MINI REVIEW

An International Evaluation of the Cancer-Preventive Potential of Nine Retinoids

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Abstract

The International Agency for Research on Cancer (IARC) convened a working Group of experts in March 1999 to evaluate the cancer preventive potential of nine retinoids and to compile the fourth volume of the *IARC Handbooks of Cancer Prevention.* The handbook provides a comprehensive review of the relevant information in the published scientific literature through March 1999 on the potential role of all-*trans*-retinoic acid, 13-*cis*-retinoic acid, 9-*cis*-retinoic acid, all-*trans N*-(4-hydroxyphenyl)retinamide, etretinate, acitretin, N-ethylretinamide, targretin and LGD 1550 in cancer prevention. Of these, the data suggest that all-*trans*-retinoic acid, 13-*cis*-retinoic acid and N-ethylretinamide are not suitable for chemoprevention of cancer in humans either because they are too toxic, may enhance cancer occurrence or are ineffective. In contrast, 9-*cis*-retinoic acid, etretinate and acitretin show some promise, but more data are required, while all- *trans N*-(4-hydroxyphenyl)retinamide is quite promising. Targretin and LGD 1550 are of interest, based on theoretical grounds, but there are no significant human and little experimental data as yet.

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Introduction

In 1997 the International Agency for Research on Cancer (IARC) initiated its series 'IARC Handbooks of Cancer Prevention' with publication of a volume in which the cancer chemopreventive effects of non-steroidal anti-inflammatory drugs were subject to evaluation (IARC, 1997). The Handbook series has much in common with the IARC Monographs on 'The Evaluation of Carcinogenic Risks to Humans' which first appeared in 1972. The first volume of the new series contained four sections, each presenting a systematic summation of published data for a specific anti-inflammatory agent (aspirin, sulindac, piroxicam and indomethacin) and culminating in an evaluation of evidence for cancer chemopreventive activity (IARC, 1997). This volume (in common with all in the series) also contained a Preamble in which the organization and principles of the evaluation process are described, together with 'General Remarks' in which features common to anti-inflammatory agents and relevant to their impact on cancer are delineated. Volume 2 concerned 'Carotenoids' (IARC, 1998a), while Volume 3 was restricted to Vitamin A (IARC, 1998b), a scenario which anticipated subsequent evaluation of certain retinoids which have been employed in the context of chemoprevention.

The retinoids are a class of compounds structurally related to Vitamin A. In the last 30 years, more than 2,500 retinoids have been synthesized and biologically tested, with the objective of identifying those with an enhanced therapeutic ratio. Within the first generation of retinoids, all trans-retinoic acid (tretinoin) and 13-cis-retinoic acid (isotretinoin) were identified. The second generation included the aromatic retinoids etretinate and acitretin with an enhanced therapeutic ratio and the third generation the poly-aromatic retinoids with or without polar end groups. The majority of the retinoids have been studied for their cancer-preventive activity in

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experimental models, and some in clinical trials in humans. On 24-30 March, 1999, a working group of international experts met in Lyon to consider the existing evidence on the cancer-preventive activity of nine retinoids. The resulting handbook details the evidence considered, and summarizes the evaluations made by the working group, and should be referred to for relevant citations relating to the statements that follow (IARC, 1999).

The retinoids subject to evaluation by the working group were:

all-trans-retinoic acid
13-cis-retinoic acid (isotretinoin)
9-cis-retinoic acid
all-trans N-(4-hydroxyphenyl)retinamide
etretinate
acitretin
N-ethylretinamide
targretin (LGD 1069)
LGD 1550

It was recognized that in view of the many retinoids which might be considered for evaluation, the designation of nine would necessitate omitting a number of compounds including those at various stages of development, or which have been studied and are no longer under clinical consideration. Some of the newer retinoids are already in preliminary human study. Those eventually found to have a better risk/benefit ratio could eventually find a role for use in humans.

Evaluation of chemopreventive effects in humans is impossible by conventional observational analytic epidemiology studies (case-control and cohort) if an agent is not in use in the general population, as is the case for all the agents to be considered in this volume. Therefore the human evidence that was considered by the working group was derived almost exclusively from randomized trials, either with development of cancer as the endpoint, or utilizing some biomarker of neoplasia. Thus almost uniquely to date in this series, with the exception of sulindac in Volume 1 of the Handbook series (IARC, 1997), the human data arose largely as a consequence of calculated and orderly usage, a characteristic normally restricted to experimental studies. This availability of clinical trial data was a considerable advantage, as it meant that bias and confounding were largely taken care of in the design of the trial. However, it has one important disadvantage: conclusions must necessarily be restricted to the population studied in the trial, and extrapolation to other groups would be tenuous at best, and potentially in serious error. Further, many of the clinical trials considered involved individuals already diagnosed with one neoplasm, and hence known to be at substantial risk of a second primary tumor, either at the same or a different site but with similar etiology. In considering these trials, the working group found that often the evidence for chemopreventive efficacy was derived from a secondary data analysis (chemoprevention of the relevant cancer site had not been the primary hypothesis of the trial), and thus the findings require independent confirmation, which has not been reported thus far.

The relationship of toxicity of an agent to its beneficial effects (the risk/benefit or therapeutic ratio referred to earlier) is critical with regard to chemoprevention because the subjects considered for this approach to cancer prevention are healthy, and it is likely (with the exception of carriers of the rare dominant cancer-susceptibility genes) that their probability of not developing cancer is substantially greater than the risk that they will develop it. These considerations are not so prominent for agents considered for use among patients at risk for second primary tumors, as patients may be prepared to tolerate side-effects in this situation, regarding this as an extension of their therapy. Toxic effects also may be less problematic when retinoids are used in dermatology, a role for which at least one of the agents considered in this handbook has become almost standard therapy (e.g. 13-cis-retinoic acid in acne). Concerns over toxicity are also likely to be less problematic when the agent is used as therapy for a malignancy (e.g. all-trans-retinoic acid in acute promyelocytic leukemia). But this is not so for most chemoprevention applications, and therefore particular attention to the toxicity of these compounds was given during the evaluation by the working group.

The influence of retinoids on plasma retinol has relevant clinical implications as regard to toxicity and it might also be relevant for the preventive effect. all-trans-Retinoic acid and all-trans N-(4-hydroxyphenyl)retinamide were first shown to inhibit the concentrations of endogenous retinol in humans and in rats. The same effect has been found to occur with 13-cis-retinoic acid, 9-cis-retinoic acid and N-ethylretinamide. Endogenous retinol level reduction has been related with the high binding affinity of retinoids bearing modifications in the area of the retinol hydroxyl end group with retinol-binding protein, the specific protein that transports retinol.

Background to retinoid action

Retinoids are involved in signal transduction. Most of their effects are believed to be mediated through two families of retinoid receptors, retinoic acid (RARs a, b and g) and retinoid X (RXRa, b and g) receptors. These receptors belong to the superfamily of nuclear receptors (NR), comprising such diverse receptors as those for steroids and thyroid hormones, retinoids and vitamin D3, which are present in vertebrates, arthropods, and nematodes. The members of this superfamily act both as ligand-modulated transcriptional activators and/or suppressors, while for a large group of so-called "orphan" nuclear receptors no ligands exist or have not yet been found. Nuclear receptors may have acquired ligand-binding ability during evolution, suggesting that the ancestral NR was an orphan. Control of gene expression by retinoid receptors, like all nuclear receptors, results from both direct modulations of the activity of cognate gene programs, the mutual interference with the activity of other signalling pathways and regulatory events that occur at the post-transcriptional level (e.g., mRNA and/or protein stabilization or

destabilization).

Retinoid receptors regulate complex physiological events that trigger key steps during development, control maintenance of homeostasis, and induce or inhibit cellular proliferation and differentiation, and cell death. Importantly, retinoid receptors display a strong differentiative and anti-proliferative activity. Each of the subtypes of retinoid receptors includes three isotypes designated a, b, and g, localized to chromosomes 17q21, 3p24,and 12q13, respectively. The RXRa, RXRb, and RXRg genes have been mapped to chromosome 9q34.3, 6p21.3 and 1q22-23, respectively. The RARs bind both all-trans-retinoic acid and 9-cis-retinoic acid, whereas the RXRs bind only 9-cis-retinoic acid. These receptors also bind a variety of synthetic retinoids, some of which exhibit RAR or RXR selectivity or preferentially bind to specific RAR isotypes.

Retinoids induce cellular differentiation or suppress proliferation in many malignantly transformed cell lines including epithelial cancers, melanoma, neuroblastoma and leukaemia, and germ cell, bone and breast cancers. The most plausible mechanism by which retinoids may affect various aspects of carcinogenesis (both early and late events) is modulation of genes whose products regulate cell growth, differentiation and apoptosis. Indeed, some genes involved in cell cycle control such as $p21^{WAF1/Cip1}$ and cyclin D1 or in differentiation are regulated by retinoid-activated receptors.

Immediate evidence that changes in retinoid receptors may be associated with carcinogenesis is based on the chromosomal translocation of RAR b in acute pro-myelocytic leukemia and loss of RAR b expression in various malignant cells and tissues.

Summary of the literature and results of the evaluations

1. all-trans-Retinoic acid

No studies have been reported of the use of all-trans-retinoic acid for the prevention of invasive cancer in humans. The results of one randomized, controlled trial indicate that topically applied all-trans-retinoic acid is effective in reversing moderate dysplasia of the uterine cervix (cervical intra-epithelial neoplasia-II) but not against more severe dysplastic lesions. It was reported to be efficacious against actinic keratosis of the skin in one randomized trial but not in another at a lower dose. Two further trials suggest that topically applied all-trans-retinoic acid is effective against dysplastic naevi.

The preventive efficacy of all-trans-retinoic acid was evaluated in experimental models of skin, liver and mammary gland carcinogenesis. The results of several experiments in mice indicated that all-trans-retinoic acid was effective against two-stage skin carcinogenesis when 7,12-dimethylbenz[a]anthracene was used as the initiator, whereas it enhanced skin carcinogenesis induced by this carcinogen alone or with ultraviolet radiation. One study in mice indicated that all-trans-retinoic acid enhanced N-nitrosodiethylamine-induced liver carcinogenesis, and in one study in rats, it was ineffective against N-methyl-N-nitrosourea-induced mammary carcinogenesis.

In vitro, all-trans-retinoic acid inhibited the transformation of normal cells by carcinogens and of immortalized cells by viral oncogenes. all-trans-Retinoic acid inhibited cell proliferation in monolayer cultures and modulated the differentiation of a large number of immortalized, transformed and tumorigenic cell types derived from trachea, skin and cervical epithelia. all-trans-Retinoic acid also suppressed the anchorage-independent growth of a variety of tumor cell lines and abnormal squamous differentiation in immortalized, transformed and arrested cells in the G_1 phase of the cell cycle.

Inhibitory effects of all-trans-retinoic acid against carcinogen-induced genotoxicity were most often observed when agents were used that required bioactivation. Inhibition of carcinogen-induced neoplastic transformation in vitro occurs when all-trans-retinoic acid is added after carcinogen exposure. Studies in animals and humans after topical application of all-trans-retinoic acid have demonstrated alterations in enzymes that mediate carcinogen metabolism.

Most studies of the effects of all-trans-retinoic acid on carcinogenesis indicate that inhibition of the post-initiation stage is the main mechanism of its putative preventive effects. The mechanisms of action may be related to growth inhibition, induction of differentiation and/or apoptosis.

It was concluded by the IARC working group that there is inadequate evidence that all-trans-retinoic acid has cancer-preventive activity in humans, and inadequate evidence that it has cancer-preventive activity in experimental animals (IARC, 1999). In addition, all-trans-retinoic acid therapy gives rise to significant toxicity, and is an established teratogen in experimental animals.

2. 13-cis-Retinoic acid

Secondary analyses of the results of one randomized trial of the use of 13-cis-retinoic acid as adjuvant therapy for cancers of the head and neck indicated a statistically significant reduction in the incidence of second primary tumours of the upper aerodigestive tract. A study of use of 13-cis-retinoic acid at high doses and in a group at inherited high risk, with no controls, suggested that this compound is effective in preventing basal- and squamous-cell cancers of the skin. Two randomized controlled trials among patients at lower risk involving lower doses of 13-cis-retinoic acid have shown no evidence of preventive efficacy.

High doses of 13-cis-retinoic acid were shown to be effective against oral leukoplakia in two randomized trials, one with controls receiving placebo and the other receiving b-carotene. One controlled trial showed no effect of 13-cis-retinoic acid in reducing cytological changes in the bronchi. Studies of molecular markers suggested that 13-cis-retinoic acid increases expression of human retinoic acid receptor b, but the relevance of these findings to cancer-preventive activity is unclear.

A single intervention study showed a decrease in micronucleus formation in cells of the buccal cavity in patients, some of whom were smokers, who had been treated with 13-cis-retinoic acid for 12 months.

13-cis-Retinoic acid is a confirmed human teratogen. The potential developmental toxicity associated with maternal therapy with this retinoid depends on the dose, the stage of gestation, the duration of treatment and the route of administration.

The preventive efficacy of 13-cis-retinoic acid has been evaluated in two-stage skin carcinogenesis models in mice and in urinary bladder carcinogenesis models in mice and rats. 13-cis-Retinoic acid was effective in most studies with both models. It was ineffective in models of tracheal, salivary gland, esophageal and renal carcinogenesis.

In vitro, 13-cis-retinoic acid inhibited proliferation in numerous cell lines. 13-cis-Retinoic acid inhibited growth in both monolayers of adherent cell cultures and in semi-solid medium (anchorage-independent growth). 13-cis-Retinoic acid also induced cell differentiation in transformed cells and triggered apoptosis in a few cell lines. In most cell lines, the response to 13-cis-retinoic acid was similar to that to all-trans-retinoic acid.

The ability of 13-cis-retinoic acid to inhibit genetic and related effects in cell cultures has been examined in a limited number of studies, and these have yielded mixed results. In two studies, a reduction in the frequency of chromosomal damage was seen in human lymphocytes exposed to radical-generating agents (bleomycin and X-irradiation) when they were pretreated with 13-cis-retinoic acid; in contrast, a third study showed an increase in the frequency of diepoxybutane-induced sister chromatid exchanges and chromosomal damage in human lymphocytes treated concurrently with the mutagen and the retinoid.

Orally administered 13-cis-retinoic acid inhibited the induction of micronucleated cells in the bone marrow of animals treated with benzo[a]pyrene and reduced the binding of this carcinogen to DNA in the liver, stomach and lung, but not the kidney. Although the mechanism of this protective effect is unknown, it might be related to alterations in microsomal enzyme activity which has been shown to occur in both liver and skin of mammals treated with 13-cis-retinoic acid.

The working group concluded that there is limited evidence that 13-cis-retinoic acid has cancer-preventive activity in humans (IARC, 1999). This evaluation was based on its effectiveness against oral leukoplakia, and preliminary evidence for prevention of second primary cancers of the aerodigestive tract. There is also limited evidence that 13-cis-retinoic acid has cancer-preventive activity in experimental animals (IARC, 1999). This evaluation was based on the observation of inhibitory effects in most but not all studies with models of skin and urinary bladder carcinogenesis.

However, 13-cis-retinoic acid has a relatively low therapeutic ratio of efficacy to toxicity, and is an established human teratogen.

3. 9-cis-Retinoic acid

No data were available to the Working Group on putative cancer preventive activity in humans.

The cancer preventive efficacy of 9-cis-retinoic acid was evaluated in two animal studies on carcinogen-induced mammary carcinogenesis, one on prostate carcinogenesis and one on colon carcinogenesis in rats. 9-cis-Retinoic acid prevented mammary and prostate tumors but not colon tumors; however, it reduced the numbers of aberrant crypt foci and adenomas in the colon.

In general, the in vitro effects of 9-cis-retinoic acid in vitro were similar to those of all-trans-retinoic acid, in that both inhibited cell proliferation and induced differentiation and apoptosis in some cell lines; however, the 9-cis isomer was more potent than the all-trans isomer in several cell systems. 9-cis-retinoic acid caused growth inhibition in normal, immortalized and malignant cell lines, often but not always in G_0 or G_1. Induction of differentiation and apoptosis were seen in several types of cells. The cells that were sensitive to 9-cis-retinoic acid responded to concentrations that are achieved in plasma with standard pharmacological doses.

The ability of 9-cis-retinoic acid to inhibit carcinogen-induced genotoxicity has not been studied in vitro or in vivo; however, two studies suggest that it might reduce carcinogen induced DNA damage by altering the activity of some cytochrome P450 isozymes both in vitro and in vivo.

9-cis-Retinoic acid suppresses cell proliferation and increases differentiation and apoptosis. The mechanisms by which proliferation is inhibited may involve antagonism of AP-1, decreased concentrations of cyclins, increased amounts of cyclin-dependent kinase inhibitor and inhibition of growth-stimulating signalling pathways. Induction of apoptosis and differentiation also appear to contribute to the putative cancer-preventive effect of 9-cis-retinoic acid.

The working group concluded that there is inadequate evidence that 9-cis-retinoic acid has cancer-preventive activity in humans (IARC, 1999). There is limited evidence that 9-cis-retinoic acid has cancer-preventive activity in experimental animals (IARC, 1999). This evaluation was based on the observation of inhibitory effects in two studies of mammary carcinogenesis and one study of prostate carcinogenesis in rats. 9-cis-Retinoic acid is a teratogen in mice.

4. all-trans N-(4-hydroxyphenyl)retinamide

In a preliminary report of a large randomized trial of use of all-trans N-(4-hydroxyphenyl)retinamide, equivocal results were obtained with regard to the development of new contralateral tumors among women previously treated for early breast cancer. There were fewer new cancers among treated pre-menopausal women but more cancers among treated post-menopausal women. A decrease in the risk for ovarian cancer was reported among all treated women in this trial.

Two studies, only one of which was randomized, of

intermediate end-points, suggested an effect of all-trans N-(4-hydroxyphenyl)retinamide against oral leukoplakia. A possible effect on ploidy in urinary bladder cells has also been described.

The chemopreventive efficacy of all-trans N-(4-hydroxyphenyl)retinamide has been evaluated in animal models of mammary gland, prostate, lung, skin, urinary bladder, and colon carcinogenesis and lymphomagenesis. It was effective in reducing tumour incidence or multiplicity in 11 of 12 studies of mammary carcinogenesis in mice or rats. The results of such studies depend critically on the experimental conditions, including the strain and age of the animals, their diet and the dose of both carcinogen and retinoid. It was effective in one study in a model of urinary bladder carcinogenesis in mice and ineffective in another and effective in one study of prostate carcinogenesis but not in two others. It was ineffective in one study of lung carcinogenesis in mice. It was effective in one study of carcinogenesis of the colon and in two studies of lymphomagenesis in mice. In one skin carcinogenesis study in mice, all-trans N-(4-hydroxyphenyl)retinamide was ineffective, or enhanced skin tumor development.

In-vitro studies suggest that all-trans N-(4-hydroxyphenyl)retinamide can affect carcinogenesis at several levels: it inhibited the transformation of cultured cells and of tissue in organ culture; it inhibited the proliferation of a variety of tumour cell lines; and it induced apoptosis but it rarely induced differentiation.

There are insufficient data to conclude whether all-trans N-(4-hydroxyphenyl)retinamide can reduce the genotoxic effects of carcinogens in vitro or in vivo. Indications that it alters the metabolism of carcinogens and thus may affect DNA damage are provided by a study showing alterations to cytochrome p450 mRNA levels in cell cultures exposed to the retinoid, and a study in which phase I and phase II enzymes were shown to be altered in the liver of animals fed this compound. The altered metabolism was associated in vivo with a reduction in the binding to tissue DNA of a carcinogen known to be metabolized by these enzymes.

Few reports indicate any activity of all-trans N-(4-hydroxyphenyl)retinamide at the initiation stage of carcinogenesis and most suggest it acts on tumour promotion. The mechanisms that may account for the cancer-preventive effects of this retinoid appear to be associated with its ability to inhibit cell proliferation by increasing the amount of a cyclin-dependent kinase inhibitor and by down-regulating cyclin D1 and inducing apoptosis which has been extensively studied. Its limited effects on differentiation raise doubts as to whether this is a mechanism for cancer prevention. The high concentrations required to induce apoptosis in vitro restrict extrapolation of these studies to infer relevance to cancer chemoprevention in vivo.

The working group concluded that there is inadequate evidence that all-trans-N-(4-hydroxyphenyl)retinamide has cancer preventive activity in humans (IARC, 1999). However, there is sufficient evidence that all-trans N-(4-hydroxyphenyl)retinamide has cancer preventive activity in experimental animals (IARC, 1999). This evaluation was based on the observation of inhibitory effects in models of mammary carcinogenesis in mice and rats and its effectiveness in a limited number of studies against prostate and colon carcinogenesis and lymphomagenesis. all-trans N-(4-Hydroxyphenyl)retinamide does not have significant toxicity in humans with the dose schedule normally used. Therefore, all-trans N-(4-hydroxyphenyl)retinamide shows promise as a cancer preventive agent in humans.

5. Etretinate

Etretinate was evaluated in six randomized trials for efficacy in preventing the recurrence of superficial tumours of the urinary bladder. None showed unequivocal evidence of an effect of treatment; efficacy was suggested in analyses of some end-points. Etretinate was not effective in preventing second primary tumours in subjects with head-and-neck cancer when compared with those given placebo. In two reports of the same study without a separate control group, etretinate was reported to reduce an index of metaplasia in bronchial biopsy samples from heavy smokers. In a randomized trial involving 150 subjects, however, etretinate showed no efficacy in reducing atypia in sputum samples when compared with placebo. In one study with no controls in which etretinate was given orally at a high dose or orally at a moderate dose plus topical application as a paste, regression of leukoplakia of the mouth was reported, more notably when topical application was added. In one study with no controls, oral treatment with etretinate appeared to reduce the severity of actinic keratotic and keratocanthoma lesions of the skin. In two double-blind cross-over trials involving patients with actinic keratosis, improvement in terms of the number and size of lesions was reported in patients treated orally with etretinate.

The cancer-preventive efficacy of etretinate has been assessed in mouse, rat and rabbit models of carcinogenesis and in relation to virus-induced tumours. It was ineffective in inhibiting UV-induced skin carcinogenesis in mice, but inhibited chemically-induced skin tumours in mice and rabbits. In one study in mice, etretinate reduced the size of skin papillomas. It was effective in various models of digestive tract carcinogenesis in mice and rats. In single studies, etretinate was ineffective in preventing either leukaemia or lung tumours but was effective in preventing urinary bladder carcinogenesis in rats. It was effective in a model of benign tumours induced in mice by Shope papilloma virus and in models of malignant tumours in hamsters and chickens induced by Rous sarcoma virus. In some experimental models, etretinate enhanced the tumorigenic effects of carcinogens.

Etretinate has been shown to modify differentiation in several models in vitro: in tracheas of hamsters, squamous metaplasia induced by vitamin A deficiency was reversed. In respiratory tracts exposed to carcinogens, etretinate inhibited loss of mucus secretion and ciliary action. In many studies with human and animal keratinocytes, etretinate

causes changes in differentiation similar to those seen after treatment with all-trans-retinoic acid. In contrast to all-trans-retinoic acid, etretinate did not induce differentiation in promyelocytic leukemic cell lines. Proliferation was inhibited in murine and human melanoma cell lines, in lymphoblastoid lines and in normal keratinocytes. Because of differences in the experimental protocols, it is not clear whether etretinate is selectively active against tumour cells. In all cases, it was less active than all-trans-retinoic acid. Etretinate has been studied in many in-vitro models of immune function, but no consistent responses were reported.

There have been no detailed studies of the mechanism of action of etretinate. Its ability to inhibit the induction of ornithine decarboxylase in keratinocytes after treatment with phytohaemagglutinin suggests that, like all-trans-retinoic acid, it acts in the promotional phase of carcinogenesis.

The working group concluded that there is inadequate evidence that etretinate has cancer preventive activity in humans (IARC, 1999). There is limited evidence that etretinate has cancer preventive activity in experimental animals (IARC, 1999). This evaluation was based on the observation of inhibitory effects in studies with models of skin cancer, and in single studies with models of digestive tract and urinary bladder carcinogenesis and in three models of virus-induced tumors. However, etretinate is toxic, and a human teratogen, and in most countries is no longer available for use.

6. Acitretin

The active form of etretinate is acitretin. In one trial with 44 renal transplant patients, acitretin reduced the frequency of occurrence of squamous-cell cancers of the skin when compared with placebo. In the same trial, the prevalence of keratotic skin lesions was also reduced by acitretin. When treatment was stopped, the numbers of cancers and keratotic skin lesions increased.

In single experimental studies, acitretin reduced the incidence of spontaneous and chemically induced liver tumors in mice and rats in conjunction with reductions in body weight.

Acitretin has been tested for its ability to inhibit proliferation or to induce differentiation of tumour and normal cells in vitro. Acitretin was more active than etretinate, and both were less active than all-trans-retinoic acid and 13-cis-retinoic acid. Acitretin had an anti-proliferative effect on some but not all tumour cell lines that were tested. Several studies with epidermal cells showed that the effects of acitretin depended on the culture conditions and/ or the proliferation rate. It did not induce differentiation of leukemic cells in vitro. In normal epidermal cells, it decreased cornified envelope formation and modified the pattern of keratin. In studies of lymphocyte proliferation in vitro, the effects depended on the concentration of acitretin and on the mitogen used to induce proliferation.

The differentiating effects of acitretin on epidermal cells may be associated with modifications in the pattern of keratin expression and membrane glycosylation. The only mechanism that has been studied in relation to the anti-proliferative activity of acitretin is inhibition of ornithine decarboxylase activity, which is more marked in hyperproliferative states. The effects of acitretin on immune function have not been studied extensively. It stimulated the production of interleukin-1 both in vitro and in vivo, which might result in activation of lymphoid cells. An effect of acitretin, which might contribute to cancer-preventive activity, is inhibition of angiogenesis which has been described in one study.

The working group concluded that there is inadequate evidence that acitretin has cancer-preventive activity in humans and there is inadequate evidence that acitretin has cancer-preventive activity in experimental animals (IARC, 1999). However, acitretin is a derivative of etretinate, and therefore probably has similar cancer preventive efficacy to etretinate. Further, its toxicity is less than etretinate, though it is a potent teratogen in experimental animals.

7. all-trans-N-Ethylretinamide

No data were available to the Working Group on the putative cancer preventive activity of all-trans-N-ethylretinamide in humans.

The cancer preventive efficacy of all-trans-N-ethylretinamide has been evaluated in models of respiratory tract and pancreas carcinogenesis in hamsters, of liver carcinogenesis, in mice, of urinary bladder carcinogenesis in mice and rats and of colon carcinogenesis in rats. Tumour incidence was enhanced in the trachea and pancreas of hamsters, in the liver in mice, and in one study, in the urinary bladder in rats. all-trans-N-Ethylretinamide had cancer preventive effects in some studies of urinary bladder carcinogenesis in mice and rats but was ineffective in models of colon carcinogenesis. all-trans-N-Ethylretinamide inhibited carcinogen-induced neoplastic transformation at concentrations similar to those at which all-trans-retinoic acid had this effect. In the hamster trachea it was less potent than all-trans-retinoic acid in reversing squamous metaplasia; when tested in chick skin for an equivalent endpoint, its activity was similar to all-trans-retinoic acid.

The working group concluded that there is inadequate evidence that all-trans-N-ethylretinamide has cancer preventive activity in humans, and there is evidence suggesting lack of cancer preventive activity in experimental animals (IARC, 1999). all-trans-N-Ethylretinamide was never approved for use in humans and is no longer being produced.

8. Targretin

No data were available to the Working Group on the putative cancer preventive activity of targretin in humans.

In a single study of three months duration, targretin was effective in preventing mammary cancer induced by N-

methyl-N-nitrosourea in rats. In two models of differentiation in human cells in vitro, targretin, which preferentially binds to the RXRs, was less active than ligands binding to retinoic acid receptors; however, in both models supra-additive activity was seen when the cells were treated simultaneously with targretin and the ligands binding to retinoic acid receptors.

The working group concluded that there is inadequate evidence that targretin has cancer-preventive activity in humans and inadequate evidence that targretin has cancer-preventive activity in experimental animals (IARC, 1999).

9. LGD 1550

No data were available to the Working Group on the potential cancer preventive activity of LGD 1550 in humans or in experimental animals. LGD 1550 inhibited proliferation in human breast cancer cells expressing retinoic acid receptor-a, but not in cells that did not express this receptor.

The working group concluded that there is inadequate evidence that LGD 1550 has cancer-preventive activity in humans and in experimental animals (IARC, 1999).

Discussion

Of the retinoids examined, none were considered by the Working Group as exhibiting sufficient evidence of cancer preventive activity in humans (IARC, 1999). Working groups have so far concluded that for chemoprevention such categorization requires findings from randomized controlled trials with malignant disease as an endpoint, as was the case for aspirin in the first evaluation (IARC, 1997). Though such an outcome is theoretically possible, the limitations of organizing such trials are now widely appreciated. It seems likely that reliance will increasingly be placed on 'intermediate effect' biomarkers for the purpose of comparing retinoids or similar agents thought to have chemopreventive activity.

One of the compounds considered, N-hydroxyphenylretinamide, is characterized by a considerable body of essentially positive experimental findings but relatively little human data. The working group considered that the experimental findings provide a positive encouragement to examining chemopreventive activity of this compound in humans (IARC, 1999). Nonetheless, this scenario highlighted a specific aspect of such experimental studies. By comparison with the massive amount of data which underpins extrapolation of carcinogenesis findings from experimental animals to humans, the amount of analogous data in relation to cancer chemoprevention is small.

The biology of retinoid action, specifically in relation to RARs and RXRs, has been subject to intense molecular analysis. However, despite the advances that have been made in this field, there were few instances when such 'mechanistic' data significantly influenced evaluation of cancer chemopreventive activity exhibited by individual retinoids.

Nevertheless, recent advances in the understanding of the biochemical and molecular mechanisms of retinoid action, raise the hope that new synthetic retinoids can be synthesized that may be useful in chemoprevention of cancer. Such compounds may target specific molecules, and may result in far fewer unwanted side-effects than those in use up till now. In this regard the new retinoid receptor subtype specific agonists hold particular promise.

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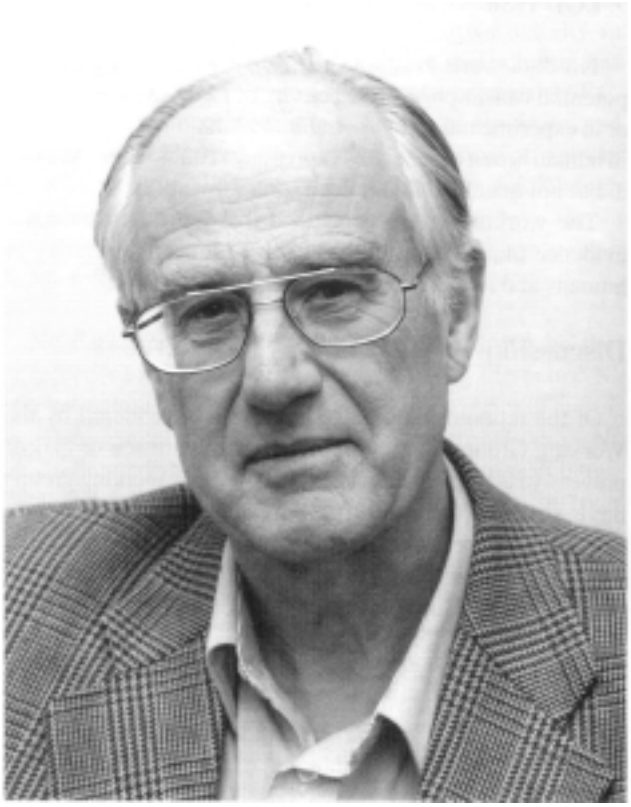
Notes: A.B.Miller was Senior Epidemiologist, International Agency for Research on Cancer, Lyon and Acting Chief, Unit of Chemoprevention, at the time of the working group meeting reported in this paper; P. Nettesheim was visiting Scientist in the Unit of Chemoprevention during the same time period, and B.W. Stewart was Chair of the Working Group that evaluated retinoids.

Appendix: List of Participants. Retinoids Meeting

J.S. Bertram, Cancer Research Center of Hawaii, USA; W.S. Blaner, Columbia University, New York, USA; R.A. Chandraratna, Allergan, Irvine, CA, USA; C. Chomienne, Hôpital Saint Louis, Paris, France; J.A. Crowell, National Cancer Institute, Bethesda MD, USA; M.I. Dawson, Molecular Medicine Research Institute, Mountain View, CA, USA; F. Formelli, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy; E.R. Greenberg (Vice-Chairman), Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA ; H. Gronemeyer, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; D.L. Hill, University of Alabama at Birmingham, AL, USA; R.J. Kavlock, Environmental Protection Agency, NC, USA; R. Lotan, M.D. Anderson Cancer Center, Houston, TX, USA; M. Maden, King's College, London, England; R.G. Mehta, University of Illinois at Chicago, IL; T.E. Moon, Ligand Pharmaceuticals Inc., San Diego, CA, USA; H. Nau, School of Veterinary Medicine Hannover, Germany; P. Nettesheim,

Personal Profile: Anthony Miller



Dr Miller qualified in Medicine from the University of Cambridge, England in 1955 and subsequently specialized in Internal Medicine. He became a Member of the Royal College of Physicians of London, England in 1964, and was elected a Fellow in 1987. In 1973 he became a Fellow of the Royal College of Physicians of Canada, in 1977 a Fellow of the Faculty of Public Health Medicine of the United Kingdom and in 1985 a Fellow of the American College of Epidemiology.

In 1962, he joined the British Medical Research Council as a member of the scientific staff of the Tuberculosis and Chest Diseases Unit. While with the Medical Research Council he was responsible for clinical trials and epidemiological studies in lung cancer and pulmonary tuberculosis.

In April 1971, he went to Canada to set up the Epidemiology Unit of the National Cancer Institute of Canada. In 1986 he became full-time in the Department of Preventive Medicine and Biostatistics of the University of Toronto where he was Director of the MSc/PHd Specialization in Epidemiology from 1986-91 and Chairman of the Department 1992-96. In 1996 he became Emeritus, and since then has served as a special expert in the Early Detection Branch, US National Cancer Institute and Senior Epidemiologist in the International Agency for Research on Cancer in Lyon. He was acting Chief of Chemoprevention in the Agency in the years 1998-99. He was seconded to the Deutsches Krebsforschungszentrum in 1999 and is currently head of the Division of Clinical Epidemiology. His current research programme continues his interests in diet and cancer, screening for cancer, and the application of knowledge to cancer control.

Dr Miller has served on numerous scientific advisory committees in Canada and the United States as well as Internationally. He has over 300 scientific publications to his credit.