Introduction

Vitamins have been thought to play a role in the prevention of cancer for decades. Now, in the 1990s, a unique class of agents—specifically, vitamin A and its analogues—has received much attention in the development of new chemotherapeutic agents. Retinoids, which are synthetic analogues of vitamin A, have been used in numerous oncology studies, and impressive data have been generated in adult leukemia studies. A unique characteristic of this class of agents is the ability to function as differentiating agents providing a novel approach to cancer treatment. An understanding of the basic molecular and cellular mechanisms of retinoid action, as well as their role in normal physiological functions, is necessary to fully appreciate the function of retinoids as potential chemotherapy agents.

Class Overview

Retinoids are naturally occurring and synthetic analogues of vitamin A (retinol) that function in the regulation of a variety of physiologic processes including growth, vision, reproduction, epithelial cell differentiation, and immune function (Fig 1). The primary dietary sources for retinol are carotenoids from vegetables and retinyl esters from animal tissues; each is then converted in intestinal cells (enterocytes) to retinol.[1] Retinol plays an important role in visual response, while most of the other functions of retinoids are thought to be mediated by its oxidized product, all-trans retinoic acid (ATRA). ATRA is derived from the intracellular oxidation of preformed retinol that has been absorbed from the gastrointestinal tract.[1,2] Intracellular isomerases may further convert ATRA to 9cisRA, 11cisRA, or 13cisRA, with 3,4-didehydroretinoic acid and 14hydroxy 3,14-retroretinol being synthesized directly from retinol (Fig 2).[1,3] Each cell produces its own pool of retinoids that remain intracellular to function as mediators rather than as hormones circulating in the bloodstream.[2,3]

Molecular and Cellular Biology

Both retinol and ATRA are highly protein bound in plasma retinol to retinol binding proteins and ATRA to albumin.[1,2] Retinol is released at the cellular membrane and rebound intracellularly to cellular retinol-binding proteins (CRBP). All-trans-RA can be metabolized immediately after binding to cellular all-trans-RA-binding proteins (CRBP) and oxidized by cytochrome P450 enzymes located in smooth endoplasmic reticulum. Alternatively, all-trans-RA or its isomers enter the cell nucleus and bind to all-trans-RA receptors (RAR) or to retinoid ‘X’ receptors (RXR). After dimerization (ie, the formation of an RAR/RXR heterodimer or an RXR/RXR homodimer), these reactivated receptors bind with high affinity to specific DNA segments (the all-trans-RA response element) and effect the transcription of messenger RNA. Ultimately, the retinoid response is mediated by primary target genes, by interference with other transcription factors, and by control of certain posttranscriptional actions. From Warren RP Jr, de The H, Wang ZY, et al. Acute promyelocytic leukemia. N Engl J Med. 1993;329:177-189. Copyright 1993 Massachusetts Medical Society. Reprinted with permission.

Nuclear retinoic acid receptors (RARs) are the primary site of ATRA activity.[2] RARs are part of a larger class of nuclear hormone receptors that includes steroids, vitamin D3, thyroid hormone, and transcription factors (ATRA and retinoid X).[4,5] Two families of RARs have been described that bind both retinoids and DNA and result in activation of transcription and expression of other important genes that mediate specific cellular functions of retinoids.[6] Three nuclear RARs (RARalpha, beta, and gamma) have been described in humans.[1,2,5,7] Once activated, RARs bind to specific DNA sites and function as a factor in transcription leading to control of target gene expression.[2] All three nuclear receptors share homology with the presumed DNA binding region of the genes. RARalpha gene maps to the q21 band of chromosome 17 that is in close proximity to the chromosome breakpoint associated with acute promyelocytic leukemia (APML).[1,5] Both RARalpha and RARbeta are expressed in a human APML cell line. [1] High levels of RARalpha and RARbeta are also found in the hippocampus, adrenals, cerebellum, hypothalamus, and testes. RARgamma messenger RNA is found almost exclusively and in high levels in skin.[1] All RAR’s are structurally similar but vary in their affinity for ATRA and synthetic retinoids.[1,5] Three other retinoid receptors recently have been identified (RXRalpha, beta, and gamma) that are only somewhat related to the RARs in their protein sequences and do not bind to ATRA, although a slight response has been seen with ATRA in high concentrations.[2,3] A stereoisomer of ATRA, 9cis RA, is a naturally occurring ligand of the RXRs and activates both RARs and RXRs.[2] It has been postulated that nuclear RARs are the final mediators of ATRA action on gene expression that may lead to cellular differentiation, inhibited growth, and
ultimately cell death.\cite{3,4} The ability of ATRA to induce apoptosis (programmed cell death) may lead to tumor regression rather than cytostatic effects.\cite{1}

**Molecular and Cellular Effects**

Retinoids have been shown to suppress carcinogenesis in various epithelial tissues in experimental animal model systems by inhibition of tumor promotion (the conversion of an affected cell to a preneoplastic or neoplastic cell).\cite{7} They also have been shown in clinical chemoprevention trials to be effective against a variety of conditions, including several types of human cancer, especially squamous cell cancers.

**Pharmacokinetics**

Most of the pharmacokinetic data for retinoids are derived from cisRA and other retinoids used for dermatologic conditions, but data for ATRA recently has been obtained.

**cisRA**

Peak plasma levels of 1.0 mu-g/mL occur 1.5 to hours following the oral administration of an 80mg dose of cisRA (cRA), with significant variation among patients.\cite{1} Metabolism of cRA is via oxidative pathways in the liver.\cite{8} With chronic cRA therapy, the 4oxo metabolite accumulates in the plasma and exhibits an area under the curve (AUC) of greater magnitude than the parent compound. Isomerization of cRA to ATRA also occurs;\cite{8} cRA has a biphasic elimination profile with a terminal half-life of 10 to 77 hours. Oral absorption of cRA is strongly influenced by drug formulation and by the relationship of administration time to food intake.\cite{1}

**AllTrans Retinoic Acid**

Contrary to what was originally proposed, the pharmacokinetics of ATRA differ significantly from those of cRA.\cite{1,8} After a 45 mg/m-squared oral dose, plasma levels peak at approximately 1 mu-g/mL.\cite{1} ATRA is rapidly eliminated from the plasma with a half-life of less than one hour.\cite{1,8} Metabolism of ATRA involves oxidation followed by glucuronidation.\cite{8} Cytochrome P450like enzyme systems are thought to be involved in metabolism, since administration of P450 inhibitors increases plasma concentrations of ATRA in animal models and patients.\cite{8} The 4oxo metabolite accounted for less than 10% of the circulating drug.\cite{1} Chronic administration of ATRA has been associated with lower peak serum concentrations and AUC in some studies.\cite{1} Although the mechanisms responsible for this phenomenon are unknown, those hypothesized include malabsorption, induction of cytochrome P450 activity by ATRA, or elevation of CRABP levels resulting in increased plasma clearance into nontarget tissues.\cite{1} This decline in ATRA plasma levels associated with chronic administration may be an important mechanism of acquired resistance to ATRA therapy seen primarily in patients with APML.\cite{1} Potential methods to overcome this phenomenon that currently are being investigated include blocking oxidation by administration of a P450 inhibitor or using an intermittent dosing schedule.\cite{8} The absorption of ATRA also is influenced by the relationship administration time to food intake and type of food. Higher absorption rates and AUC are seen when the drug is administered with foods that have a high fat content.

**Clinical Applications of Retinoids**

The unusual mechanism of action by retinoids leads to a unique role for these agents in the treatment of cancer. Preclinical studies of retinoidic acid suggest potential roles of these agents for (1) direct induction of differentiation either alone or in combination with other agents, (2) growth inhibition without differentiation, and (3) induction of apoptosis.\cite{1,2} Their activity as differentiating agents seems to be the most significant. Differentiation therapy differs from chemotherapy in that differentiation is not cytotoxic and may require prolonged periods of time before a response is noted. When neoplastic cells are exposed to a differentiating agent, the cell stops its abnormal replication and becomes a mature, differentiated cell. These cells are no longer capable of multiple divisions and undergo apoptosis. Compared with conventional chemotherapy, differentiation therapy may produce fewer severe side effects. However, when treatment with this therapy is discontinued, the effect on the cancer cell may be lost. Trials investigating the use of these agents in many malignant diseases currently are underway. A sample of current studies involving retinoids is outlined in Table 1.

Table 1. Malignancies in Which Retinoic Acid Compounds Are Being Tested

<table>
<thead>
<tr>
<th>Adult solid tumors (various)</th>
<th>Chronic myelogenous leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric solid tumors (various)</td>
<td>Mycosis fungoides/Sezary syndrome</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Squamous cell carcinoma of the head and neck</td>
<td>Malignant glioma</td>
</tr>
<tr>
<td>Malignant glioma</td>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>Germ cell tumors</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Myeloma</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Small cell lung cancer</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>Liver cancer</td>
</tr>
</tbody>
</table>

**Applications in Hematologic Malignancies**

**Acute Promyelocytic Leukemia**

APML (FAB M3) accounts for approximately 10% of the adult acute myeloblastic leukemias. Distinguishing features of APML include distinctive morphology, younger age at onset, specific chromosomal abnormality, associated consumptive coagulopathy, lower peripheral leukocyte count, higher complete remission rate, more favorable prognosis, and achievement of complete remission without bone marrow hypoplasia.\cite{2,6} Conventional chemotherapy for APML, which includes an anthracycline in combination with cytarabine, is reported to induce complete remission in 60% to 80% of patients. Fiveyear survival is reported to be approximately 40%-\cite{2} Low survival rates are primarily due to early mortality during induction therapy of APML secondary to bleeding complications and sepsis.\cite{2}

Retinoids offer a unique approach to the treatment of APML by eradicating cells containing the chromosomal abnormality unique to APML without the toxicities seen with conventional chemotherapy. The chromosomal abnormality associated with APML is the translocation t(15;17)(q22;q1221), which has been shown to involve the RARalpha on the long arm of chromosome 17 and the PML gene on chromosome 15. This translocation results in a unique fusion mRNA (PML/RARalpha) clinically sensitive to ATRA.\cite{2,6} Studies have shown that retinoic acids (both cis and trans) can induce granulocytic differentiation in the HL60 cell line.\cite{2,6} Secondary to complete differentiation, apoptosis occurs and thus eliminates the cells containing the abnormal PML/RARalpha gene. Structureactivity studies that have been conducted in APML cells comparing the cis and trans isomer of retinoic acid indicated that the trans isomer (ATRA) had greater cytodifferentiation.\cite{6} In 1990, an investigational new drug treatment was filed to further
Several investigations have reported clinical benefits during clinical trials of ATRA in the treatment of APML, all showing evidence for high efficacy and low toxicity.[9] In the initial report from Huang et al.[10] 23 of 24 patients with APML achieved both partial remission (PR) and complete remission (CR) without developing bone marrow hypoplasia. Patients were treated with 45 to 100 mg/m²-squared per day orally until remission was achieved. CR occurred between 20 and 199 days. Six patients were maintained on ATRA alone (20 to 30 mg/m²-squared per day orally). At the time of submission of the original report, a oneyear followup showed 15 patients remaining in CR (three patients having been previously treated with conventional chemotherapy).

### Table 2. Event-Free Survival in APML Patients Treated With ATRA Vs Chemotherapy Plus ATRA

<table>
<thead>
<tr>
<th>Group</th>
<th>Complete Remission</th>
<th>Early Death</th>
<th>Resistant Leukemia</th>
<th>EFS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRA (N=46)</td>
<td>41 (91%)</td>
<td>5 (9%)</td>
<td>0</td>
<td>79%</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>38 (81%)</td>
<td>4 (8%)</td>
<td>5 (10%)</td>
<td>50%</td>
</tr>
</tbody>
</table>

*AFS (event-free survival) was reported at 12 months (P=0.001).

A multicenter, randomized trial (APL91 Trial)[11] was conducted in Europe in patients aged 65 years or younger in whom APML was newly diagnosed. This study compared treatment with chemotherapy alone as daunorubicin 60 mg/m²-squared per day for three days plus cytosine arabinoside (araC) 200 mg/m²-squared per day for seven days for courses 12, followed by daunorubicin 60 mg/m²-squared per day for three days plus araC 1 g/m²-squared every 12 hours for four days for course 3 (chemotherapy group) to treatment with ATRA (45 mg/m²-squared per day orally) administered until CR or a maximum of 90 days, followed by the same chemotherapy protocol as the chemotherapy group (ATRA group). Eventfree survival (EFS) was the major endpoint of the study ("event" defined as failure to achieve CR, relapse, or death in CR). The study was terminated after the first interim analysis as EFS was significantly higher in the ATRA group (Table 2). The difference in CR rate between the two groups is not statistically significant, but the duration of coagulopathy is significantly reduced in the ATRA group compared with that of the chemotherapy group. The results of this study show a significant improvement of EFS in newly diagnosed APML by combining ATRA with conventional chemotherapy compared with chemotherapy alone. The authors hypothesized that the improvement in EFS is a result of lower incidence of relapses in patients receiving both ATRA and chemotherapy, suggesting that ATRA and chemotherapy could act synergistically to reduce the tumor burden in APML. These findings raise the argument that ATRA should be incorporated in the frontline therapy of newly diagnosed APML in association with conventional chemotherapy.[11]

The New York Study is a phase II evaluation and comparison with a historical control to evaluate the safety and efficacy of ATRA in inducing CR and to examine the effects of ATRA on duration of remission and survival in patients with APML.[12] Fiftysix patients with newly diagnosed disease (n=34) and those who had relapsed following chemotherapy (n=22) were eligible and treated during a twoyear period with ATRA (45 mg/m²-squared per day by mouth divided into two doses after meals).[12] Newly diagnosed patients discontinued ATRA therapy 30 days after achieving CR. Patients then received three courses of consolidation chemotherapy with idarubicin (12 mg/m²-squared per day intravenously for three days) and araC (200 mg/m²-squared per day intravenously for five days); further treatment was continued every three to six weeks with idarubicin (the same daily dosage for two days) and araC (the same daily dosage for four days).[12] Patients who were treated previously with chemotherapy and then relapsed were given ATRA to induce remission; ATRA was given until they relapsed. Of 51 patients having the PML/RARA alpha genearrangement, 14 (86%) achieved a CR with ATRA therapy.12 In patients with the gene rearrangement, no difference in response rates was seen between newly diagnosed (26 of 30) and previously treated (18 of 21) patients. In patients with no gene rearrangement (n=5), three (newly diagnosed patients) achieved remission with chemotherapy. Twentytwo of 26 newly diagnosed patients with the gene rearrangement who achieved remission went on to receive consolidation chemotherapy and achieved a median relapsefree survival greater than 28 months. When these patients were compared to historical control patients, overall survival was higher in the patients treated with ATRA for remission induction compared with patients treated only with chemotherapy. The incidence of death during induction chemotherapy was not significantly different between the two groups. Thirteen patients (three newly diagnosed) were treated with ATRA alone as both induction and maintenance of remission. All relapsed within 10 months of starting treatment with a median duration of remission of 3.5 months (range 1.5 to 23 months).[12] Ten patients who had previously relapsed from a chemotherapyinduced remission relapsed again during a subsequent remission while still taking ATRA. These patients were continued on ATRA at 90 mg/m²-squared per day with none going into remission.[12] Nine patients who had previously relapsed from a CR induced by ATRA were treated again with ATRA at some future time after discontinuing ATRA therapy (median duration off ATRA therapy was six months); three of these patients achieved a second CR with ATRA (two patients died prior to evaluation and four patients were not responding).[12]

Adverse events seen with ATRA therapy were an initial leukocytosis (nonfunctional cells), followed by a transient leukopenia seen after three to five weeks of treatment.[12] Thirteen (23%) patients developed the characteristic “retinoic acid syndrome.” Other adverse effects included headache (90%), skin reaction/chelitis (65%), nausea/vomiting (51%), and bone pain (25%).[12] The results of this study indicate that ATRA is an effective agent for inducing remission in patients with APML.[12] The remission is of short duration, with resistance rapidly developing when ATRA is used alone.[12] ATRA used as a singular inducing agent, followed by consolidation with conventional chemotherapy, is associated with longer survival times when compared with historical controls treated with chemotherapy only.[12]

### Acute Myelogenous Leukemia

The role of ATRA in other subtypes of acute myelogenous leukemia (AML) is unclear. Several studies have shown a decreased sensitivity of ATRA in nonAPML cells lines.[1] Clinical experience with ATRA in other subtypes of AML has been limited and is usually seen in combination with lowdose chemotherapy with or without colonystimulating factors.[5] In a study involving 18 patients with relapsed or refractory AML,[13] 11 achieved CR (61%) when treated with ATRA 45 mg/m²-squared per day and lowdose cytarabine 20 mg subcutaneously twice daily for 10 days administered every 28 days. Median duration of CR was 10 months and median survival was 12 months. Studies are needed to determine if ATRA provides any additional benefit in combination with other agents, since results from combination studies are similar to those seen with single agents. A phase II trial of ATRA alone in elderly AML patients demonstrated no objective response in 12 assessable patients.[1]

### Myelodysplastic Syndromes

Many studies have explored the use of retinoids in myelodysplastic syndrome.[5] The results of these studies are difficult to interpret due to the differences in agents, doses, and schedules, as well as the use of additional agents. The results thus far have been disappointing, with a response rate of 38% in 69 patients participating in clinical trials of cRA at doses of 20 to 160 mg/m²-squared per day.[14] While these agents may have a future role in myelodysplastic syndrome and future study is warranted, the current opinion is that these agents are not useful in this disease state, especially when used alone.[2,5]

### Table 3. Toxicity of Systemic Retinoid Therapy

<table>
<thead>
<tr>
<th>Major/Common</th>
<th>Chelitis</th>
<th>Headache</th>
<th>Xerosis/inflammation</th>
<th>Lethargy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fatigue
Minor/Uncommon
- Anorexia
- Dry mucous membranes
- Nausea/vomiting
- Visual disturbances
- Pruritus
- Epistaxis
- Pseudotumor cerebri
- Hair thinning
- Psychological changes

Toxicity Associated With Treatment of APLM

Transient hyperleukocytosis
Retinoid acid syndrome:
- fever
- dyspnea
- diffuse pulmonary infiltrates
- pleural effusions
- peripheral edema
- weight gain
- hypotension
- pericardial effusions

Toxicities of Systemic Retinoid Therapy

The benefit of retinoid therapy, as compared to traditional chemotherapy, is an improved side effect profile. The common toxicities of systemic therapy with retinoids are outlined in Table 3. The most common toxicities include cheilitis, headache, xerosis/inflammation, lethargy, and fatigue.[1,14] Other mucocutaneous side effects usually are minor and can be treated with lotions and creams. Due to their high teratogenic potential, retinoids generally are avoided during pregnancy; however, there are case reports of the successful use of ATRA during the late second and third trimesters in patients with APM.[17,18]

ATRA seems to cause more neurologic side effects than cRA.[1] The effects of ATRA on the central nervous system seem to be dose related. The incidence of headaches and dizziness is higher in patients receiving systemic ATRA doses of 70 to 100 mg per day when compared with those patients receiving 30 mg per day (50% vs 4%, respectively).[1] The treatment of children may result in toxicities not seen as often in adults. Children appear to be more sensitive to the neurotoxicity of ATRA and should probably receive lower doses of ATRA.[19]

Toxicity During Treatment of APM

The treatment of APM with retinoids has resulted in unique and sometimes fatal complications due to the differentiation of atypical promyelocytes in addition to the more common side effects seen in other disease states. Unique toxicities of ATRA in APM include Sweet syndrome,[20] bone marrow necrosis,[21] and thromboembolism when combined with antithrombinolytic therapy.[22] Hyperleukocytosis has been reported in both the APL91 and New York trials.[11,12] Huang et al[10] did not report hyperleukocytosis in any of their patients. The hyperleukocytosis is due to an increased amount of circulating maturing atypical cells that have undergone terminal differentiation rather than lysis in traditional chemotherapy. Hyperleukocytosis (leukocyte count >20 X 10^9 per 10^9 cells/L) seems to occur between days 6 and 20 of therapy in 25% to 40% of patients, especially in patients with an initial leukocyte count >5 X 10^9 per 10^9 cells/L.[5,23] Leukapheresis may be required in some patients with rapidly increasing leukocyte counts.[23]

The hyperleukocytosis may precede the development of retinoic acid syndrome (RAS). RAS is characterized by fever, dyspnea, respiratory distress, interstitial pulmonary infiltrates, pleural effusions, fluid overload, weight gain, and organ infiltration by leukemic cells on autopsy.[23] This syndrome is seen in 25% of APM patients undergoing induction therapy with ATRA; however, it is not seen in patients who have taken retinoid acid for other diseases and is seen only rarely in other myeloid leukemias.2 RAS has been reported as occurring between the second day and third week of therapy.[2] RAS should not be confused with other similar complications that APM patients may experience secondary to the disease or other potential adverse effects of ATRA.[24]

Although its cause is unknown, RAS clinically resembles the capillary leak syndrome described with interleukin2 due to release of cytokines.[23] Organ infiltration by leukemic cells could be due to the maturation of leukemic cells resulting in abnormal cells with certain properties of normal neutrophils such as migration and extravascular adhesion.[23] The onset of RAS has been recently related to the expression of CD13, which is associated with a poor outcome in AML.[2]

Management of RAS includes the institution of highdose steroids (dexamethasone 10 mg every 12 hours for three to five days) at the development of dyspnea and weight gain.[23] The European experience with ATRA in APM has advocated the use of chemotherapy in patients with high initial or rapidly increasing leukocyte counts to decrease the incidence of RAS.[11] However, in the New York study, over half of the patients developed leukocytosis but less than a quarter of these patients actually developed RAS.[12] In addition, several patients who developed the syndrome did not have leukocytosis, and one patient did not have APM.[23] It appears that the actual leukocyte count does not consistently predict those patients who may be at risk for developing RAS. The risk vs benefit of adding chemotherapy to induction therapy with ATRA must be carefully considered as the risk of fatal coagulopathy increases.[23] In addition, decreasing the dose from 45 to 25 mg/m-squared does not affect the incidence of RAS.[25]

Conclusions

Retinoids have established differentiation therapy as an effective means of cancer treatment. Understanding the molecular and cellular biology of these drugs has allowed us to appreciate the cellular mechanisms of carcinogenesis and application in cancer treatment. Studies support the use of ATRA as a frontline agent in the induction therapy of APM (CR 80% to 90%) at doses of 45 mg/m-squared per day orally. Remissions obtained with ATRA are rapid but of short duration. No role for ATRA has been demonstrated in maintaining remission; conventional chemotherapy remains the treatment of choice for consolidation. The overall incidence of fatal coagulopathies associated with APM is reduced when ATRA is used as the inducing agent; however, this therapy is not without toxicity. The most significant adverse effect is RAS, which occurs in approximately 25% of patients undergoing induction therapy with ATRA.

Application has been made to the Food and Drug Administration for approval of ATRA (Vesanoid) with the indication for use as second line therapy of APM refractory to standard therapy. Approval is anticipated for the latter part of 1995. Further data are needed to support the use of ATRA as frontline induction therapy of APM. A final consideration is the potential reduction in costs associated with ATRA compared with conventional induction therapy for APM and its complications. Primarily, cost reductions may be seen by administering the majority of induction therapy with ATRA on an outpatient basis with the potential avoidance of lifethreatening coagulopathies and management costs associated with conventional chemotherapy.

References


