Review

Chemopreventive Drug Development: Perspectives and Progress

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Abstract

Chemoprevention drug development has the goal of identifying safe and effective chemopreventive agents for clinical use. Several distinctive strategies are pursued in developing chemopreventive agents: (*a*) identifying and validating predysplastic and early dysplastic lesions that can be used instead of cancers as endpoints for measuring chemopreventive activity; (*b*) identifying and testing candidate agents based on considerations of mechanisms of action; (*c*) evaluating combinations of agents with potential for maximizing efficacy and minimizing toxicity; and (*d*) applying a systematic methodology for identifying and ranking candidate agents at each stage of development to ensure discovery of the best agents and most effective use of available resources.

This article discusses 22 drugs and three drug combinations which have reached an advanced stage of development as chemopreventive agents. The first generation of drugs are the most advanced, now being in Phase II and Phase III clinical trials. These drugs include several retinoids [vitamin A, 13-*cis*-retinoic acid, all-*trans*-*N*-(4-hydroxyphenyl)retinamide], calcium, β-carotene, tamoxifen, and finasteride. The second generation drugs are those in Phase I clinical trials. From most to least advanced, these drugs are 2-difluoromethylornithine, sulindac, piroxicam, oltipraz, *N*-acetyl-*l*-cysteine, aspirin, ibuprofen, carbenoxolone, 18β-glycyrrhetinic acid, and the combination of 2-difluoromethylornithine with piroxicam. The third generation includes agents with significant evidence of chemopreventive activity in animal models. These agents are now in preclinical toxicity testing. They are *S*-allyl-*l*-cysteine, phenhexyl isothiocyanate, curcumin, ellagic acid, fumaric acid, fluasterone, and the combinations of all-*trans*-*N*-(4-hydroxyphenyl)retinamide with oltipraz and all-*trans*-*N*-(4-hydroxyphenyl) retinamide with tamoxifen.

Introduction

As the understanding of the process of cancer increases, preventive intervention is becoming scientifically practical for many cancers. While prevention of exposure and changes in diet may someday alter incidence, chemical intervention offers an attractive approach with potential for more immediate results. The NCI's[2] chemoprevention drug development program, which has been described previously (1–3), has as a goal the identification of safe and effective chemical agents for the prevention of human cancers. This program is an applied drug development science effort with clinical trials as the endpoint. It begins with the identification of candidate agents for development and the characterization of these candidates for efficacy using *in vitro* and animal screens. Promising agents are then further tested in animal models to evaluate the design of regimens for clinical testing and use. Agents judged to have potential as human chemopreventives are subjected, as appropriate, to preclinical toxicity and pharmacokinetic studies. The most successful agents then progress to clinical trials. The purpose of this article is to discuss the strategies, perspectives, and progress of chemoprevention drug development, often using examples from the NCI program. As evidence of progress, the status and rationale for development of agents that currently appear to be most promising (Table 1) are reviewed.

Strategies for Chemopreventive Drug Development

Epithelial Lesions that Are Targets for Chemopreventive Agents. The rational design and development of chemopreventive agents requires a clear understanding of the epithelial lesions that are targets for the action of these agents. Fig. 1 diagrams the early development of epithelial neoplasia. A major target epithelial lesion is that of histologically visible neoplasia very early in its development, long before it invades across the basement membrane. This "intraepithelial neoplasia," as it is called at this stage, begins as a monoclonal focus near the basement membrane and expands upward and laterally. When it finally becomes invasive across the basement membrane, it is at this point termed "cancer." Prior to invasion, the morphological changes of intraepithelial neoplasia are collectively termed "dysplasia." It is the consensus of pathologists that carcinoma *in situ* and severe dysplasia form an indistinguishable continuum (4). The other major target lesion for chemopreventive agents may be termed "predysplasia," which is the stage of neoplastic development after initiating DNA mutational changes have occurred but before the onset of dysplasia, when the tissues still

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[2] The abbreviations used are: NCI, National Cancer Institute; AFB_1, aflatoxin B_1; B(*a*)P, benzo(*a*)-pyrene; DFMO, 2-difluoromethylornithine; DHEA, dehydroepiandrosterone; DMBA, 7,12-dimethylbenz(*a*)anthracene; DMH, 1,2-dimethylhydrazine; G6PDH, glucose-6-phosphate dehydrogenase; GSH, glutathione; 4-HPR, all-*trans*-*N*-(4-hydroxyphenyl)retinamide; MNU, *N*-methyl-*N*′-nitrosourea; MTD, maximum tolerated dose; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NOEL, no observed effect level; NSAID, nonsteroidal antiinflammatory drug; ODC, ornithine decarboxylase; PAH, polycyclic aromatic hydrocarbon; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Table 1 Cancer chemopreventive agents under development

	Preclinical toxicology	Clinical trials		
		Phase I	Phase II	Phase III
First generation				
Retinoids				
Vitamin A[a]	+[b]	+	+ (3)[c]	+ (2)
13-*cis*-retinoic acid[a]	+		+ (2)	+ (2)
4-HPR[a]	+	+	+	+
Calcium	+	+ (3)	+ (2)	
β-Carotene		+	+ (6)	+
Tamoxifen[a]	+	+	+	
Finasteride[a]	+			+
Second generation[d]				
DFMO	+	+	+ (2)	
Sulindac[a]	+		+ (2)	
Piroxicam	+	+		
Oltipraz	+	+		
N-acetyl-*l*-cysteine	+	+		
Aspirin	+	+		
Ibuprofen	+	+		
Carbenoxolone	+	+		
18β-Glycyrrhetinic Acid	+	+		
DFMO + Piroxicam	+	+		
Third generation[d]				
S-Allyl-*l*-cysteine	+	+		
Phenhexyl Isothiocyanate	+			
Curcumin	+			
Ellagic acid	+			
Fumaric acid	+			
Fluasterone	+			
4-HPR + Oltipraz	+			
4-HPR + Tamoxifen	+			

[a] Previous development by the pharmaceutical industry.
[b] +, Testing is completed or in progress.
[c] For Phase II and III studies, numbers in parentheses, number of trials.
[d] Second- and third-generation agents are listed in order from most to least advanced in development.

appear morphologically normal. The natural history of intraepithelial neoplasia in the major human epithelia, with implications for chemopreventive strategy, has been recently reviewed (5). The field of chemoprevention rests on the fundamental concept that the progression of neoplasia will be much easier to slow or eliminate with chemopreventive agents at the predysplastic and dysplastic stages of neoplastic development than with chemotherapeutic agents at the postinvasive, cancer stage of neoplastic development. The scientific basis for this concept is the progression of cells from a normal homogeneous state to more and more heterogeneity as carcinogenesis evolves. Therefore, more cells can be affected by treatment in early stages of carcinogenesis when they are more homogeneous than in later heterogeneous stages. An important element of chemopreventive drug development is the identification and validation of markers for predysplasia and dysplasia that can serve as endpoints for chemopreventive activity.

General Mechanisms of Chemopreventive Activity. Another important aspect in the development of chemopreventive agents is the mechanism(s) by which they inhibit cancers. As is evident in the discussions of individual agents below, the knowledge of mechanisms of chemoprevention is far from complete, and the multiple possible chemoprevention-associated activities that any agent may have (*e.g.*, the retinoids) confounds the discovery of the most important mechanisms. Nevertheless, the known pharmacological properties of the agents being evaluated and the experimental testing data on various classes of agents provide very useful insights into mechanism that may lead to the development of more effective chemopreventive drugs. In some cases, the pharmacological activity of a compound suggests very specifically the target tissues and cancers against which a chemopreventive drug may be active. For example, an antiestrogen such as tamoxifen would be developed for use against estrogen-sensitive cancers such as those of the breast. In other cases, the testing of classes of compounds known to have a very general chemopreventive activity may lead to the discovery of tissue specificities, such as those of antiinflammatories in colon and bladder.

Table 2 presents a working classification of the chemopreventive agents listed in Table 1 arranged according to structure or pharmacological effects associated with chemopreventive activity. Note that many of the agents fall into more than one class. Moreover, the list of classes is undoubtedly incomplete.

In Table 2, the specific chemopreventive activities and structures are grouped into three general classes. The first is inhibitors of cellular proliferation (antiproliferatives), manifested in such specific mechanisms as ODC inhibition, protein kinase C inhibition, and antiestrogenic activity. Antiproliferatives include retinoids, polyphenols, antihormones, calcium, DFMO, and the DHEA analogue, fluasterone. A second general class is carcinogen blocking agents. Blocking is often produced by enhancing the carcinogen-detoxifying enzymes, especially the Phase II metabolic enzymes, including GSH *S*-transferases, which perform conjugation and other reactions. Wattenberg and Talalay (6–8) have emphasized the desirability of selecting chemopreventive agents which induce mostly Phase II metabolic enzymes as opposed to compounds which induce both Phase I mixed function oxidases and Phase II enzymes. Induction of mixed function oxidases carries the potential of activating procarcinogens.

A third general class of inhibitors is antioxidants, such as *S*-allyl-*l*-cysteine, curcumin, *N*-acetyl-L-cysteine, NSAIDs, and polyphenols. These agents trap electrophilic sites on activated carcinogens, scavenge oxygen-free radicals and organic free radicals, and terminate lipid peroxidation. These activities may be either a direct or indirect effect of the antioxidant agent. Examples of agents with indirect antioxidant effects are those that enhance the Phase II metabolizing enzymes, thereby elevating electrophile trapping potential via increased GSH production and induction of the enzyme GSH peroxidase. The antioxidant mechanism is reputed to be both antimutagenic (9) and antiproliferative (10).

Combinations of Agents. At least two factors limit the potential usefulness of chemopreventives in the clinic. One is that cancers are not reduced to zero by administration of these agents. The second is toxicity. Several very promising agents are toxic at efficacious doses. The simultaneous or sequential administration of multiple inhibitors can increase the efficacy of chemopreventive agents and reduce toxicity. Such an approach uses differences in the mechanisms of cancer inhibition among the agents to increase the inhibitory activity. Further, the increased efficacy achieves desirable levels of cancer inhibition at lower and presumably less toxic doses of the individual agents.

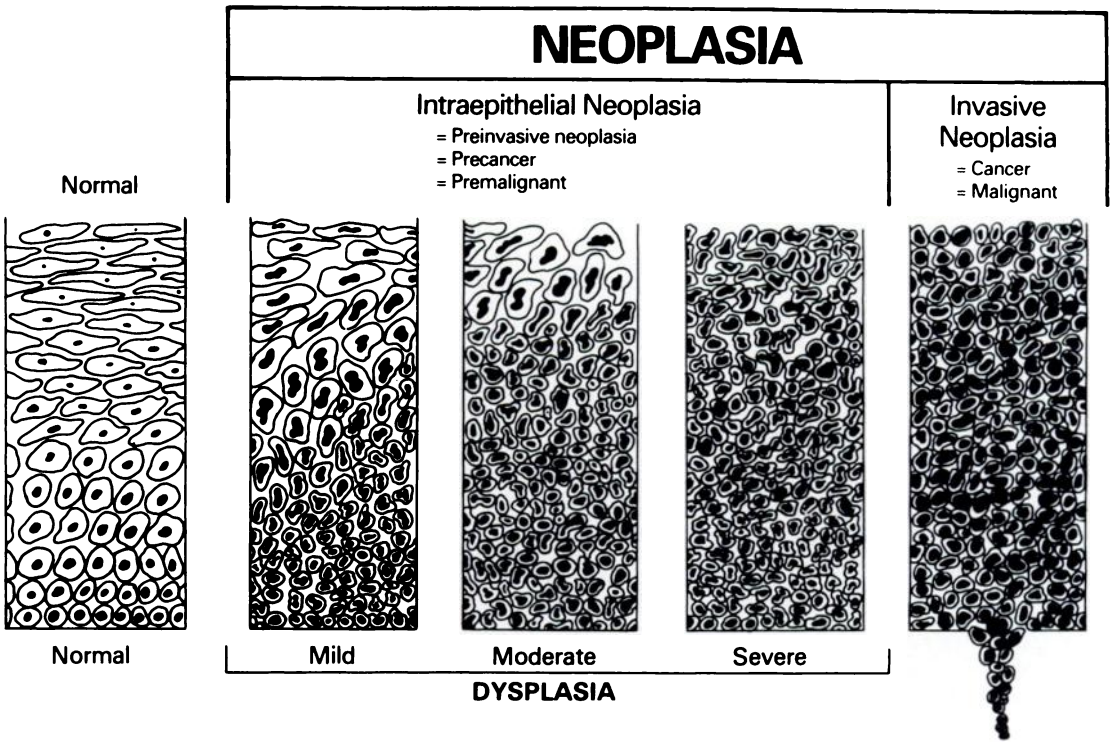

Fig. 1. Development of Epithelial Neoplasia. In estimating the severity of intraepithelial neoplasia (dysplasia), the extent of the lesion as well as the deviation from normal cellular morphology is used. Adapted from Boone *et al.* (5), with permission.

Positive effects have been demonstrated in animal models using combinations of two chemopreventive agents. Several of the combinations have shown synergism; *i.e.*, the inhibitory potency of the combinations of agents was greater than the sum of the potencies of the single agents. Synergistic chemopreventive activity has been reported for DFMO and piroxicam in rat colon (11, 12) and for 4-HPR and tamoxifen in rat mammary (13, 14). In other studies (data not shown), synergistic activity has been observed in hamster lung for β-carotene with 4-HPR and with vitamin A and for 4-HPR with oltipraz. Likewise, combinations of DFMO with 4-HPR and with oltipraz and 4-HPR with oltipraz have synergistic activity in the bladder. Three of these combinations—DFMO and piroxicam, 4-HPR and oltipraz, and 4-HPR and tamoxifen—are currently undergoing toxicology testing in the NCI chemopreventive drug development program. These three combinations are proceeding because of the promise they and their single agent components have against cancers in colon and bladder, lung and bladder, and breast, respectively. The other combinations, which include either β-carotene or vitamin A, are scheduled for further testing by the NCI in the coming year.

Prioritization of Newer Chemopreventive Agents. Because of the many compounds under consideration for development, a systematic methodology for identifying and ranking candidate chemopreventive agents is essential to find the best agents and to make the best use of available resources. In the NCI program, this activity ranges from selecting candidates for initial testing in efficacy screens, as well as those most appropriate for further efficacy evaluation, to identifying those agents for preclinical toxicity and Phase I clinical trials. At each stage, criteria are applied to set priorities for the agents. In most cases, these priorities are based on four factors: efficacy; toxicity; commercial availability; and possible mechanisms of action.

Efficacy is judged by previous animal studies, *in vitro* results (*e.g.*, inhibition of cell transformation and antimutagenesis), epidemiology studies, and anecdotal reports in humans. Toxicity is determined by formal toxicology studies in animals, case reports in humans, and, when available, previous clinical experience. The commercial availability of large amounts of agents at reasonable cost is an important factor. Cost is generally related to ease of synthesis or isolation, the amount of agent manufactured for other commercial uses, and the interest of the manufacturer in promoting the development of the agent as a cancer chemopreventive. Possible mechanisms of action may be used to lower the ranking of a candidate when other agents with the same mechanism of action and range of chemopreventive activity are already under development, or to promote a candidate agent that is desirable because it has multiple mechanisms by which it may produce its chemopreventive effect.

Other factors that may be considered, particularly for agents ready for toxicology and Phase I clinical trials, are pharmacokinetics, the availability of appropriate dosage formulations, and regulatory status for clinical use (*e.g.*, a candidate agent that already was approved by the U.S. Food and Drug Administration for human use would likely be ranked higher than one without such approval).

Table 2 Working pharmacological and chemical structural classification of promising chemopreventive agents

- Antiproliferatives
  - Retinoids/carotenoids
    - β-carotene, 4-HPR, 13-*cis*-retinoic acid, vitamin A
  - Antihormones
    - Finasteride, tamoxifen
  - Antiinflammatories
    - Aspirin, carbenoxolone, curcumin, 18β-glycyrrhetinic acid, ibuprofen, piroxicam, sulindac
  - G6PDH inhibitors
    - Fluasterone
  - ODC inhibitors
    - Aspirin, carbenoxolone, curcumin, DFMO, 18β-glycyrrhetinic acid, 4-HPR, ibuprofen, *N*-acetyl-*l*-cysteine, piroxicam, 13-*cis*-retinoic acid, sulindac, vitamin A
  - Protein kinase C inhibitors
    - Carbenoxolone, 18β-glycyrrhetinic acid, 4-HPR, tamoxifen
  - Other
    - Calcium
- Blocking agents
  - Phase II metabolic enzyme inducers
    - *S*-allyl-*l*-cysteine, *N*-acetyl-*l*-cysteine, oltipraz, phenhexyl isothiocyanate
  - Polyphenols
    - Ellagic acid
  - Other
    - Curcumin, fluasterone
- Antioxidants/electrophile scavengers
  - Antiinflammatories
    - (See under "Antiproliferatives")
  - Antioxidants
    - β-carotene, curcumin, ellagic acid, fumaric acid, *N*-acetyl-*l*-cysteine
  - Phase II metabolic enzyme inducers
    - (See under "Blocking agents")
  - Thiols
    - *S*-allyl-*l*-cysteine, *N*-acetyl-*l*-cysteine, oltipraz

Status of Chemopreventive Agents Currently Under Development

Table 1 presents the 22 drugs and three drug combinations that currently have reached an advanced level of development as chemopreventives. Based on the status of their development, the chemopreventive drugs in Table 1 are grouped into three generations. Chemopreventive agents in the first generation are well documented clinically or epidemiologically and are now in Phase II or Phase III clinical trials. Second-generation drugs are those which have demonstrated chemopreventive efficacy in animal studies, have been through toxicological testing in rats and dogs, and are now in Phase I clinical trials. Third-generation agents are those which have demonstrated chemopreventive efficacy in animal studies and are now undergoing toxicology testing.

First-Generation Agents. Several first-generation agents already have progressed significantly as chemopreventive agents; these are the retinoids, β-carotene, and calcium. Many animal efficacy studies have been completed on retinoids, which demonstrate chemopreventive activity in mammary glands, bladder, and skin (reviewed in Refs. 15 and 16). Several epidemiology studies have examined the relationship between blood levels of vitamin A and cancer risk (*e.g.*, Refs. 17–22). Retinoids are active in the proliferation and progression stages of carcinogenesis (23). Retinoids inhibit several activities involved in tumor promotion, including induction of ODC; they probably participate in signal transduction via cellular receptors. They induce terminal differentiation in selected cells, and this activity may be mediated by binding to receptors. They stimulate intercellular communication and are immunostimulants. Unfortunately, there is also significant potential toxicity associated with retinoids. For example, vitamin A and many of its analogues accumulate in liver and cause hepatic damage; they also can cause eye damage, and they are teratogens (24). Although the toxicity of certain of the efficacious synthetic retinoids such as 4-HPR appears to be less severe than that of vitamin A, there is still concern. Because of this potential toxicity, clinical trials of the synthetic retinoids have been limited to patients with previous cancers and those at high risk for cancer.

The rationale for the development of β-carotene as a chemopreventive agent is based on case-control epidemiology data from lung cancer patients (21, 25–33), a chemical structure indicating ability to scavenge free radicals, and bioconversion to vitamin A. Unlike the retinoids, there is little concern about toxicity; however, there are only scattered animal efficacy results. Several clinical trials of β-carotene are ongoing which include well subjects at increased risk for cancer (*e.g.*, chronic smokers) as well as patients with precancerous lesions (*e.g.*, colon polyps).

These clinical trials are not yet completed, except that there has been one negative result from a randomized trial of β-carotene in skin cancer (34). Although this outcome appears to be conclusive, the dosage used may have been too low to be effective in skin. Encouraging results have been obtained from a preliminary trial of β-carotene in patients with oral leukoplakia, which is associated with increased risk of oral cancer and, particularly in the presence of dysplasia, is considered a premalignant lesion (35, 36). Of 24 patients treated with 30 mg β-carotene/day, 17 showed signs of lesion regression within 3 months (35). Meanwhile, the activity of β-carotene in animal efficacy experiments is providing insight for interpreting the clinical data. One difficulty with obtaining reliable results in animal efficacy studies has been poor absorption of dietary β-carotene in rodents (see, for example, Ref. 37). Recently, chemopreventive efficacy has been observed in studies with injectable forms of β-carotene where adequate blood levels of the agent have been obtained. For example, injectable β-carotene inhibited the induction of mammary carcinoma induced in rats by MNU (data not shown). Also, this form of the agent proved to be efficacious against carcinogen-induced lung tumors when administered in combination with vitamin A, oltipraz, or DFMO; under the conditions of these experiments, none of the agents was effective when administered alone (38).

Prominent among ongoing clinical trials of first generation drugs is the six-center CARET study, which is testing the chemopreventive effect of a combination of 25,000 international units vitamin A and 30 mg β-carotene/day in preventing lung cancer in heavy smokers and workers exposed to asbestos (39). Trials of 13-*cis*-retinoic acid in preventing oral leukoplakia (36, 40, 41), second primaries of the upper aerodigestive tract (42), and bronchial dysplasia in

Table 3 NCI chemoprevention drug development program: chemopreventive efficacy in animal models (second- and third-generation agents)[a]

Agent	Hamster		Colon		Rat mammary		Mouse bladder	Mouse skin
	Lung	Trachea	Mouse	Rat	DMBA	MNU		
DFMO	–[b]	–	–	+	+	+	+	
Piroxicam				+		+	+	+
Oltipraz	+	+	+	+	+	+	+	+
N-acetyl-*l*-cysteine		+		+		+	+	
Aspirin				+		–		
Ibuprofen				+			+	
Carbenoxolone				+	–	+		
β-Glycyrrhetinic acid		–	+	–		+		+
Curcumin			+			+		
Ellagic acid		–		+	–	–	+	
Fumaric acid		+				+		
Fluasterone				+		+		

[a] Agents listed in order from most to least advanced in development.
[b] +, Chemopreventive activity observed; significant at $P < 0.05$; –, no significant chemopreventive activity observed.

chronic smokers (43) are underway. Positive results have been obtained in studies of oral leukoplakia (36, 40) and in prevention of second primary head and neck tumors (42). 4-HPR is now being tested for chemopreventive effect on cancer in the opposite breast of patients who have undergone mastectomy for breast cancer (24).

Phase II clinical trials of calcium in preventing adenomatous polyps of the colon are in progress. The chemopreventive potential of calcium was first shown by its protective effect against proliferation in the colon of patients at high risk for cancer (44–46). Calcium has shown chemopreventive activity at the cellular level (44, 45, 47, 48), in animals (49–51), and clinically (48). A total dose of 2000 mg elemental calcium/day has been proposed to be the likely efficacious and highest nontoxic dose that can be recommended currently (48).

Tamoxifen is a well known antiestrogen used in the adjuvant therapy of breast cancer (52). This clinical use initially was based on its efficacy in causing the regression of carcinogen-induced mammary tumors in rats, as well as its ability to prevent new tumors in the same animals (53, 54). Recently a Phase III trial of tamoxifen for the prevention of breast cancer began (55). Finasteride is an inhibitor of testosterone 5α-reductase. Interest in it as a potential chemopreventive agent arose because of its efficacy in treatment of proliferative disease in prostate, benign prostatic hyperplasia (56). A Phase III trial of finasteride for the prevention of prostate cancer has recently begun.

Second- and Third-Generation Agents. For second- and third-generation agents, much evidence establishing their efficacy as chemopreventive agents has come from preclinical efficacy studies. Table 3 shows the efficacy of some of these agents in the animal models of carcinogenesis that are part of the NCI chemopreventive drug development screening process. The models have been described in detail previously (1, 3, 57). The chemopreventive activities and rationale for developing these agents is summarized below.

DFMO alkylates and irreversibly blocks ODC, preventing conversion of ornithine to putrescine. This is the first and rate-limiting step in polyamine synthesis, which is closely linked to cell proliferation (58–60). ODC is believed to be important in tumor promotion (61, 62), and its inhibition thus may be a mechanism for inhibiting carcinogenesis. DFMO has chemopreventive activity in mouse skin (61, 63, 64), mouse colon (65), rat colon (11, 12, 66), rat (67) and mouse (68) urinary bladder, and rat mammary gland (68–72). Previous clinical trials of DFMO involving cancer patients established a p.o. MTD for DFMO of 9–12 g/m^2/day (approximately 230–300 mg/kg body weight/day) (73, 74). The dose-limiting side effects observed included diarrhea, anemia, leukopenia, thrombocytopenia, and loss of hearing acuity. Chronic (1-year) p.o. toxicity studies in rats and dogs found NOELs at 400 mg/kg body weight/day and <50 mg/kg bw/day (the lowest dosage tested), respectively. A recently completed Phase I cancer prevention clinical trials showed drug effect with no toxicity, particularly otoxicity, in patients treated with a low dose level of 500 mg/m^2/day (13 mg/kg body weight/day) for 10–12 months (75), suggesting that this dose level is appropriate as a starting point for further clinical studies. Additional animal studies are characterizing the otoxicity.

Four of the second generation chemopreventive agents are NSAIDs, sulindac, piroxicam, aspirin, and ibuprofen. A prominent biological activity of the NSAIDs is inhibition of the synthesis of prostaglandins and other eicosanoids, particularly inhibition of fatty acid cyclooxygenase (*e.g.*, Refs. 76–78). Epidemiological and experimental data strongly suggest that carcinogenesis in epithelial tissues may be modulated by inhibiting some aspects of the prostaglandin biosynthetic cascade (*e.g.*, Refs. 76, 77, 79, 80). The mechanism(s) may involve reductions not only in growth-promoting tissue prostaglandin levels but also in suppressed immune surveillance (81, 82) and in oxidation (activation) of proximate carcinogens (80, 83, 84).

In animal studies, NSAIDs have chemopreventive activity in numerous tissues. They reduce formation of both colon polyps and carcinomas in laboratory animals given carcinogens (11, 66, 85–95). They also inhibit the induction of tumors in rat urinary bladder (96, 97), hamster buccal pouch (98, 99), rat mammary gland (100–103), mouse skin (104–108) and duodenum (88), and hamster esophagus (109), pancreas (110), and uterine cervix (111).

In animal efficacy screens carried out under the NCI chemopreventive drug development program (Table 3), the NSAIDs were active in the rat colon (aspirin, ibuprofen, piroxicam), rat mammary (piroxicam), mouse bladder (ibuprofen, piroxicam), and mouse skin (piroxicam). Sulindac has not been tested in the NCI screens but has demonstrated efficacy against DMH-induced colon tumors in mice (95). In

preliminary clinical studies, sulindac has also shown dramatic effects in causing the total or almost total regression of colorectal adenomatous polyps in patients with familial adenomatous polyposis and Gardner's syndrome (112, 113). In one of these studies, regression was seen in 9 patients with familial adenomatous polyposis in less than 4 months of treatment (113). A recent major epidemiological study also suggests that NSAIDs have promise in the clinic as chemopreventives. Regular aspirin use (16 times/month or more often) has been reported to reduce the relative risk of death from colon cancer by 40% (79). For clinical use as chemopreventives, the goal is to identify dosages/regimens which are efficacious in cyclooxygenase inhibition and nontoxic with respect to the gastrointestinal upset, ulcers, and nephropathy which limit NSAID usage in other applications.

Oltipraz is a synthetic dithiolthione related to naturally occurring 1,2-dithiolthiones found in cruciferous vegetables. It is a schistosomicidal drug that has demonstrated chemopreventive efficacy in many animal model systems. Oltipraz inhibited the induction of forestomach and pulmonary tumors in mice by B(*a*)P, *N,N'*-diethylnitrosamine and uracil mustard (114). It also protected against AFB_1-induced liver cancer (115), azaserine-induced pancreatic cancer,[3] and spontaneous hematopoietic tumors (113) in rats. As shown in Table 3, oltipraz has been highly effective in animal screens carried out under the NCI chemopreventive drug development program; positive results have been seen in hamster lung and trachea, mouse and rat colon, rat mammary, mouse bladder, and mouse skin. The activity against azoxymethane-induced colon cancer in rats has been reported in the literature (117).

Although the mechanism of this activity is not fully understood, the anticarcinogenic potential of oltipraz was first suggested by its chemoprotective, radioprotective, and antimutagenic properties. Ansher *et al.* (118) demonstrated that oltipraz protected against hepatotoxicity in mice induced by acetaminophen and carbon tetrachloride. The agent also inhibited AFB_1-induced hepatotoxicity and DNA adduct formation in rat liver (119). Oltipraz administered p.o. increases liver GSH levels and induces enzymes involved in electrophile detoxification, *i.e.*, GSH *S*-transferases, epoxide hydrolase, and NAD(P)H:quinone oxidoreductase (118–120). GSH is present in high concentrations in most cells, where it functions to inactivate electrophilic carcinogens and scavenge oxygen-free radicals. It also reacts with hydrogen peroxide catalyzed by glutathione peroxidase and prevents the formation of other more reactive oxygen compounds (121). The chemopreventive and chemoprotective efficacy of oltipraz in liver has been attributed to these activities (115, 118, 122). There also is some evidence that oltipraz may have antiproliferative effects that may or may not be directly related to modulation of GSH and the Phase II metabolic enzymes. For example, in efficacy studies in rat colon (123) and mammary cited in Table 3, the agent was effective even when it was administered only after treatment with the carcinogen had been completed.

Besides the wide spectrum of its efficacy, oltipraz is an interesting candidate for further development as a cancer chemopreventive agent because of its apparent low toxicity. Early studies of acute and subacute toxicity of oltipraz in animals demonstrated that the drug is well tolerated on p.o. administration (124). In chronic (1-year) toxicity studies in rats and dogs carried out under the NCI drug development program, NOELs were established at 10 and 15 mg/kg body weight, respectively, with minimal toxic effects present at 60 mg/kg body weight (the highest dosage tested). Observation of schistosomiasis patients in clinical trials also indicated that oltipraz is tolerated on p.o. administration (125, 126). Despite these encouraging results, much work remains to be done to determine the appropriate chronic clinical dosage regimen for chemoprevention studies. The agent has shown significant toxicity in certain clinical settings. For example, acute administration at high doses (up to 2 g) in schistosomiasis therapy was discontinued due to delayed side effects, especially phototoxicity (127). In a 6-month Phase I clinical trial at 125 and 250 mg/day, side effects included photosensitivity, heat intolerance, gastrointestinal discomfort, neurological abnormalities, and an altered taste; the lower dose was considered to be in excess of the MTD (128).

In animal screens cited in Table 3, *N*-acetyl-*l*-cysteine had chemopreventive activity in the hamster trachea, rat colon, rat mammary, and mouse bladder models. Published studies indicate that *N*-acetyl-*l*-cysteine prevented urethane-induced lung tumors in mice (129) and DMH-induced colon tumors in rats (130). Like oltipraz, *N*-acetyl-*l*-cysteine stimulates intracellular production of GSH and activity of GSH *S*-transferases; it is readily deacetylated to form cysteine in the body, which enhances GSH synthesis (131). These activities may be the basis of its chemopreventive potential. Toxicity is considered low and the drug has been marketed for years as a mucolytic agent (Mucomyst) and for treatment of acetaminophen poisoning. Chronic toxicity studies in rats and dogs at dosages up to 1 g/kg body weight/day and 300 mg/kg body weight/day, respectively, did not show any significant toxicities (132). Phase I cancer prevention trials are currently planned for 1.6 g/m^2/day, possibly escalating to 6.4 g/m^2/day (or 42 to 169 mg/kg body weight/day).

18β-Glycyrrhetinic acid is found in licorice root and has antiinflammatory effects by mechanisms that appear to differ from the NSAIDs. It has been used at concentrations up to 2% in ointments for the treatment of various skin diseases (133). Carbenoxolone is the succinic acid ester of 18β-glycyrrhetinic acid and is also a potent antiinflammatory; it is used in the treatment of peptic ulcers. It appears to act locally on the stomach, possibly by stimulating the production of protective mucus (133).

Both 18β-glycyrrhetinic acid and its saponin parent, glycyrrhizin, have shown chemopreventive activity in various animal models. Glycyrrhizin inhibited the development of liver tumors in mice and rats (reviewed by Nishino in Ref. 134) and 18β-glycyrrhetinic acid inhibited tumor promotion in mouse skin (134, 135). In studies cited in Table 3, 18β-glycyrrhetinic acid exhibited chemopreventive efficacy in the mouse colon, mouse skin, and rat mammary models. Carbenoxolone was efficacious in the rat mammary model. The mechanism of chemopreventive activity of these agents is not well understood, but is believed to be related to their antiinflammatory potential, as evidenced by the inhibition by 18β-glycyrrhetinic acid of the inflammation associated with tumor promotion in mouse skin (134). 18β-Glycyrrhetinic acid also inhibits numerous other biological activities associated with tumor promotion, especially those mediated by signal transduction via protein kinase C [reviewed by Nishino (134)].

Although Phase I clinical trials with 18β-glycyrrhetinic acid are still underway and those for carbenoxolone are still

[3] B. D. Roebuck, unpublished results (116).

in the planning stages, previous studies with carbenoxolone indicate that these two agents will be well tolerated in a chemoprevention dosage regimen. As an antiulcer and antiinflammatory agent, carbenoxolone has undergone extensive clinical testing at doses up to 300 mg/day (136). At doses above 100 mg/day, severe side effects have been observed, consisting mainly of diastolic hypertension, edema, and hypokalemia. Although these side effects were still present at 100 mg/day, they were seen in only a few percent of patients. Therefore, it appears that 100 mg/day would be the MTD for carbenoxolone in the context of cancer chemoprevention. This dosage is still much higher than the estimated chemopreventive dose in humans. The dose for humans equivalent to the chemopreventive dose in the rat mammary model, calculated on the basis of relative surface area, is only 10 mg/day. For 18β-glycyrrhetinic acid, our chronic toxicity studies in rats and dogs established NOELs in the rat at 1000 mg/kg body weight/day and in the dog at 300 mg/kg body weight/day. Phase I clinical trials are evaluating doses up to 500 mg/m^2/day (approximately 13 mg/kg body weight/day).

S-Allyl-*l*-cysteine is a water soluble organosulfur compound found in garlic. For many years, there has been a high level of interest in the potential chemopreventive effects of garlic, onion, and their components. Studies of these compounds have been reviewed recently (137). Epidemiological studies have shown inverse correlations between gastric cancer incidence and consumption of vegetables in the *Allium* genus (138, 139). Garlic oil has shown chemopreventive activity in mouse skin (140) and cervix (141), and several of its volatile, lipophilic components (particularly, diallyl sulfide) have shown chemopreventive activity in mouse colon and stomach (142–144). Diallyl sulfide also inhibited skin cancer induced in mice by DMBA (145), esophageal cancer induced in rats by *N*-nitrosobenzylmethylamine (146), glandular stomach cancer induced in rats by *N*-methyl-*N*′-nitrosoguanidine (147). In an NCI-sponsored study in hamsters, diallyl disulfide strongly inhibited the induction of tracheal tumors in hamsters by MNU (data not shown).

The volatility and pungency of the lipophilic garlic compounds make them difficult to test and unpalatable. These disadvantages have led recently to interest in the water soluble, less aromatic components such as *S*-allyl-*l*-cysteine. On p.o. administration the compound inhibited DMH-induced colon tumors in female C57BL mice (148).

The mechanism of action of the garlic sulfur compounds is not well understood but appears to be related to electrophile detoxification. Like oltipraz and *N*-acetyl-*l*-cysteine, *S*-allyl-*l*-cysteine (148) and other garlic sulfur compounds (143) enhance the activity of GSH *S*-transferases. Also, diallyl sulfide and, probably, other garlic sulfides inhibit cytochrome P450IIE1 which is involved in metabolic activation of carcinogens such as DMH and *N*-methyl-*N*′-nitrosoguanidine (137, 149). Preclinical acute and subchronic (90-day) toxicity evaluations of *S*-allyl-*l*-cysteine in rats and dogs are currently underway. Human toxicity of the agent has not been characterized but is anticipated to be very low.

Several arylalkyl isothiocyanates have been shown to inhibit mammary, forestomach, and lung tumors induced by PAHs and nitrosamines in rats and mice (150–154). Tobacco smoking is a significant risk for several major human cancers including those in the lung, other sites in the upper aerodigestive tract, and bladder (*e.g.*, Ref. 155). Chemopreventive agents that potentially can counter the effects of smoking are therefore of high interest. Arylalkyl isothiocyanates, and phenhexyl isothiocyanate in particular, may be such agents in the lung. A series of arylalkyl isothiocyanates with alkyl chains ranging from two (phenethyl isothiocyanate) to six (phenhexyl isothiocyanate) carbons inhibited lung tumors induced in mice by the tobacco-specific carcinogen NNK (152, 153). In these studies, the length of the alkyl chain in the isothiocyanates proved to be an important determinant of the potency of chemopreventive activity. Chemopreventive efficacy increased as the alkyl chain was elongated. Thus, phenhexyl isothiocyanate, the most potent of the agents tested, was 50–100 times more potent than phenethyl isothiocyanate (154, 156). Although the reasons for this structure-activity relationship have not been elucidated, increased lipophilicity and stability have been suggested (157).

The available evidence indicates that a primary mechanism of the inhibition of NNK carcinogenesis by arylalkyl isothiocyanates is prevention of NNK-DNA adduct formation (157). Preliminary results of toxicology testing in rats indicate low toxicity. Anticipated toxicities include minor weight loss and fatty changes in the liver, as seen in F344 rats fed phenethyl isothiocyanate at doses of 3 or 6 μmol/g (approximately 490 or 980 mg/kg) diet for 13 weeks (153, 158).

Curcumin is the major yellow pigment in turmeric and curry and is obtained from the rhizome of the plant *Curcuma longa*. It is of high interest both because of its potential for chemopreventive activity and its apparent low toxicity; *i.e.*, it is already a common dietary component. In animal cancer screens cited in Table 3, curcumin had chemopreventive activity in mouse colon and MNU rat mammary models. In other studies, the agent had tumor inhibitory activity in the two-stage DMBA/TPA mouse skin model (159–161) and in the induction of skin tumors by B(*a*)P (162).

Curcumin may have chemopreventive activity via multiple mechanisms. It is a potent antiinflammatory agent (162–165). It inhibited arachidonic acid metabolism in CD-1 mouse skin by blocking both the lipoxygenase and cyclooxygenase pathways (162, 166). There is also evidence that it inhibits phospholipase A_2 (162). Curcumin exhibits strong antioxidant activity (167, 168), being an effective scavenger of superoxide radicals (169). On topical application, curcumin inhibited TPA-induced DNA synthesis in mouse skin as measured by tritiated thymidine incorporation, demonstrating the inhibitory effect of curcumin on proliferation (160). It also may inhibit the metabolic activation and DNA binding of PAH carcinogens (162, 170, 171). As noted above, curcumin is not expected to exhibit much toxicity in humans. Toxic effects of chronic exposure in humans have not been characterized apart from respiratory symptoms and allergic dermatitis in spice factory workers (172). Ulcerogenic effects have been reported in rats (173). In our acute toxicity study in rats, curcumin was not toxic; *i.e.*, 50% lethal dose >3.5 g/kg body weight, the highest dose that reasonably could be administered p.o. An acute toxicity study in dogs and subchronic (90-day) toxicity evaluations in rats and dogs are currently underway.

Ellagic acid represents the naturally occurring polyphenols which have recently received much attention as potential chemopreventives (*e.g.*, Refs. 174–181). Besides ellagic acid, this class of agents includes the green tea catechins and various flavonoids. Ellagic acid itself is found in a number of fruits and vegetables, including grapes, strawberries, raspberries, and nuts (182). Conney *et al.* (183) first demonstrated its potential chemopreventive activity by its

inhibition of the mutagenicity of PAHs. In animal studies it has shown chemopreventive activity against tumors induced by PAHs in mouse skin on topical administration (184–188) and in mouse lung on i.p. administration (184, 187, 188). It also inhibited nitrosamine-induced esophageal papillomas on p.o. administration (189). In animal screens cited in Table 3, ellagic acid reduced tumor multiplicity in rat colon and carcinoma incidence in mouse bladder when fed in the diet.

The chemopreventive activity of ellagic acid may be related generally to its antioxidant potential, but it also appears to be related specifically to its ability to prevent metabolic activation of carcinogens and binding of the activated carcinogens to DNA. For PAHs, its chemopreventive activity has been attributed to inhibition of mixed function oxidases involved in activating the carcinogens and to binding to the activated form of the carcinogens (190). For nitrosamine carcinogens, its activity has been attributed to site-specific binding to DNA, thereby preventing reaction of the carcinogens with DNA (191). As is likely the case for all polyphenols, chemopreventive activity of ellagic acid at sites other than colon may be limited by its poor absorption on p.o. administration (190, 192). Uses in cancer prevention might require formulations facilitating absorption (193). Oral toxicity studies have not been completed; however, in chemoprevention studies cited in Table 3, no significant toxicity was seen at dose levels up to 6 g/kg diet/day. Ellagic acid is known to be pharmacologically active by other routes of administration. For example, at low i.v. dosages (0.22 mg/kg body weight) to human cancer patients, ellagic acid activated the intrinsic blood coagulation system (194).

Fumaric acid has good potential for further development because of its lack of toxicity, as well as its chemopreventive activity. It is a metabolic intermediate in mammalian tissues (citric acid and urea cycles) and is a generally recognized as safe substance used commercially in food and beverages as an antioxidant, acidulant, flavoring agent, feed additive, and cure accelerator (195, 196). The chemopreventive efficacy of fumaric acid was shown first by Kuroda and associates. They identified fumaric acid as the component of the herb *Capsella bursa-pastoris* responsible for its antiproliferative and antiinflammatory properties by which the herb inhibited the growth of transplanted tumors in mice (197) and gastric ulcers in rats (198). It also reduced the liver toxicity of the carcinogens mitomycin C and AFB_1 (199). In a series of studies, they showed that fumaric acid had chemopreventive activity in mouse forestomach (200), rat liver (201, 202), and mouse lung (200). Subsequently, the agent demonstrated chemopreventive effects in studies in MNU hamster trachea and MNU rat mammary models (see Table 3).

The mechanism of the chemopreventive action of fumaric acid has not been elucidated, but may be related to its antioxidative potential. On the basis of the studies cited above, fumaric acid appears to be active in later stages of carcinogenesis. For example, in the studies in mouse forestomach, rat liver, and mouse lung cited above, it was active when given after treatment with the carcinogen was completed. Also, in the rat mammary study cited in Table 3, fumaric acid significantly increased tumor latency but did not decrease tumor incidence or multiplicity.

From studies reported in the literature, fumaric acid appears to have little toxicity (196, 203–205). In 6-week dose tolerance studies preparatory to the chemoprevention screens cited in Table 3, no toxicity was observed at the doses tested, which ranged from 0.4 to 20 g/kg diet. It should be noted that the high dose tolerance might be related to poor absorption from the gastrointestinal tract (cited in Ref. 205). Additional preclinical acute and subchronic toxicity evaluations are scheduled in rats and dogs.

Schwartz *et al.* (206–216), as well as other investigators, have demonstrated the chemopreventive activity of the androgen DHEA in numerous animal models. DHEA is a potent inhibitor of G6PDH. The primary function of this enzyme is catalysis of the formation of extramitochondrial NAD(P)H and ribose 5-phosphate. Schwartz has hypothesized two ways in which inhibition of G6PDH may mediate the chemopreventive activity of DHEA (216). First, DHEA inhibits the activity of carcinogens such as B(*a*)P, AFB_1, and DMBA which require metabolic activation via mixed function oxidases (216–219). Mixed function oxidases require NAD(P)H as a cofactor. Thus, since inhibition of G6PDH reduces the formation of NAD(P)H, it consequently reduces the activity of mixed function oxidases and the activation of certain carcinogens. Secondly, DHEA also inhibits tumor promotion and proliferative activity induced by TPA (210, 220). Cell proliferation requires NAD(P)H-dependent DNA synthesis, and DNA synthesis in mouse epidermis and mammary tissue also is inhibited by DHEA (221). Accordingly, reduction of the NAD(P)H pool by inhibition of G6PDH could inhibit carcinogen-induced cell proliferation.

Unfortunately, the chemopreventive potential of DHEA is compromised by some undesirable pharmacological effects: potent hormonal (222), liver-enlarging (223), and peroxisome-proliferating activities (223, 224). To eliminate these side effects while preserving chemopreventive activity, Schwartz designed several analogues (216, 223, 225). One of these analogues, fluasterone (16α-fluoro-DHEA; DHEA analogue 8354) is particularly promising and is being developed in the NCI chemopreventive drug program. Fluasterone does not have the androgenic or liver toxicity of DHEA (223). It was a more potent inhibitor of tumor initiation and promotion in the DMBA/TPA mouse skin model than DHEA (220), and, in animal studies cited in Table 3, it was effective in the rat mammary gland against MNU-induced cancers (226) and in rat colon against azoxymethane-induced tumors (12).

Subchronic studies in rats (up to 1 g/kg body weight/day) and dogs (up to 250 mg/kg body weight/day) have established a NOEL of 250 mg/kg body weight for fluasterone in both species; no target organs with histopathology were identified in either study. Effects seen at the high doses tested included dose-related weight loss (>10% at 1 g/kg body weight/day) and hypocholesterolemia (at 500 mg/kg body weight/day and 1 g/kg body weight/day) in the male rats. The relevance of these effects to the potential of fluasterone for clinical use has not yet been evaluated. Particularly, the minimal effective doses of fluasterone have not been determined. Pharmacokinetic evaluations are currently underway and chronic toxicity studies are planned. Like some of the other compounds discussed, fluasterone may require formulations designed to augment bioavailability.

Future Directions

The progress that has been made to date indicates that chemoprevention research will soon begin to yield practical applications for the reduction of cancer incidence. Nonetheless, the time and resources required to carry out a full clinical evaluation of a chemopreventive agent in a cancer incidence reduction study is of great concern. As stated above, the success of chemoprevention rests on the ability

to limit the progression of neoplasms before they become frank cancers. To address this goal, as well as the concern for time and resources, the role of Phase II clinical studies has been expanding to evaluate markers in predysplastic and dysplastic tissue as endpoints for evaluation of chemopreventive agents. Studies in patients with dysplastic lesions—cervical dysplasia, oral leukoplakia, superficial bladder cancers, and actinic keratoses—have been initiated recently. These and other Phase II studies will be used to search for and validate earlier markers that are endpoints for chemoprevention. In these studies, various potential markers of abnormal cellular proliferation and differentiation and genetic changes such as abnormal gene expression (including oncogenes and tumor suppressors), altered DNA content, and chromosome structural changes may be evaluated. More experimentation in animal models related to the validation of markers also has begun. Recently, in the NCI chemoprevention drug development program, studies of potential markers have been initiated in mouse and rat colon, hamster buccal pouch, hamster pancreas, hamster and mouse lung, and rat bladder.

New technologies are also expected to benefit chemoprevention research, especially detection and validation of early markers. Particularly interesting are techniques, such as fine needle aspiration and the polymerase chain reaction, that will allow early and rare lesions to be detected relatively noninvasively. Likewise, as knowledge in molecular biology and the basic cellular processes in carcinogenesis increases, chemopreventive agents that are directed to repair or suppress early genetic lesions and control cellular growth mechanisms (*e.g.*, programmed cell death, angiogenesis) may be possible.

To date, chemoprevention research efforts have focused primarily on cancers of the colon, lung, breast, and bladder. In the NCI drug development program, models for evaluating potential chemopreventive agents in prostate and pancreas are being investigated. Other cancers with high incidence or mortality are expected to be addressed within the next few years—particularly, brain cancers, leukemia and non-Hodgkin's lymphomas, and melanoma.

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