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RETINOIDS AND CANCER PREVENTION

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Perspectives

Prevention of cancer may soon be a possibility for people who, because of genetic disposition or environmental exposure to carcinogens, have a high risk for developing the disease. Compounds representing several diverse chemical classes are being tested in animals and in humans for their effectiveness in combatting cancer (chemoprevention). One such class is the ret-

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inoids, which consists of the natural vitamin A compounds [retinol, retinal, and all-*trans*-retinoic acid (RA)] and their derivatives and analogs (Figure 1).

When administered to humans, retinoids have demonstrated activity in preventing cancers of the skin (67, 71, 101), head and neck (59), lungs (98), and bladder (129). Further, they are effective in the treatment of leukoplakia (58), a preneoplastic disease; acute promyelocytic leukemia (60); and myelodysplastic syndromes (12). At present, in six different clinical trials, retinoids are being evaluated in patients with the preneoplastic diseases of cervical dysplasia, asbestosis, and actinic keratoses and in high-risk groups including cigarette smokers, women previously treated for breast cancer, and patients with a predisposition to develop basal cell carcinomas (18). The promise of retinoids in such trials is based, to some extent, on animal experiments, in which these compounds demonstrate activity in preventing cancer of the skin, forestomach, liver, mammary gland, and bladder (see 88).

To detect preventive activity of retinoids, chemical carcinogens are ordinarily employed to induce cancer in experimental animals. These carcinogens vary in structure. There are polycyclic hydrocarbons, such as 7,12-

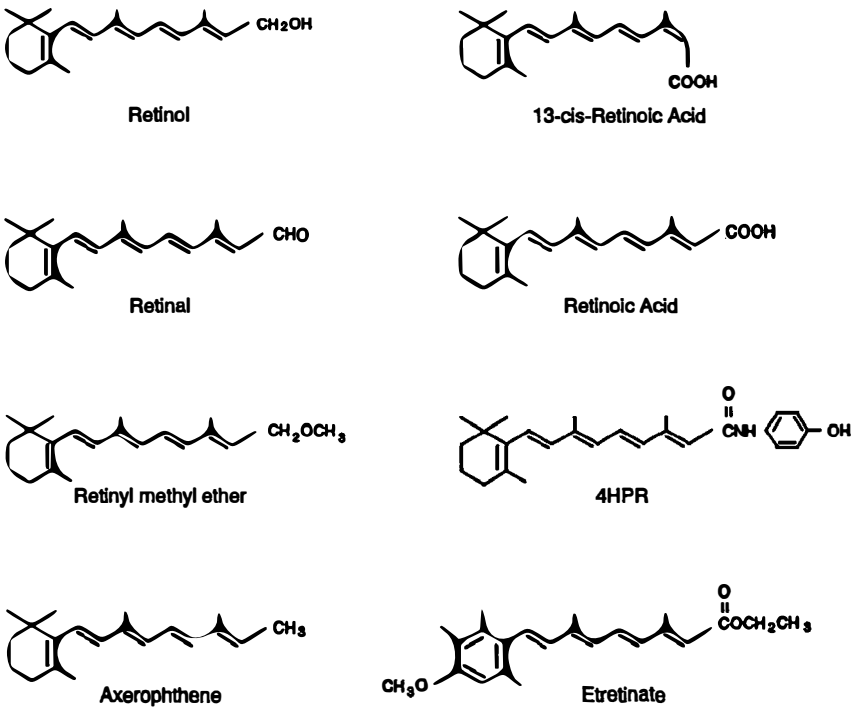
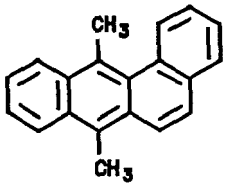
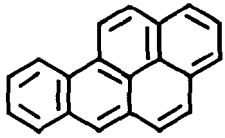


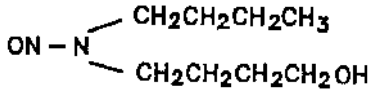
Figure 1 Structures of selected retinoids.



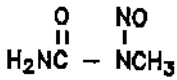
DMBA



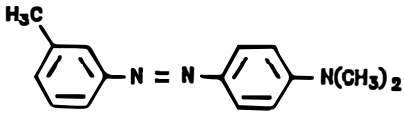
BP



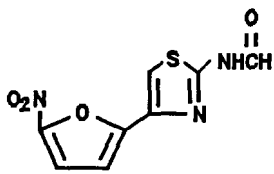
HO - BBN



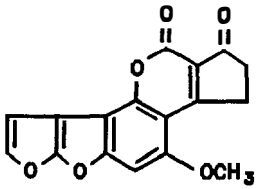
MNU



3'-MeDAB



FANFT



AFLATOXIN B_1

Figure 2 Structures of selected carcinogens.

dimethylbenz(α)anthracene (DMBA) and benzo(α)pyrene (BP); nitrosamines, such as *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (HO-BBN); nitrosoureas, such as methylnitrosourea (MNU); azobenzenes, such as 3'-dimethylaminoazobenzene (3'-MeDAB); and chemicals with complex structures, such as *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) and aflatoxin B_1 (Figure 2).

Carcinogenesis occurs in cells through a process involving initiation, promotion, and progression. During initiation, an irreversible change takes place, involving production of a mutation in the genome usually as a result of interaction of the carcinogen with cellular DNA. Promotion occurs when initiated cells are converted to the tumor phenotype by a chemical that is not an initiator and, by itself, not a carcinogen. In the final stage, the tumor becomes highly malignant and grows rapidly.

The action of retinoids in preventing cancers of the skin and mammary gland is generally thought to be accomplished via an antipromoting effect (46, 138), but their mechanism of action in preventing other types of cancer is not yet known. Retinoids are also immunostimulants, a fact that may account for their observed activity against established cancers (see 55). Both cell-mediated cytotoxicity and that of natural killer cells are enhanced by RA. In some systems, retinoids cause leukemic cells to differentiate, irreversibly converting them to morphologically mature granulocytes with the same functional markers as the mature neutrophil (19).

At the molecular level, retinoids seem to modify gene expression through the mediation of intracellular-binding proteins and nuclear receptors (111). The mode of action of retinol and RA in the control of differentiation and tumorigenesis apparently involves cellular retinol-binding protein (CRBP) and cellular RA-binding protein (CRABP) (6, 109). These proteins may be involved in the transport of retinoids to nuclear sites, where they interact with their receptors (RARs) (45, 111). Binding of retinoids to CRABP and to RARs requires a free terminal carboxyl group (109); a hydroxyl or lipophilic terminal group is required for binding to CRBP (56, 97).

In general, retinoids that are active in the prevention of murine leukemias and lymphomas and murine papillomas and carcinomas of the skin have a sidechain that possesses, or is readily convertible to, a free carboxylic acid group. The presence of such a group appears to be necessary, but not sufficient, for chemopreventive activity in the skin. In contrast, retinol and its derivatives and other retinoids lacking a terminal functional group are most active in preventing cancer of the mammary gland. However, to exert their activity, these retinoids are not likely to be converted to compounds with free carboxyl groups, because RA and 13-*cis*-RA have demonstrated little chemopreventive activity against mammary cancer (90).

Because retinamides show activity in preventing breast and bladder cancer, do not bind to either of these proteins, and are not readily converted to RA (118), other metabolic conversions may be involved in the formation of active metabolites of these compounds. Alternatively, there may be a separate class of binding proteins or receptors for retinamides and other retinoids that do not bind to the known RARs.

We conclude that at least three distinct classes of retinoids, each with its

own biochemical properties, are active in chemoprevention: one characterized by a terminal carboxylic acid group that is active in preventing skin cancer, one by a hydroxyl or nonpolar terminal group that is active in preventing mammary cancer, and one by a terminal amide group that is active in preventing bladder and mammary cancer.

Pharmacokinetics also appears to be a factor in the chemopreventive potential of retinoids (see 55, 56, 62). RA, 13-*cis*-RA, *N*-(4-hydroxyphenyl)retinamide (4-HPR), and *N*-(2-hydroxyethyl)retinamide are effective in the prevention of bladder cancer, and, in this tissue, relative to other tissues, they have prolonged half-lives. For chemoprevention of lung cancer, however, neither 13-*cis*-RA, *N*-(2-hydroxyethyl)retinamide, nor 4-HPR has appreciable activity; 13-*cis*-RA is likewise inactive in the prevention of colon cancer. Accordingly, loss of these compounds from the respective tissues is relatively rapid. Further, retinyl methyl ether, 4-HPR, and axerophthene, none of which is readily converted to a structure with a free carboxyl group, show activity in preventing breast cancer and accumulate in breast tissue. *N*-(4-Methoxyphenyl)retinamide, the major metabolite of 4-HPR, also accumulates in the mammary gland of rats dosed with this metabolite. Accumulation of retinoids in the mammary gland is not related entirely to their lipophilicity, for retinyl butyl ether, which is less polar than retinyl methyl ether but has less chemopreventive activity than retinyl methyl ether, does not accumulate to the same extent. In the following sections we discuss the cancer preventive activity of retinoids in various tissues and organs.

Skin

A model system involving Swiss or CD1 mice is used extensively in evaluation of retinoids for the prevention of papillomas and basal cell carcinomas. In this system, DMBA is applied to the skin, and later the test retinoid is applied along with croton oil or the active ingredient in this oil, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Retinoids active in this system are etretinate, RA, retinal, retinol, retinyl acetate, retinyl palmitate, 13-*cis*-RA, 5,6-epoxy-RA, 5,6-dihydro-RA, various arotinoids (see 30, 55, 136, 139, 140), and various 3-substituted 4-oxoretinoic acids (117); other retinoids tested have little or no effect. Administered in the feed of mice, retinyl palmitate, but not 13-*cis*-RA, is active in preventing papillomas (42). With DMBA as an initiator and either anthralin (31) or 7-bromomethylbenz(α)anthracene (138) as a promoter, RA applied topically reduces the frequency of papilloma formation. When BP or *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine is the initiator and no promoter is given, however, topically applied 13-*cis*-RA does not prevent papilloma formation (40). Similarly, when BP is administered without a promoter, orally administered retinyl palmitate and RA are not effective in preventing skin papillomas and carcinomas (114). After BP has been

applied to the skin of mice as an initiator and TPA and 13-*cis*-RA have been applied for several weeks, inhibition of tumor promotion is stable in the absence of further promotion by TPA (41).

When DMBA is applied without a promoter to the skin of CD1 mice, RA does not reduce the frequency of papilloma formation (136). In fact, the frequency of papillomas can increase (51). In contrast, when 3-methylcholanthrene (3MC) is administered without a promoter, retinyl palmitate and 13-*cis*-RA are effective in reducing the frequency of skin papillomas and carcinomas (1). Several experiments have been performed with SENCAR mice, which develop cancer more readily than other strains. In the model system involving DMBA and TPA, RA is active in reducing the frequency of papillomas (30). RA is also active in this system in which mezerein is substituted for TPA (137). When no promoter is administered along with DMBA, however, RA and 13-*cis*-RA, given in the feed or applied topically, increase the frequency of papilloma formation (78). Administered in the feed, 4-HPR decreases the frequency of papillomas; however, applied topically, it has no effect on the frequency (77). When SENCAR mice are dosed with BP and anthralin as initiator and promoter, respectively, 13-*cis*-RA has no effect on the frequency of papilloma formation (40).

A related system involves induction of papillomas with DMBA and croton oil or TPA on the skin of Swiss mice and treatment of the established tumors with retinoids administered orally or intraperitoneally. Retinoids with demonstrated activity in this system are RA, retinyl palmitate, etretinate, motretinide, ethyl retinoate, acitretin, 7,8-dihydro-RA, 8,9-dihydroacitretin, 4,5-dihydroacitretin, various arotinoids, and various fluorinated aromatic retinoids (see 22, 55, 74, 99, 100). Other retinoids tested have little or no effect. Regression of established basal cell carcinomas has been noted in mice treated with etretinate (16).

When administered orally to mice previously exposed to ultraviolet light, neither retinyl palmitate, etretinate, RA, nor 13-*cis*-RA has any effect on the frequency of formation of papillomas and squamous cell carcinomas (63, 65, 144). Further, when RA is applied to the skin of these mice, an increased frequency of squamous cell carcinomas is noted (34, 36). Although a recent report (39) indicates that retinyl palmitate administered continually before, during, and after exposure to ultraviolet light, reduced the total volume of papillomas that are formed, retinoids show little promise in the prevention of skin cancers caused by ultraviolet light.

For humans, topical application of RA can cause the regression of dysplastic nevi (33) and cutaneous lesions of malignant melanoma (69). Some basal cell carcinomas (101), squamous cell carcinomas (see 71), and cutaneous lesions of malignant melanoma (87) respond to oral administration of 13-*cis*-RA. Patients with xeroderma pigmentosum develop skin cancers at a slower

rate when they are dosed orally with 13-*cis*-RA (67); and humans with actinic keratosis, a premalignant lesion, show therapeutic responses to etretinate (91).

Oral, Head, and Neck Cancer

Reports are conflicting about the efficacy of retinyl esters applied directly to the cheek pouch of hamsters in which buccal tumors are induced by administration of DMBA. Some reports indicate that these compounds increase the incidence of oral tumors (see 84), but one indicates that retinyl palmitate delays the induction of such tumors (64). Overall, the results are not promising for further experiments involving topical application of retinyl esters. Conversely, three reports note that oral administration of 13-*cis*-RA can produce favorable results. Not only can the incidence of buccal tumors induced by DMBA be reduced (120), but the same result can be obtained for those tumors induced by application of this carcinogen to the tongue (43, 119). These results demonstrate that selection of the appropriate retinoid and the appropriate route of administration are important factors in experiments involving chemoprevention by retinoids.

For humans, 13-*cis*-RA is used to treat oral leukoplakia, a disease that has a low, but definite, incidence of malignant transformation. The observed responses, 9 of 11 (116) and 16 of 24 (58), demonstrate the compound is moderately effective. In a recent and promising development, patients who had been successfully treated for head and neck cancer were placed in a clinical trial to evaluate the preventive effect of 13-*cis*-RA versus that of a placebo (59). Although no difference was noted between the two groups in recurrence of the tumor at the primary site, there were significantly fewer occurrences of second primary tumors (2 of 49 in the treated group versus 12 of 51 in the control group).

Leukemias and Lymphomas

The demonstration that retinoids can have an effect on cancers of the blood in experimental animals and humans is a relatively recent development. C57B1/10W mice exposed to X rays and placed on a diet containing 13-*cis*-RA developed fewer thymic lymphomas than did controls (103); on a similar diet, AKR mice, in which thymic lymphomas spontaneously appear, developed fewer lymphomas than did controls (104). In both cases, however, the administered dose prevented the weight gain seen in controls, which led to the conclusion that the observed effect on lymphomas could be related to retinoid toxicity. In mice, intraperitoneal administration of etretinate reduced the incidence of spontaneously occurring lymphoma (20); and administration of retinyl palmitate to newborn Swiss mice, the mothers of which had been exposed to ethylnitrosourea, prevented the development of leukemias in about

half of the offspring (150). In the latter report, there was no mention of retinoid toxicity. Retinyl palmitate, applied to the skin of mice after application of an extract of pepper, reduced the number of lymphomas developing in the spleens of these animals; oral administratin of retinyl palmitate was ineffective following skin application or feeding of the pepper extract (121). No decrease in the incidence of leukemias was noted in Long-Evans rats dosed with etretinate, during and/or after dosing with DMBA (11).

More promising results have been demonstrated in treatment of a type of human leukemia. All 24 patients with acute promyelocytic leukemia, some of whom were unresponsive to chemotherapy, experienced complete responses after receiving doses of RA (60). In subsequent experiments, 14 complete responses in 22 patients (21) and 9 complete responses in 11 patients were noted (142). Similarly, dosing with 13-*cis*-RA produced responses in about one third of patients with myelodysplastic syndromes (see 12). Nevertheless, dosing with 4-HPR produced no responses in 14 patients with myelodysplastic syndromes and may, in fact, have enhanced the leukemic progression in these individuals (38). 13-*cis*-Retinoic acid is apparently ineffective in the treatment of acute myeloid leukemia in elderly patients (68) and acute nonlymphocytic leukemia in pediatric patients (9). These results demonstrate that retinoids can be active in blood diseases but that different retinoids can have differing activities, even in humans. The retinamides especially should be administered with caution.

Mammary Gland

Reports from various laboratories demonstrate prevention by retinoids of mammary tumors induced in rats by either of several different carcinogens. With DMBA as the carcinogen, retinyl acetate and retinyl methyl ether are reasonably effective (see 55). Other retinoids that cause a reduction in frequency of mammary cancers are 4-HPR (17, 46, 79); temarotene, an arotinoid without a functional end group on the sidechain (131); and either of two other arotinoids (49). 13-*cis*-RA, at nontoxic doses, has little or no chemopreventive effect for mammary cancer (2). One report indicates that 4-HPR is not effective in reducing the frequency of mammary tumors induced by either DMBA or MNU (122), but these negative results may be due to the fact that the investigators used a rat chow different from that used by others.

With MNU as the carcinogen, retinyl acetate and retinyl methyl ether are effective in preventing mammary cancers (see 55, 117, 146). Other active retinoids are 4-HPR (see 46, 55); retinyl propynyl ether (117); and axerophthene, the hydrocarbon analog of retinol (133). Retinoids reported to be without activity are the methyl ether analog of etretinate and 13-*cis*-RA (117, 134). Another report demonstrates that retinyl acetate, fed prior to but not after MNU, causes an increase in the number of adenocarcinomas (46).

Nevertheless, when this retinoid is fed continuously, the number of adenocarcinomas that develop is greatly reduced.

Retinyl acetate is also effective, in rats, in reducing the frequency of adenocarcinomas that are induced by exposure to X rays (128), by BP (80), or by estrogens (57) as well as those that occur spontaneously (128).

In contrast to the encouraging results with rats, mice that have been dosed with DMBA develop mammary tumors that are not prevented by either retinyl acetate or 4-HPR (145). Further, retinyl acetate is not effective in preventing the spontaneous mammary cancers that develop in C3H-A mice (76). If mice are on a diet containing retinyl acetate during the time that they are being dosed with estrogen plus progesterone, the frequency of mammary tumors is increased (147).

In the mammary gland, the retinol-binding protein, CRBP, may be involved in mediating the chemopreventive activity of retinoids, for most compounds that are active in this system also bind to this protein (56). Although 4-HPR is a notable exception, this discrepancy may not invalidate the relationship, since an unidentified metabolite, which may bind to CRBP, is thought to be the active form of 4-HPR (85).

Lung

An early report indicated that retinyl palmitate, when administered to hamsters, prevented tumors induced in the respiratory tract by instillation of BP + ferric oxide (108). Although this experiment has not been repeated exactly, attempts to demonstrate a response with retinyl acetate in similar situations were unsuccessful (8, 124, 125). Another observation not confirmed is that 13-*cis*-RA prevented the formation of lung cancer caused by BP + ferric oxide (102). In this experiment, the number of cancers was small, and no detailed description of the results has appeared.

Attempts to use retinoids to prevent tracheal cancer caused by instillation of MNU into hamsters were not successful (see 55). In contrast, hamsters dosed with diethylnitrosamine (DEN) developed fewer lung carcinomas when they were placed on a diet containing 4-HPR (86), and hamsters dosed with 2,6-dimethylnitrosomorpholine (DMNM) developed fewer lung adenomas and carcinomas when they were placed on a diet containing 13-*cis*-RA (127).

An attempt to prevent lung cancer caused by administration of dibutylnitrosamine to rats by weekly dosing with retinyl palmitate was unsuccessful (114), and development of lung adenomas in the offspring of mice dosed with ethylnitrosourea (ENU) on day 14 of pregnancy was not affected by placing retinyl palmitate in the drinking water (150). Mice dosed with ethyl carbamate, however, developed fewer lung tumors when they are also administered *N*-homocysteine thiolactonyl retinamide (82) or *N*-homocysteine thiolactonyl retinamido cobalamin (83).

Although the experiments with animals have not demonstrated great promise for the use of retinoids in the prevention of lung cancer, two clinical trials have been encouraging. Etretinate, administered over a six-month period, reduced the degree of bronchial metaplasia in humans with extensive exposure to cigarette smoke (44); and patients with resected stage 1a lung cancer had fewer recurrences if they were dosed with retinyl palmitate for 14 months (98).

Liver

Retinoids may be active in preventing hepatic cancer. Retinyl acetate reduced the incidence of spontaneous liver tumors developing in C3H-A^{vy} mice (76), and RA reduced the incidence of liver tumors induced in Sprague-Dawley rats by 3'-MeDAB (29). Acitretin prevented spontaneous hepatomas in C3H/HeNCrj mice and 3'-MeDAB-induced tumors in rats (94), and retinyl acetate delayed the elevation of hepatic γ-GTPase and the appearance of preneoplastic nodules (75). 4-HPR did not reduce the incidence of spontaneously developing liver tumors in C3H/He mice, but did reduce the incidence of tumors induced by diethylnitrosamine in BALB/c mice (61). RA did not prevent liver tumors in BDF mice dosed with diethylnitrosamine (81). In Lewis rats, *N*-(2-hydroxyethyl)retinamide reduced the incidence of liver cell carcinomas (27).

Some disturbing reports regarding retinamides and liver cancer have appeared. Female rats dosed with the carcinogen azaserine and either of three retinamides had an increased incidence of liver cancer (72). Further, after 72 weeks of administration of 13-*cis*-*N*-ethylretinamide, hepatocellular carcinomas and adenomas developed in mice dosed with this compound only or with HO-BBN and the retinoid (54). No such results, however, were seen when *N*-ethylretinamide was administered for 1–2 years (53). In a separate experiment, both *N*-ethylretinamide and 13-*cis*-N-ethylretinamide increased the incidence of liver tumors in mice (81). In hamsters, *N*-(2-hydroxyethyl)retinamide may have prevented (14) or, along with other retinamides and 13-*cis*-RA, enhanced (13) the development of liver adenomas.

Whether or not only retinamides cause such toxicity and whether or not such an effect is limited to rodents remains to be determined. Nevertheless, for prevention of cancer, retinamides should be used with caution. Considerable experimental work must be performed before a biochemical understanding of these results can be achieved.

Pancreas

Various retinamides reduce the incidence of pancreatic carcinomas in rats dosed with azaserine (see 27). Retinamides reduce the frequency of acidophilic foci, which are thought to be preneoplastic lesions, in the pancreata of these

rats (106). The results are not as convincing, however, for hamsters dosed with *N*-nitrosobis(2-oxopropyl)amine (BOP). In these animals, retinamides may reduce the incidence (73), have no effect (14), or may enhance (13) the frequency of pancreatic carcinomas. Nevertheless, 13-*cis*-RA may reduce the incidence of pancreatic tumors in hamsters dosed with DMNM (127). Again, retinamides should be used with caution in experiments designed to prevent cancer.

Bladder

The activity of retinoids in preventing carcinogen-induced bladder cancer was established by studies in several different laboratories, which involved two species of animals and two different chemical carcinogens. Various retinoids were used to reduce the occurrence of papillomas, transitional-cell carcinomas, and squamous-cell carcinomas induced in rats and mice by HO-BBN (see 52, 53, 55, 89). The most effective retinoids in rats dosed with MNU were 13-*cis*-RA, RA, retinyl acetate, 4-HPR, and some alkyl retinamides; other alkyl retinamides had no measurable activity (126, 130). A nine-week delay in starting the feeding of 13-*cis*-RA to rats dosed with HO-BBN did not diminish its inhibition of bladder carcinogenesis (7). One group of investigators found that *N*-(2-hydroxyethyl)retinamide had chemopreventive activity in the bladders of rats (132); another group could not confirm such activity (105). Likewise, etretinate was reported to be both active (93) and inactive (113) in rats.

In contrast, transitional cell neoplasms induced by FANFT were not responsive to either 13-*cis*-RA, retinyl palmitate, *N*-ethylretinamide, or *N*-(2-hydroxyethyl)retinamide (see 26), and bladder cancers induced in rats dosed with dibutylnitrosamine were not prevented by retinyl palmitate (114).

In humans, oral administration of RA resulted in 4 complete and 7 partial remissions in 15 patients with recurrent papillomas of the bladder (35); oral administration of etretinate produced 6 complete and 5 partial remissions in 15 similar patients (5). In a double-blind, randomized clinical trial that had proceeded for 24 months, etretinate reduced the incidence of recurrent bladder tumors (129). Recurrences were observed in 9 of 16 patients dosed with a placebo but in only 4 of 14 dosed with etretinate. Retinamides have not yet been tested in humans for such activity. In view of the hepatocarcinogenicity of retinamides on long-term administration to rodents, these compounds should not be tested further until it is determined if such an effect is limited to rodents.

Colon

The most encouraging reports regarding the prevention of colon cancer by retinoids have shown a reduction in the number of tumors per rat in rats dosed

simultaneously with 1,2-dimethylhydrazine (DMH) and retinyl palmitate (107); a modest delay in the time to tumor development in rats dosed simultaneously with DMH and 13-*cis*-RA (96); and a reduction in the frequency of colon tumors in rats dosed simultaneously with DMH and 13-*cis*-RA (95). In another test (123), oral administration of retinyl acetate or 4-HPR to rats dosed with DMH caused a decrease in the number of tumor-bearing rats compared to that of controls, but retinoid-dosed animals showed decreased food consumption and decreased body weight gain owing to the toxicity of the retinoids. In the same study, several other retinoids, administered in the feed to rats dosed with either MNU or DMH, did not reduce the frequency of colon adenomas or carcinomas, and intrarectal administration of retinyl palmitate before dosing with MNU increased the percentage of tumor-bearing rats compared to that of controls. Further negative results were reported in several attempts to prevent colon cancer in rats by administration of retinoids (see 32). Thus, although several different retinoids have been tested for the prevention of colon cancer, the promise for success is modest.

Other

Retinyl palmitate added to the feed reduced the incidence of sarcomas induced in mice by Moloney murine sarcoma virus (115), and intraperitoneal administration of etretinate reduced the incidence of sarcomas induced in hamsters by Rous sarcoma virus (37). Intramuscular etretinate also reduced the incidence of papillomas induced on rabbit skin by application of Shope papilloma virus (37). Retinyl palmitate, administered orally and simultaneously with BP, had no effect on the production of spindle-cell sarcomas in rats (114); but intraperitoneal administration of RA to mice reduced the incidence of fibrosarcomas produced by 3MC (23). The effects of retinoids in preventing sarcomas may be related to their immunoenhancing effects. Topical application of retinoids in such experiments can not be recommended.

Orally administered retinyl palmitate reduced the incidence of forestomach papillomas induced in hamsters by intratracheal application of BP + ferric oxide (108, 124, 125). Further, hamsters dosed with DMBA or BP and retinyl palmitate developed fewer forestomach carcinomas than did those dosed with the carcinogen alone (24). Nevertheless, 13-*cis*-RA had no effect on the formation of forestomach papillomas in hamsters dosed with DMNM (127); etretinate did not reduce the incidence of forestomach papillomas that occur spontaneously in mice (151); and retinyl acetate, administered in the drinking water, increased the incidence of forestomach papillomas produced by butylated hydroxyanisole (50). Further experiments are required before a general conclusion can be drawn about the effectiveness of retinoids in these model systems.

Neither 13-*cis*-RA nor motretinide reduced the incidence of kidney tumors

in rats dosed with DMN (48); and 13-*cis*-RA was inactive in reducing the incidence of kidney tumors in hamsters dosed with DMNM (127). Neither RA nor retinyl palmitate demonstrated activity in preventing tumors induced by DMBA in the salivary glands of rats (3, 4). Retinyl palmitate (114) and 13-*cis*-RA (47) were also ineffective in preventing tumors induced by MNU in the central and peripheral nervous systems of rats.

In regard to prevention of esophageal cancer by retinoids, a promising report stated that administration of retinyl palmitate to hamsters also dosed with DMBA reduced the incidence of esophageal lesions (24), but the experiments described have yet to be confirmed. Attempts by others to demonstrate preventive effects of retinyl acetate, 13-*cis*-RA, and etretinate for esophageal tumors produced by N-nitrosomethyl benzylamine (NMBA) have been uniformly unsuccessful (see 28, 141). A randomized intervention trial in China showed, for a combination of retinol, riboflavin, and zinc, no effect on premalignant esophageal lesions in humans (92).

Retinyl palmitate reportedly reduced the incidence of carcinomas of the cervix and vagina of hamsters dosed with DMBA (24), but apparently no further experiments of this type have been performed. In a clinical trial, however, RA, topically applied to the cervix, produced responses in 12 of 36 patients with cervical dysplasia (143).

Summary and Conclusions

As indicated above, in some cases the effects of retinoids appear to be species-specific. Although retinyl acetate and 4-HPR are ineffective in preventing mammary cancer induced by DMBA or occurring spontaneously in mice (76, 145), these retinoids prevent carcinogen-induced mammary cancer in rats. In contrast, retinoids have modest chemopreventive activity for bladder cancer in various strains of both mice and rats and may have some therapeutic and preventive effects in human bladder (see 129). Retinyl palmitate is reported to reduce the incidence of esophageal lesions in hamsters (24); however, retinyl acetate may increase the incidence of esophageal tumors in rats (141). Although 13-*cis*-RA reduces the incidence of spontaneous thymic lymphomas in AKR mice and C57B1/10W mice exposed to X rays (103, 104) and has some therapeutic effect on myelodysplastic syndromes in humans (see 12), 4-HPR may enhance leukemic progression in patients with this syndrome (38). For treatment of this syndrome, selection of the proper retinoid appears to be important. Topically applied retinyl palmitate reduces the incidence of cervical cancer in hamsters (24), and topically applied RA has a therapeutic effect on cervical dysplasia in humans (143). Retinamides have a modest chemopreventive effect against pancreatic cancer in rats dosed with azaserine (see 27); these compounds are reported both to increase and to decrease the incidence of pancreatic cancer in hamsters (13, 73).

Retinoids may, or may not, be carcinogen-specific in different species. Some are effective in preventing mammary cancer in rats, regardless of which carcinogen is used (see 55). Applied to mouse skin, retinoids are active with either DMBA or BP as the carcinogen and 12-tetradecanoyl phorbol-13-acetate (TPA) as the promoter (41, 135). Nevertheless, retinoids are not effective in preventing skin papillomas and carcinomas caused by UV light (see 65). There is no comparable system for humans, although retinoids demonstrate activity against basal cell carcinomas (101), squamous cell carcinomas (67, 71, 87), and actinic keratoses (91) on the skin of humans. Fewer bladder tumors develop in rats dosed with HO-BBN when they are put on diets containing certain retinoids (see 55), but those dosed with FANFT are not affected (26). Similarly, retinyl acetate is reported to be active against liver tumors induced by 3'-MeDAB but not against those induced by aflatoxin B_1 (29, 95). In contrast, forestomach carcinomas induced in hamsters by either DMBA or BP are prevented by retinyl palmitate (24, 108, 124, 125).

The route of administration of retinoids may also be important. In the prevention of skin tumors, retinoids are effective when administered topically, intraperitoneally, or orally (15, 100, 135). 4-HPR, however, must be administered orally, apparently so that it can be converted to an active metabolite (77). If retinyl palmitate or retinyl acetate is administered topically, an increased frequency of cancer in the buccal pouch of hamsters dosed with DMBA is observed (see 84); however, the frequency of cancer is decreased with oral administration of 13-*cis*-RA (43, 119, 120). Administration of retinyl palmitate or 4-HPR intrarectally increased the incidence of rectal tumors; administered orally, these retinoids had no effect (123).

The time and extent of dosing with retinoids is often important. To be most effective in preventing skin cancer, retinoids should be applied shortly before the promoter (135). Administration of retinoids prior to, but not after, the carcinogen, can result in more mammary tumors than if no retinoid is administered (46). Continued administration of retinoid, relative to administration of no retinoid, however, greatly reduces the number of tumors that appear.

Retinoids are often tissue-specific in their effects. As described above, retinoids appear to be effective in preventing cancer of the skin, oral cavity, blood, mammary gland, pancreas, and bladder. Convincing data is lacking, however, to indicate that retinoids have substantial effect in preventing cancer of the lung, esophagus, or colon. Data for the liver and forestomach are equivocal. A major limitation to the use of retinoids in humans is their toxicity, which includes embryopathy (10). Administered to mice and rats, retinoids with a free carboxyl group and the all-*trans* configuration are generally more toxic than others without these structural features (70, 110). Also, in general, the retinoids with teratogenic activity are those with the

all-*trans* configuration and with either a free carboxyl group or a structure that can readily be transformed to such a group (67a, 112, 148). Thus, all-*trans*- and 13-*cis*-*N*-ethylretinamide and 13-*cis*-*N*-(2-hydroxyethyl)retinamide have little teratogenic activity, and 13-*cis*-4-HPR has only 1/20 the embryotoxicity of RA (148, 149). 4-HPR also has limited teratogenic activity (66).

We now know more about the types of retinoids that are needed to achieve better chemoprevention. The results obtained to date are encouraging. In humans, retinoids appear to be effective in preventing cancer in some individuals. More success will likely be realized by continued development of short-term tests that are predictive of preventive activity in human organs with the highest cancer incidence; better procedures for classification of retinoids according to their mode of action; and more effective and less toxic analogs of retinoids with established activity. Of nearly 400 reported tests of retinoid activity in preventing cancer, about three fourths have involved one of six common retinoids: retinyl palmitate, retinyl acetate, retinoic acid, 13-*cis*-retinoic acid, etretinate, or 4-HPR, each of which has some undesirable toxic effects. New, effective, and less toxic retinoids are needed.

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