Retinoids in cancer prevention and therapy

W. Bollag & E. E. Holdener
Department of Clinical Research, Division of Oncology & Hematology, F. Hoffmann-La Roche Ltd, Basel, Switzerland

Summary. Retinoids are a class of compounds structurally related to vitamin A. In preclinical studies, all-trans retinoic acid (tretinoin), 13-cis retinoic acid (isotretinoin) and the aromatic retinoids etretinate and acitretin have preventive and therapeutic effects on carcinogen-induced premalignant and malignant lesions. Clinically, chemoprevention with isotretinoin and etretinate has been tested with some degree of success in such indications as basal cell carcinomas, squamous cell carcinomas, superficial bladder tumors and second primary tumors in patients with squamous cell carcinoma of the head and neck. Limited therapeutic success has also been achieved with retinoid treatment of precancerous and cancerous conditions of the skin, oral cavity, larynx, lung, bladder and vulva. Dramatic therapeutic effects have been observed in the treatment of acute promyelocytic leukemia with tretinoin, which leads to very high rate of complete remission. Excellent results were recently reported in the treatment of squamous cell carcinomas of the skin and cervix with a combination of isotretinoin and recombinant interferon alfa-2a (rIFN alfa-2a, Roferon®-A). The mechanism of action of retinoids is through modulation of cell proliferation and differentiation. Retinoids vary in their capacity to induce differentiation and to inhibit proliferation in a series of human transformed hematopoietic and epithelial cell lines. Some cytokines potentiate the retinoid-induced cell differentiation and act synergistically with retinoids to inhibit cell proliferation. The pattern of synergism is dependent upon the combination and tumor cell line tested. The discovery of nuclear retinoid receptors has contributed substantially to the understanding of the mechanism of action of retinoids at the molecular level. Further understanding of the molecular biology of retinoids is expected to contribute to a rational design of new retinoids in the future, which in turn may result in improvements in the prevention and therapy of cancer.

Key words: retinoids, cancer prevention, cancer therapy, cytokines, combination therapy, mechanism of action

Introduction

The retinoids are a class of chemical compounds structurally related to vitamin A and comprised of natural and synthetic analogs. A correlation between vitamin A and cancer was first noted in the nineteen twenties, when experimentally-induced vitamin A deficiency was shown to lead to hyperplastic, metaplastic and dysplastic tissue changes, i.e.preneoplastic lesions and ultimately neoplasms [1-4]. Forty years later, a preventive effect of vitamin A on the development of chemically-induced tumors was demonstrated in animal models [5-9]. Further experiments showed that, in addition to its preventive action, vitamin A also had a therapeutic effect in cancer [10, 11]. The antitumor effect was not only associated with vitamin A (retinol) but also with the natural metabolite vitamin A acid (all-trans retinoic acid), as well as other synthetic retinoids [10-27]. This was the basis for the clinical use of retinoids in the prevention and therapy of a variety of precancerous and neoplastic diseases.

In the 1940s, preclinical studies suggesting preventive and therapeutic antitumor effects of vitamin A led to clinical trials of systemic vitamin A in the treatment of precancerous lesions, e.g. actinic keratosis and oral, cervical and vulval leukoplakia [10, 17]. However, the success rate was low because the high doses required led to undesirable side effects, classic symptoms of hypervitaminosis A. The objective of Roche research was to identify and develop retinoids with an improved risk/benefit ratio. In 1968 Roche began synthesizing retinoids by chemical modification of the vitamin A molecule [17, 18, 22]. The screening model initially used was chemically-induced papilloma in the mouse [11, 14]. Since this time, more than 2,500 retinoids have been synthesized and biologically tested. The compounds with the most promising therapeutic index have been subject to clinical trials. Within the first generation of retinoids, all-trans retinoic acid (tretinoin) and 13-cis retinoic acid (isotretinoin) were identified as promising candidates, the second generation of retinoids included the aromatic retinoids etretinate and acitretin and the third generation produced the polyanilic androgens with or without oral end groups. These compounds were found useful in the treatment of various dermatological conditions, such as acne, psoriasis and other disorders of keratinization and lichen ruber planus [17, 18, 22, 28]. In addition, some of them clearly showed activity in the treatment of malignant diseases [17, 18, 20, 23, 29, 30].
Nuclear retinoic acid receptors and cytoplasmatic retinoic acid binding proteins

Studies on the mechanism of action of retinoids have progressed in the last few years with the discovery of the nuclear retinoic acid receptors. In the past, retinoid binding proteins such as the cytosolic cellular retinoic acid binding proteins I and II (CRABPI and CRABPII) were well characterized [31, 32] although previously mistaken for receptors. However, these binding proteins are responsible for the intracellular transport of retinoids and the regulation of retinoid concentration. Furthermore, they may play a role in the metabolism of retinoic acid. Different affinities for CRABP are suggested for the various retinoids.

To date, six nuclear retinoic acid receptors have been identified [33–44]. They fall into two subclasses: the RAR receptors α, β and γ which have a high affinity for all-trans retinoic acid and the RXR receptors α, β and γ which do not bind to this ligand. However, 9-cis retinoic acid has been shown to be a ligand for RARα [45, 46]. All these receptors belong to the superfamily of steroid-thyroid hormone receptors which share sequence homology in the DNA-binding domain. The homology within the amino acid sequence (Fig. 1) in the DNA and ligand binding domains is very high among the receptors in the same subgroup (RAR or RXR). These receptors presumably function in the following way (Fig. 2): Retinoic acid or other retinoids first bind to the ligand-binding domain E of the receptor, perhaps inducing a conformational change of the receptor. The activated receptor then activates or represses transcription of genes containing a retinoic acid response element (RARE) (recognized by the DNA-binding domain C of the receptor). These transcriptional events result in the synthesis of mRNAs and proteins which might be responsible for biological functions such as cell proliferation and differentiation. It is not known, however, whether individual receptors have specific biological functions. Two or more receptors might cooperate in the transcription of responsive genes and thus enable a specific biological function [41–43]. Recently the formation of heterodimers between RXRs and RARs as well as other members of the superfamily of nuclear hormone receptors has been demonstrated. The variety of specific heterodimers, each one having specific transcriptional properties, might explain the pleiotropic action of retinoids [47]. The tissue distribution of the different receptors varies considerably. The RARα, for example, is expressed in many adult tissues, whereas the RARγ receptor shows a preferential expression in adult skin. It is quite possible that normal and aberrant receptors have different activities once bound. This might explain the differences among retinoids regarding their ‘specific’ preventive and therapeutic effects on precancerous and cancerous lesions.

Mechanism of antitumor action

Development and maintenance of normal tissue depends on an adequate balance between growth and differentiation as well as cell renewal and cell loss. In malignancies, this equilibrium is disturbed. There is good evidence that the anti-tumor activity of retinoids as well as of cytokines interacting with retinoids is at least partially due to either induction of cellular differentiation [48–54] and/or inhibition of cell proliferation [50, 55–57]. The effect of retinoids on cell proliferation and differentiation based mainly on investigations using human transformed tumor cell lines is discussed below.

Induction of differentiation

Tretinoin and other retinoids can induce differentiation in certain malignant cell lines in mice and humans, such as acute promyelocytic leukemia HL-60 [19, 48, 49], histiocytic lymphoma U 937 [48, 58], neuroblastoma LA-N-1 [52], teratocarcinoma F9 [53], and embryonal carcinoma [54, 59, 60]. This differentiation can be ascribed to either morphologically or by means of specific markers characteristic of the differentiated cell. The oncologists’ and biologists’ hope was to force the malignant cell to give up its properties of autonomous proliferation and aggressive growth and, through differentiation, resume its original physiological function. Complete clinical remissions have recently been achieved in patients with acute promyelocytic leukemia, whereby the proliferating, non-differentiated leukemic promyelocyte matures into a non-proliferating,
differentiated granulocyte [61–72]. This clinical model of a differentiation therapy has generated tremendous interest in the 'physiologic' therapeutic approach to cancer. Systematic investigations on the differentiation-inducing effect of various retinoids and of a panel of cytokines as well as of their combination [73, 74] have recently been performed using human transformed hemopoietic cell lines such as HL-60 (human acute promyelocytic leukemia) and U937 (human histiocytic lymphoma). The effect of differentiation was determined by the induction of an oxidative burst, as measured by the capacity to reduce nitroblue tetrazolium (NBT). The characteristic property of granulocytes and/or monocytes/macrophages to produce oxygen radicals was taken as an index of their status of differentiation.

Tretinoin  
All-trans-retinoic acid

Isotretinoin  
13-cis-retinoic acid  
ROACCUTAN®/ACCUTANE®

9-cis retinoic acid

Acitretin  
NEOTIGASON®/SORIATANE®

Etretinate  
TIGASON®/TEGISON®

Ro 13-7410

Ro 13-6307

Temarotene

Ro 14-6113

Fig. 3. Chemical structure of retinoids.

A variety of synthetic retinoids were selected for investigation using all-trans retinoic acid as a prototype. These retinoids have widely differing chemical structures (Fig. 3) and possess divergent pharmacological properties. The following retinoids have been examined, the three retinoic acid isomers, tretinoin (all-trans retinoic acid), isotretinoin (13-cis retinoic acid), and 9-cis retinoic acid; the active metabolite of etretinate, acitretin [all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid]; Ro 13-7410 [p-(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propanoic acid]; temarotene [1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-(E)-3-methylstyryl]napthalene]; the active metabolite of temarotene Ro 14-6113 [p-(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propanoic acid] and Ro 13-6307 (all-E)-3-methyl-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2,4,6-octatrienoic acid].

The effect of retinoids on the differentiation induction of HL-60 cells is summarized in Fig. 4. The aromatic retinoid with a triene side chain, Ro 13-6307, was the most potent inducer of differentiation among the compounds tested. Tretinoin also induced a high level of differentiation, whereas temarotene and its metabolite Ro 14-6113 failed to induce differentiation. These results suggest that the carboxylic acid end group may play a critical role in the capacity of retinoids to act as differentiation inducing agents. This is substantiated by the observation that the carboxylic acid end group is a crucial factor for retinoids to bind and/or activate the retinoic acid receptors RAR α and β [75]. Recently, 9-cis retinoic acid, the specific ligand for the retinoic acid receptors RXRa, β and γ was compared with all-trans and 13-cis-retinoic acid regarding their differentiation inducing effect (Bollag and Brockhaus, unpublished). 9-cis retinoic acid was markedly superior (100-fold) to the other isomers in inducing differentiation (Fig. 5).

Ro 13-6307 was also the most active of the retinoids tested in U937 cells. In contrast to HL-60 cells, the induction of differentiation in U937 cells could not be seen following treatment with acitretin or the arotinoid Ro 13-7410. Again, no activity was observed with retinoids lacking a carboxylic acid end group, such as Ro 14-6113.

Cytokines, e.g. interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4),
interferon-α (IFNa), interferon-β (IFNβ), interferon-γ (IFNγ), tumor necrosis factor-α (TNF-α), granulocyte-colony stimulating factor (G-CSF), epidermal growth factor (EGF), transforming growth factor-β1 (TGFβ1), and transforming growth factor-β2 (TGFβ2) have been shown to modulate differentiation of cells of the immune system, hematopoetic progenitor cells or a variety of other cell types [50, 57]. Several of these cytokines have previously been described to have little or no differentiation-inducing potential in transformed cell lines when used alone. However, they showed synergistic or a potentiation of activity in combination with all-trans retinoic acid [73, 74, 76–83].

In HL-60 cell lines, only retinoids containing a carboxylic acid end group such as tretinoin, isotretinoin, acitretin, Ro 13-7410 and Ro 13-6307, caused an increase in differentiation when combined with cytokines. IFNa, IFNγ, IL-1α, TNF-α and G-CSF increased the differentiation effect of all tested retinoids, whereas IFNβ, IL-1β and IL-4 were only active when given in combination with a particular retinoid. The highest degree of HL-60 differentiation was achieved with IFNγ and G-CSF in combination with retinoids (Fig. 6).

Similar results were observed with U937 cells which are, however, less sensitive to retinoids alone as well as to the combination of retinoids with cytokines.

Inhibition of proliferation

In addition to differentiation induction, the anti-tumor effect of retinoids has been associated with a direct antiproliferative effect. In 1980, the antiproliferative effect of all-trans retinoic acid was demonstrated in a series of transformed cell lines, including mammary, melanoma, lymphoid, fibroblastic and other cell lines from various species [55]. Potent antiproliferative activities were later observed in a number of squamous cell carcinoma cell lines either with retinoids as single agent [84–88] or in association with cytokines [80, 81, 84, 85, 89, 90].

In HL-60 cells, inhibition of proliferation is directly related to differentiation induction; the induction of an oxidative burst potential precedes the inhibition of proliferation by approximately 48 hours (Fig. 7), determined by the incorporation of tritiated thymidine. Inhibition of proliferation might therefore be a consequence of the induction of a terminal maturation process resembling that of normal granulocytes. In squamous cell carcinoma (SCC), retinoids seem to work through a direct antiproliferative action, and not by induction of differentiation. The assessment of differentiation induction in SCC is complicated by the absence of reliable biomarkers correlating with cell maturity. The inhibition of keratinization following treatment with retinoids is interpreted as a reflection of lack of differentiation [86–88]. Four human transformed epithelial cell lines, MCF7, mammary carcinoma, SCC4 and SCC15 squamous cell carcinomas of the tongue and A431 squamous cell carcinoma of the vulva were used to test inhibition of proliferation by retinoids. Proliferation rates were determined by the capacity of viable cells to reduce MTT dye [3-(4,5-di-
methylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide], an index of mitochondrial enzyme activity [84].

Tretinoin, isotretinoin and acitretin inhibited the proliferation of all four human transformed epithelial cell lines. High concentrations ($3 \times 10^{-5}$M to $3 \times 10^{-6}$M) were necessary to achieve inhibition of proliferation. Each cell line had its own profile of proliferative inhibition with the various retinoids. The arotinoid Ro 13-7410, containing a carboxylic acid end group, showed less inhibition of proliferation than the forementioned retinoids. Arotinoids lacking the carboxylic acid end group, such as temarotene and its metabolite Ro 14-6113, were devoid of any antiproliferative activity. Similar to differentiation-induction effects, a carboxylic acid end group is required for the antiproliferative activity of retinoids. This parallels the capacity of these latter retinoids to bind and/or activate the retinoic acid receptors RAR α and β [75]. In a recent investigation, the antiproliferative effects of all-trans, 13-cis and 9-cis retinoic acid were compared. There was no marked difference in the antiproliferative effect on SCC15 and A431 cells (Bollag and Brockhaus, unpublished).

A series of cytokines, including growth factors, are known to stimulate or inhibit the proliferation of a variety of either normal or transformed cell types [50]. We have examined the growth modulating effect of various cytokines, e.g. IFNα, IFNγ, TNF-α, IL-1, EGF, TGFβ and G-CSF, on four human transformed epithelial cell lines. In MCF7 cells, TNF-α inhibited cell proliferation to a greater extent than did TGFβ. SCC4 squamous cell carcinoma cell proliferation was inhibited in the following descending order of cytokine activity: IFNα > TGFβ > IFNγ > EGF. SCC15 cells were most markedly inhibited by IFNα, whereas only EGF significantly inhibited the proliferation of A431 cells.

A synergistic effect on the inhibition of proliferation was demonstrated when retinoids and cytokines were administered in combination. Tretinoin and isotretinoin were slightly more active than acitretin in combination with cytokines when tested with MCF7 mammary tumor and various squamous cell carcinoma cell lines (SCC4, SCC15 and A431). In the presence of retinoids, MCF7 cell proliferation was most markedly inhibited by TGFβ and TNF-α and to a lesser degree by IFNα followed by IFNγ. IFNα elicited the most marked synergism with retinoids in SCC4 cells (Fig. 8), followed in descending order of activity by EGF, TGFβ and IFNγ. IFNα was the only cytokine which showed significant synergistic activity in combination with retinoids against SCC15 cells (Fig. 9), whereas in A431 cells (Fig. 10), EGF elicited the strongest synergistic response followed by IL-1, IFNα and IFNγ. In summary, every cell line had a unique profile of proliferation inhibition by a particular cytokine when combined with retinoids. Recently, 9-cis retinoic acid, the specific ligand to RXRs, was investigated in combination experiments. 9-cis retinoic acid when combined with IFNα exerted the same synergistically inhibiting effect on proliferation of SCC15 and A431 cells as the other isomers all-trans and 13-cis retinoic acid (Bollag and Brockhaus, unpublished).

Clinical use of retinoids in cancer

Prevention of cancer

Clear preventive and inhibitory effects of retinoids on carcinogenesis have been shown in animal experiments.
with chemically-induced tumors, e.g. skin [13, 15], breast [24], bladder [26], lung and others [20, 23, 25]. This has generated increasing interest in the use of retinoids for tumor prevention in man. However, to carry out cancer prevention trials in a healthy population not belonging to a high cancer risk group, only those retinoids with virtually no acute and chronic toxicity would be acceptable. Unfortunately, no such retinoid is currently available for testing. It is only in individual patients with a high genetic or environmental cancer risk (e.g. patients with xeroderma pigmentosum, individuals with family history of high susceptibility to cancer, cancer patients with an increased chance to develop second primary tumors within a relatively short time after removal of first primary, or individuals who are constantly exposed to carcinogenic substances) that prophylactic treatment with retinoids can be seriously considered and has proven to be beneficial. The risk-benefit ratio must, however, be taken into account.

Cancer prevention studies have been carried out in indications such as xeroderma pigmentosum, basal cell carcinomas, squamous cell carcinoma of the skin, superficial bladder tumors and second primary tumors in head and neck carcinoma.

Another type of prevention of cancer is represented by the therapy of already existing precancerous conditions.

**Xeroderma pigmentosum**

Patients suffering from this genetic disorder tend to develop premalignant and malignant skin lesions with a 1000 times higher frequency compared to the general population; therefore these are candidates in which to study the cancer preventive effect. Eretinate and isotretinoin have shown preventive effects on premalignant and malignant skin lesions, including actinic keratoses, keratoacanthomas, basal cell carcinomas, squamous cell carcinomas and melanomas [91–94]. In a study of 5 patients with xeroderma pigmentosum developing a total of 121 tumors during a 2 year period before retinoid treatment, the number of new tumors including basal cell carcinoma and squamous cell carcinoma was reduced to 25 during a 2 year treatment period with 2 mg/kg/day isotretinoin. Discontinuation of isotretinoin resulted in an increase of the number of new tumors to the same level as prior to the retinoid treatment [92].

**Basal cell carcinoma**

In a few patients with a history of multiple basal cell carcinomas, isotretinoin (1.5 mg/kg/day) was able to prevent the occurrence of new lesions [95, 96].

**Squamous cell carcinoma of the skin**

Patients with organ transplants have shown a 20-fold increased risk of skin cancer in Australia. Four male immunosuppressed renal transplant recipients developed a total of 23 squamous cell carcinomas (SCC) of the skin in a 12 month period before retinoid treatment. During a treatment period of 8–13 months with 50 mg etretinate daily the number of newly diagnosed SCC decreased to six, whereas in the following 12 months without retinoid treatment there was again an increase to 34 SCC [97]. Prevention of SCC can apparently only be maintained by continuous therapy with the retinoid.

**Superficial bladder tumors**

In patients with superficial bladder tumors removed by transurethral electrocoagulation or resection, etretinate led to a reduced incidence of new bladder tumors. These results, derived from two double-blind, placebo-controlled studies, included complete prevention with a dosage of 0.3–0.5 mg/kg/day etretinate administered for 1 to 2 years. In the group receiving etretinate, 72% (21 of 29 patients) had no recurrences compared to 35% (11 of 31 patients) in the placebo group [98, 99].

**Second primary tumors in head and neck carcinomas**

Patients with squamous cell carcinoma of the oral cavity, pharynx or larynx who have been cured via surgery and/or radiotherapy have an increased risk of developing a second primary tumor not only in the head and neck area but also in the esophagus or lung. The incidence of these second primary tumors has been markedly reduced by preventive treatment with isotretinoin. In a prospective, randomized, placebo-controlled study [100] 100 patients with squamous cell carcinoma of the oral cavity (44 patients), pharynx (20 patients) or larynx (36 patients) – after completion of surgery or radiotherapy (or both) – were randomly assigned to receive either isotretinoin in a dosage of 50 to 100 mg/m²/day or placebo for 12 months. Both groups showed no difference in the recurrence rate of the primary tumor. However, after a median follow-up of 2½ years, only 2 patients (4%) in the isotretinoin group had second primary tumors compared to 12 (24%) in the pla-
There are a series of premalignant conditions which eventually develop into cancer. Retinoids have clearly shown inhibitory effects on these lesions. These effects have particularly been observed in skin lesions (actinic keratosis, keratoacanthoma, epidermodysplasia verruciformis), lesions of the head and neck area (oral leukoplakia, dysplasia and papillomatosis of the larynx), bronchial lesions (bronchial metaplasia and dysplasia), breast lesions (mammary dysplasia), lesions of genital organs (cervical dysplasia and vulval dystrophy), bladder papillomas and in patients with myelodysplastic syndrome. In the majority of these studies, retinoids were given orally. Details regarding individual studies are given in Table 1.

**Table 1. Retinoids in precancerous and cancerous diseases.**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Retinoid oral</th>
<th>Patient number</th>
<th>Complete response</th>
<th>Partial response</th>
<th>Minimal response</th>
<th>Unchanged</th>
<th>Progression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinic keratosis</td>
<td>Etretinate</td>
<td>105</td>
<td>45 (43%)</td>
<td>49 (47%)</td>
<td>0</td>
<td>11 (10%)</td>
<td>0</td>
<td>104–106</td>
</tr>
<tr>
<td>Keratoacanthoma</td>
<td>Isotretinoin</td>
<td>3</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>107–109</td>
</tr>
<tr>
<td>Epidermodysplasia verruciformis</td>
<td>Etretinate</td>
<td>14</td>
<td>13 (93%)</td>
<td>1 (7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>104, 110, 111</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>Isotretinoin</td>
<td>12</td>
<td>270*</td>
<td>43 (16%)</td>
<td>55 (20%)</td>
<td>118 (44%)</td>
<td>54 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Etretinate</td>
<td>40</td>
<td>3 (7.5%)</td>
<td>28 (70%)</td>
<td>0</td>
<td>9 (22.5%)</td>
<td>0</td>
<td>104, 143</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Isotretinoin</td>
<td>9</td>
<td>2 (22%)</td>
<td>2 (2%)</td>
<td>0</td>
<td>2 (22%)</td>
<td>0</td>
<td>144, 145</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>Etretinate</td>
<td>4</td>
<td>1 (25%)</td>
<td>1 (25%)</td>
<td>0</td>
<td>2 (50%)</td>
<td>0</td>
<td>104</td>
</tr>
<tr>
<td>Oral leukoplakia</td>
<td>Etretinate</td>
<td>5</td>
<td>8 (17%)</td>
<td>29 (60%)</td>
<td>0</td>
<td>11 (23%)</td>
<td>0</td>
<td>118–121</td>
</tr>
<tr>
<td>Larynx dysplasia</td>
<td>Isotretinoin</td>
<td>42</td>
<td>3 (67%)</td>
<td>11 (26%)</td>
<td>0</td>
<td>3 (7%)</td>
<td>0</td>
<td>123–125</td>
</tr>
<tr>
<td>Larynx papilloma</td>
<td>Isotretinoin</td>
<td>6</td>
<td>3 (50%)</td>
<td>1 (17%)</td>
<td>0</td>
<td>2 (33%)</td>
<td>0</td>
<td>126</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Isotretinoin</td>
<td>38</td>
<td>1 (2.5%)</td>
<td>4 (10.5%)</td>
<td>0</td>
<td>33 (87%)</td>
<td>0</td>
<td>145, 155</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial metaplasia</td>
<td>Etretinate</td>
<td>40</td>
<td>----- 30 (75%)</td>
<td>0</td>
<td>2 (5%)</td>
<td>8 (20%)</td>
<td>0</td>
<td>128, 129</td>
</tr>
<tr>
<td>Bronchial dysplasia</td>
<td>Etretinate</td>
<td>3</td>
<td>3 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>127</td>
</tr>
<tr>
<td>Non small-cell lung carcinoma</td>
<td>Isotretinoin</td>
<td>45</td>
<td>0</td>
<td>1 (2%)</td>
<td>2 (4.5%)</td>
<td>6 (13.5%)</td>
<td>36 (80%)</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary dysplasia</td>
<td>Retinol</td>
<td>12</td>
<td>1 (8%)</td>
<td>4 (33%)</td>
<td>0</td>
<td>7 (59%)</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical dysplasia</td>
<td>Tretinoin</td>
<td>74</td>
<td>24 (32%)</td>
<td>4 (5%)</td>
<td>41 (56%)</td>
<td>5 (7%)</td>
<td>0</td>
<td>131–133</td>
</tr>
<tr>
<td>Vulva</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vulval dystrophy</td>
<td>Etretinate</td>
<td>26</td>
<td>0</td>
<td>16 (62%)</td>
<td>0</td>
<td>7 (27%)</td>
<td>3 (11%)</td>
<td>134, 135</td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder papilloma</td>
<td>Tretinoin</td>
<td>15</td>
<td>4 (27%)</td>
<td>7 (47%)</td>
<td>0</td>
<td>3 (20%)</td>
<td>1 (6%)</td>
<td>136</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>Isotretinoin</td>
<td>69</td>
<td>4 (6%)</td>
<td>22 (32%)</td>
<td>0</td>
<td>43 (62%)</td>
<td>0</td>
<td>137–142</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>Tretinoin</td>
<td>228</td>
<td>195 (85.5%)</td>
<td>12 (5.5%)</td>
<td>0</td>
<td>0</td>
<td>21 (9%)</td>
<td>61–72</td>
</tr>
</tbody>
</table>

* Tumor no.

cebo group. Four patients in the placebo group had multiple second primary tumors. Besides one acute myeloid leukemia, all tumors were of the squamous cell carcinoma type. Twelve patients treated with placebo had 17 new tumors, 59% in the head and neck area, 12% in the esophagus and 24% in the broncho-pulmonary area. Patients treated with isotretinoin developed only two new tumors, one head and neck, and one esophageal cancer. The authors conclude from the significant suppression of second primary tumors by isotretinoin that these results provide the basis for further chemoprevention studies in subjects at high risk for all tobacco-related epithelial cancers.

**Therapy of precancerous lesions**

There are a series of premalignant conditions which eventually develop into cancer. Retinoids have clearly shown inhibitory effects on these lesions. These effects have particularly been observed in skin lesions (actinic keratosis, keratoacanthoma, epidermodysplasia verruciformis), lesions of the head and neck area (oral leukoplakia, dysplasia and papillomatosis of the larynx), bronchial lesions (bronchial metaplasia and dysplasia), breast lesions (mammary dysplasia), lesions of genital organs (cervical dysplasia and vulval dystrophy), bladder papillomas and in patients with myelodysplastic syndrome. In the majority of these studies, retinoids were given orally. Details regarding individual studies are given in Table 1.

Actinic keratosis is a common precancerous condition in elderly people. About 5% of the cases show malignant transformation. A particular therapeutic
problem is posed by multiple actinic keratoses, which do not respond well to conventional therapies. Actinic keratosis responds to topical treatment with tretinoin ointment [101–103]. Oral treatment of 105 patients with etretinate at doses of 0.5–1 mg per kg produced complete remission in 45 (43%) and partial remission in 49 (47%) [104–106]. To maintain remission, additional intermittent or long-term therapy is usually necessary.

Keratoacanthoma is a benign hyperkeratotic lesion, with similar histological features to those of squamous cell carcinoma. A tendency to spontaneous regression makes it difficult to evaluate the effects of drug treatment. Keratoacanthoma was successfully treated with isotretinoin [107–109] and in particular with etretinate [104, 110, 111]. The proportion of complete remissions is very high at 93% for etretinate.

Epidermodysplasia verruciformis is a genetic disorder and is characterized by multiple, flat, wartlike skin changes with a tendency to develop multiple squamous cell carcinomas. Papilloma viruses – of various types – play an important etiological role. Remissions obtained by treatment with etretinate are usually (in 92% of cases) only partial [112–116]. However, the quality of life of patients suffering from this disease is clearly improved by preventing new lesions.

Oral leukoplakia, a precancerous condition, does not respond well to other forms of treatment and is therefore frequently treated with tretinoin [117] and other retinoids. Of 97 patients who received isotretinoin [118–121] or etretinate [119, 120, 122], 17 had a complete regression of the lesions and 63 showed partial regression. The latter is still important for the patients as they usually benefit from the complete disappearance of subjective symptoms. Relapse follows after treatment discontinuation and maintenance therapy is advisable.

Forty-two patients with dysplasia of the larynx, a precancerous stage of carcinoma of the larynx, have been treated with etretinate. Twenty-eight (67%) of the patients achieved a complete remission and eleven (26%) showed partial regression [123–125]. Positive results were obtained with isotretinoin in recalcitrant papillomatosis of the larynx [126].

Three patients with bronchial dysplasia showed complete disappearance of their lesions after 6 months treatment with etretinate [127]. In a trial involving 40 heavy smokers with bronchial metaplasia, treatment with etretinate produced some regression of the bronchoscopically and histologically confirmed lesions of the bronchial mucosa in 30/40 patients [128, 129]. The interpretation of these results is difficult because of the lack of a placebo group.

In 12 patients, mammary dysplasia was treated with vitamin A (retinol) at an oral dose of 150’000 IU per day. In 5 patients there was objective remission and in 9 patients a subjective remission [130]. These data still await confirmation via controlled clinical trials with vitamin A or other retinoids.

Cervical dysplasia has been treated topically with tretinoin. Of 74 evaluable cases, 24 responded with complete and 4 with partial remission. In 41 patients there was very little response [131–133]. Although topical tretinoin has a certain degree of activity, this method of treatment is not advisable because of the practical difficulties and the superior clinical results that can be obtained with other therapies.

Vulval dystrophy, which includes lichen sclerosus et atrophicus of the vulva, has been treated with etretinate. In 16 of the 26 patients treated, a clear objective and subjective improvement was obtained [134, 135]. In view of the limited success by other treatments, etretinate may be a good alternative.

Oral therapy of papilloma of the bladder with etretinate was successful. In a group of 15 patients with papillomatosis of the bladder, complete and partial remission rate was 27% and 47% respectively [136]. In view of clearly superior conventional therapy these results are of limited clinical value.

Myelodysplastic syndromes only marginally responded to isotretinoin with 6% complete responses and 32% partial responses in 69 patients [137–142].

**Therapy of cancerous lesions**

**Single agent treatment**

The major types of malignant disease treated with retinoids as single agents are basal cell carcinomas, cutaneous squamous cell carcinomas (CSCC), melanomas, cutaneous T-cell lymphomas (CTCL), squamous carcinomas of the head and neck area, non-small cell lung cancer and acute promyelocytic leukemia (Table 1). Combination treatments have been used in CTCL, CSCC and cervical cancer.

Basal cell carcinoma has been treated both topically and systemically. The clinical results of oral treatment of basal cell carcinoma with isotretinoin [95, 96] and etretinate [104, 143], with complete remission in less than 20% and partial remission in 70% of patients are only of limited clinical interest, particularly in view of superior conventional treatment.

Only a small number of patients with CSCC have been treated with isotretinoin [144, 145] or etretinate [104]. These results are of more interest because refractory cases responded to this therapy with 3 of 13 patients exhibiting complete remission. Treatment of melanoma with isotretinoin was not successful (only 3 partial remissions in 20 patients [29]). In CTCL, a total of 107 patients – in various stages of the disease and some of them pretreated – were treated with isotretinoin [146–152] or etretinate [150, 153, 154]. Eighteen complete and 48 partial remissions were observed which compared favorably with conventional therapy.

Very high daily doses of 3 mg per kg isotretinoin produced remission in a few patients with squamous cell carcinoma of the head and neck area [145, 155]. In a group of 38 patients, 1 complete and 5 partial remissions were obtained, with very short-lasting remissions
and side effects at the limit of acceptability. Forty-five patients with non-small cell carcinomas of the lung did not respond to treatment with isotretinoin [29, 156].

Acute promyelocytic leukemia represents the first clinical model where a high percentage of complete remissions has been achieved with single agent retinoid therapy. Cell culture work has shown that retinoids induce differentiation in a human promyelocytic leukemia cell line (HL-60) [19, 48, 49]. In the first clinical studies in acute promyelocytic leukemia 13-cis retinoic acid was used and only occasional responses were observed [157]. All-trans retinoic acid (tretinoin) was initiated by Huang et al. [61] in Shanghai with spectacular results (95% of patients achieving complete remission). These results were later confirmed by Castaigne et al. [62], Degos et al. [63] and Warrell et al. [64]. These patients with acute promyelocytic leukemia, AML M3 (FAB), and the typical chromosomal translocation (t15;17) have been treated with a daily oral dose of 45 mg/m² tretinoin, which induced the differentiation of leukemic promyelocytes to granulocytes. Complete remissions have been achieved within 1–3 months, including the disappearance of blast cells, Auer rods and the cytogenetic marker. Furthermore, disseminated intravascular coagulation (DIC) disappeared and peripheral cytopenias have been normalized within 30–60 days. In contrast to chemotherapy, the patient did not go through a dangerous phase of treatment-induced bone marrow aplasia and deterioration of DIC. The most frequent side effects – those associated with hypervitaminosis A syndrome – were dry skin, chelitis, headache, bone pain, increase of transaminases, cholesterol and triglycerides and were of usually mild degree. However, in 10%–15% of the patients, serious complications occurred, mostly within the first 5–15 days after initiation of tretinoin therapy, consisting of hyperleukocytosis, fever, acute respiratory distress, pulmonary infiltrates, pleural or pericardial effusions, peripheral edema, impaired myocardial contractility, hypotension, renal failure, leucostasis syndrome, thrombosis, embolism and hemorrhages. These complications are probably caused by various factors such as the tretinoin-induced elevation in peripheral white blood cell count, organ infiltration by maturing leukemic cells, release of vasoactive cytokines and other factors. These complications are successfully treated either with chemotherapy [65] and/or corticosteroids [66].

To date, 228 cases of APL treated with tretinoin have been published, of which 195 experienced a complete remission (85.5%) [61–65, 67–72]. This confirms the 85% complete remission obtained in 787 patients cited in a summary report from China [69]. Tretinoin therapy does not lead to a cure. The duration of complete remission varies between 2 and 30 months [61, 62, 67]. The mechanism of ‘resistance’ is not yet clear. Decreasing plasma levels of tretinoin during the course of treatment may play a role [68].

The mechanism of action of tretinoin in APL is not yet completely elucidated. It is, however, known that in APL the coding region of the gene of the retinoic acid receptor α (RARα) is rearranged through the t(15;17) translocation. An abnormal transcript is expressed [158–161] resulting from the fusion with a new gene myl/PML located on chromosome 15. The abnormal receptor could repress granulocytic differentiation and be one step in the leukemogenesis of these cells. The differentiation induction in APL patients may require, however, high doses of tretinoin due to differences in the transactivation potential of the recombinant myl/PML receptor compared to the unaltered normal RARα [70, 162].

Retinoids combined with cytotoxic agents or interferon α. A few clinical trials have been carried out using the combination of retinoids with cytotoxic agents. However, these trials were of little or no success [163–166]. Only recently have clinical trials been performed with the combination of retinoids and interferon α (IFNα) with some promising results. Cutaneous T-cell lymphoma responded favorably to the combination of IFNα with either isotretinoin or etretinate [167–170]. However, due to the small number of patients, further investigations are required in order to prove a superiority of the combination over therapy with single agents.

Twenty-eight patients with heavily pretreated advanced inoperable cutaneous squamous cell carcinoma have been treated with oral isotretinoin (1 mg/kg/day) and IFNα subcutaneously (3 million units per day). Seven (25%) patients experienced a complete remission and 12 (43%) a partial remission [171]. The response rate was 93% (13/14) in advanced local disease, 67% (4/6) in regional disease, and 25% (2/8) in distant metastatic disease. The median response duration was 6+ months (range 1–21+ months). The study showed that the combination treatment generated about two-fold higher response rates than that achieved with higher doses of either agent used alone in advanced local skin squamous cell carcinoma. The treatment was well tolerated, fatigue being the most disturbing adverse effect in this elderly patient population with a median age of 67 years.

Twenty-six patients with previously untreated, locally advanced (≥ stage II in 21 patients) squamous cell carcinoma of the cervix have been treated with oral isotretinoin (1 mg/kg/day) and IFNα subcutaneously (6 million units per day [172]). The overall therapeutic effect was 50% objective responses with one complete remission. The response rate was 58% (11/19) in patients with ≥ stage IIB disease and 66% (10/15) in patients with bulky disease. The marked reduction in tumor size was linked with resolution of associated symptoms such as vaginal bleeding, lower abdominal pain and back pain. Adverse effects were relatively mild (usually only WHO grade I or II) and this consisted of signs and symptoms of hypervitaminosis A caused by isotretinoin as well as fatigue as a side effect of IFNα. Nevertheless, this was an outpatient treat-
ment. Similar remission rates have been reported by conventional chemotherapy with cytotoxic agents, but with much higher toxicity. These results need confirmation and the final role of this treatment vis-à-vis radiotherapy and cytotoxic agents requires randomized, prospective trials.

Outlook

While retinoids have already established themselves in the successful treatment of various dermatological diseases such as acne, psoriasis and other keratinizing dermatoses, the clinical use of retinoids in oncology is still in its infancy. Results from preclinical studies and clinical trials have resulted in cautious optimism that retinoids may prove to be useful in prevention and therapy of cancer. The discovery of new retinoids with a more favorable therapeutic index is, however, mandatory to make these treatments more acceptable.

Improved therapeutic results can also be expected from combinations of retinoids and other antitumor agents (cytokines, cytostatics, cytotoxics) generating a synergism between antiproliferative, differentiation-inducing and immunomodulatory effects. However, many questions still require answers, e.g. which retinoid is the best choice when combined with specific cytokines and/or cytostatic/cytotoxic agents in order to achieve maximal antitumor effect and minimal toxicity.

Differentiation therapy of cancer appears to be a particularly promising approach, since it represents a departure from the 'pharmacological' principle of cell destruction to the 'physiological' principle of conversion of neoplastic cells into differentiated, phenotypically normal cells.

In view of the large number of nuclear retinoid receptors and their differential tissue distribution, efforts are being made to develop receptor-specific retinoids. The tools being used include the measurement of binding affinity of new retinoids to the various receptors, as well as the activation of these receptors in cultured cells [75, 173–175]. It is hoped that such receptor-specific retinoids will exert selective anti-tumor effects and/or fewer side effects.

In spite of enormous progress in the field of nuclear retinoid receptors, we still know very little about the specific biological function assigned to each receptor. It has not yet been demonstrated that the transcriptional events mediated by one receptor are responsible for one single biological function, or even a series of functions. The search for retinoids as specific ligands to a certain receptor with the help of binding affinity and activation assays is expected to contribute to the solution of this problem. The new tools afforded by molecular biology may help in the design of tailor-made retinoids for specific diseases. For the time being, serendipity will certainly still play a role in our efforts to improve the therapeutic results with retinoids in cancer.

References

524


109. Shaw JC, White CR. Treatment of multiple keratoacanthomas


111. Mensing H, Wagen G. Ettreinat-Therapie bei solitären Kera-


Received 9 April 1992; accepted 27 April 1992.

Correspondence to: E. E. Holdener, M.D.
Department of Clinical Research
Division of Oncology & Hematology
F. Hoffmann-La Roche Ltd
CH-4002 Basel
Switzerland