal IGF-1 signaling (31). Given that both IGF-1 and IGFBP-3 are produced mainly in the liver and released as circulating proteins in plasma, and a higher ratio of IGF-1 to IGFBP-3 in the circulation is associated with an increased risk for the development of lung cancer and other cancers (breast, prostate, and colon), we carried out an in vivo study using the ferret model to systemically investigate the effect of lycopene on IGF-1/IGFBP-3 signaling (7). Plasma IGF-1 concentrations did not differ in ferrets exposed to smoke alone and those exposed to smoke with or without lycopene supplementation. In contrast, compared with controls, ferrets receiving smoke exposure but no lycopene had substantially lower plasma IGFBP-3 concentrations. However, ferrets exposed to smoke and supplemented with lycopene had similar plasma IGFBP-3 concentrations to those supplemented with lycopene alone. The expression of IGFBP-3 was upregulated greatly in the livers of ferrets supplemented with lycopene, whereas the expression of IGF-1 remained unchanged. Furthermore, the changes of IGFBP-3 by lycopene supplementation in plasma of the ferrets substantially affected apoptosis and cell proliferation in the lungs. Smoke exposure alone substantially decreased cleaved caspase 3 protein expression by 74% and increased PCNA by 4-fold, relative to controls, in the lungs of ferrets. In contrast, lycopene supplementation at either a low dose or a high dose

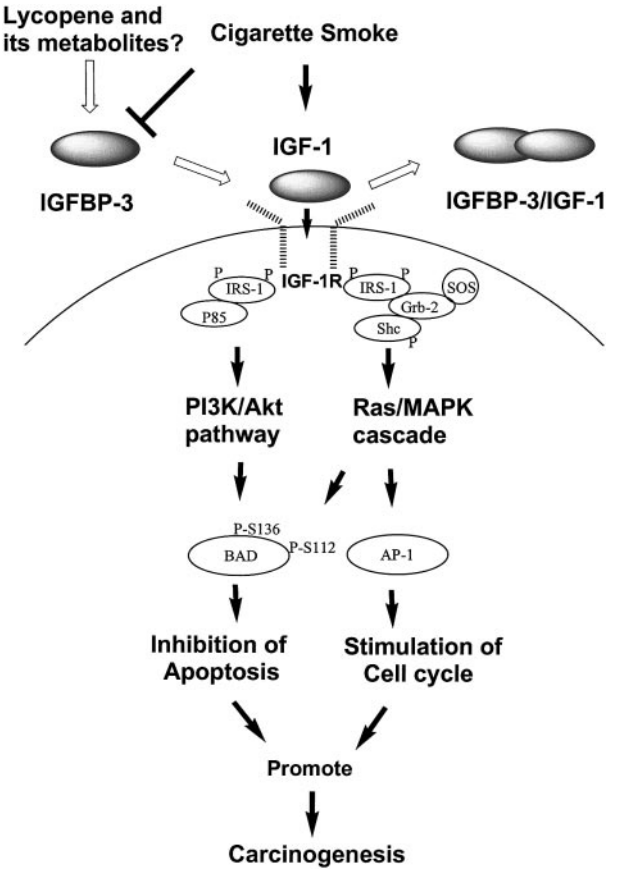

FIGURE 2 IGF binding protein-3 (IGFBP-3) regulates bioactivity of IGF-1 by sequestering IGF-1 from its receptor in the extracellular milieu, thereby inhibiting the mitogenic and anti-apoptotic action of IGF-1 and reducing cancer risk. One hypothesis is that lycopene or lycopene metabolites exert their protective effects against smoke-induced lung carcinogenesis through upregulating IGFBP-3, interrupting the signal transduction pathway of IGF-1, downregulating phosphorylation of BAD, promoting apoptosis, and inhibiting cell proliferation, thereby preventing lung carcinogenesis (31).

reversed the reduction in plasma IGFBP-3 and lung cellular apoptosis as well as hyperproliferation induced by smoke exposure. We then examined BAD (Fig. 2), which has the potential to be a chemopreventive or therapeutic target because it has a central position between growth factor signaling pathways and apoptosis (32) in lung tissue of ferrets after 9 wk of treatment. Lycopene supplementation at either a low dose or a high dose prevented the smoke-induced BAD phosphorylation at both Ser 112 and Ser 136. Our observation is in agreement with a previous study, which reported that IGFBP-3 can inhibit both PI3K/Akt/PKB and MAPK signaling pathways in nonsmall cell lung cancer (33) because the PI3K appears to mediate survival factor–induced phosphorylation of BAD Ser 136, whereas MAPKs are thought to mediate survival factor–induced phosphorylation of BAD Ser 112. These results demonstrate the importance of IGFBP-3 in the regulation of smoke-induced lung lesions, proliferation, and apoptosis, suggesting that IGFBP-3 is a molecular target of lycopene for the prevention of lung cancer. The mechanism by which lycopene increases the level of IGFBP-3 remains to be elucidated.

The ferret model may be superior to other animals (e.g., mice and rats) because the ferret lung architecture is similar to that of humans and has been used as a model for studies in inhalation toxicology, smoke-induced oxidative DNA damage, induction of phase I enzymes, and activation of and the function of tumor

suppressors (such as retinoic acid receptor β and p53). Recently, induction of various lung precancerous lesions (e.g., squamous dysplasia and atypical adenomatous hyperplasia) and tumor production (both squamous cell carcinoma and adenocarcinoma) in the lung by the combination of smoke-exposure with the tobacco-specific chemical carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, has been established in the ferret model (34). The development of both preneoplastic lesions and gross lung tumors in ferrets provides a unique model for studying lung cancer chemoprevention with dietary agents such as lycopene and for studying the molecular mechanisms of lycopene against the earlier stages of smoke-related lung cancer (35).

CONCLUSIONS

In summary, a better understanding of the biological functions of lycopene and its metabolites in cancer prevention is needed to uncover the underlying mechanisms of lycopene in human health. Comprehensive research on the effects of metabolites of lycopene is crucial in directing further research into identifying the active form(s) of this compound and the mechanism by which certain cancers are prevented by lycopene. The beneficial vs. detrimental effects of lycopene may be related to the lycopene dose administered in vivo, the accumulation of lycopene in a specific organ, the interaction of lycopene with tobacco and alcohol, the lycopene metabolites, and their effects on cell signaling pathways and molecular targets. These questions must be addressed using an appropriate animal model, which mimics humans closely in terms of lycopene absorption, metabolism, biological action, and carcinogenesis. The ferret provides an excellent and unique model for studying lung cancer chemoprevention with lycopene as well as any mechanistic studies attempting to understand molecular changes relevant to lycopene metabolism and lung cancer in humans.

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