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Perspectives and Commentaries

Prevention and Therapy of Tumors with Arotinoids

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INTRODUCTION

BOLLAG AND HARTMANN [1] have reported recently that the arotinoid Ro 15-0778 inhibits the incidence and growth of mammary adenocarcinoma induced in rats by the carcinogen dimethylbenz(a)anthracene (DMBA). This is but one of a very large number of reports illustrating the chemopreventive and chemotherapeutic effects of retinoid analogs in animal tumor model systems. This report is notable, however, because Ro 15-0778 lacks a functional end group that is chracteristic of retinoids and also fails to elicit characteristic hypervitaminosis A symptoms in rats, even when administered in large doses for protracted periods. The purposes of this commentary are to consider arotinoid structure within the context of retinoid analogs, to speculate on mechanisms of arotinoid action, to review in a general way the effectiveness of retinoids as chemopreventive and chemotherapeutic agents in both hematopoietic and solid tumor model systems, to evaluate possible limitations on the use of retinoids, and particularly arotinoids, as anticancer drugs and to discuss prospects for clinical applications.

AROTINOID STRUCTURE

The most abundant, and best known, of the naturally occurring retinoids are the all-*trans*- and various *cis*-isomers of retinol, retinal and retinoic acid. Innumerable analogs of these three retinoid classes have been designed and synthesized. Although retinol and retinal satisfy all of an animal's requirements for vitamin A, dietary retinoic acid cannot substitute for these other retinoids in the maintenance of vision and fertility. On the other hand, in many experimental systems retinoic acid is considerably more potent than retinol or retinal in regulating cell proliferation and differentiation, processes of fundamental importance in therapeutic areas such as oncology, dermatology and immunology. Accordingly, a great many of the retinoids synthesized have been analogs of retinoic acids.

Retinoic acid is characterized by its possession of a 1,1,5-trimethyl cyclohexene ring, a conjugated double-bond, 9-carbon side chain and a terminal carboxylic acid moiety. All of these moieties have been modified in the design of analogs.

An early innovation in modification of retinoids was to substitute various aromatic rings in place of the cyclohexenyl moiety. The arotinoids are, in a way, second-generation aromatic compounds insofar as they possess multiple rings. The rings replace most of the side chain so as to provide a more rigid structure [2]. By placing a carboxylic acid residue in the appropriate position on one of the aromatic rings, it is possible to obtain a compound, variously called Ro 13-7410 or TTNPB, that is actually quite analogous to retinoic acid with respect to geometry and function. In fact, such arotinoids are often considerably more active than retinoic acid in assays that assess effects on growth and differentiation [2–5].

MECHANISM OF ACTION

Despite the similarities between arotinoids and naturally occurring retinoids, the former compounds are more complex, and it is not trivial to ask whether arotinoids indeed act as retinoids and only as retinoids. Retinoids are pleiotropic and often have paradoxical activities (reviewed in [6]);

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for example, they can promote differentiation of some cell types but interfere with differentiation of others and although they commonly inhibit proliferation of several cell types under standard culture conditions, they can promote cell division of the same cell types *in vitro* under other circumstances. Many investigators have been studying the role of plasma and cellular retinoid-binding proteins in retinoid action (see Ref. [6]). Some arotinoids have been shown to bind with high affinity to cellular retinoic acid-binding protein [3]. However, it is far from clear that all retinoid functions require interaction with retinoid-binding proteins [6]. One of the more definitive ways in which to assess whether arotinoids act as retinoids is to administer these compounds to rats in place of vitamin A. However, these are difficult experiments to interpret because only some of the hypovitaminosis symptoms could be expected to be prevented by administration of acidic arotinoids. Perhaps the most suggestive published evidence is that excessive doses of acidic arotinoids such as Ro 13-7410 elicit hypervitaminosis A-like symptoms [2] and that mutant embryonal carcinoma cells selected for inability to differentiate in the presence of retinoic acid also fail to respond to Ro 13-7410 [3]. The even more difficult question of whether arotinoids possess extra-retinoid activities is essentially unanswered. The issue of the mechanism of action of Ro 15-0778 is perhaps relevant to this question. This arotinoid does not bind to retinoid-binding proteins [3], it fails to induce differentiation of embryonal carcinoma [3, 4] and promyelocytic leukemia [4] cells, it has little effect on chemically induced skin tumors [2] and minimal, if any, hypervitaminosis A activity [2]. The potency of Ro 15-0778 against carcinogen-induced mammary adenocarcinomas led Bollag and Hartmann to test the compound for antiestrogenic properties but none were found [1]. Nevertheless the pharmacological activity of Ro 15-0778 and other arotinoids might be broader than that of retinoid effects alone.

SOLID TUMORS

Moon and Itri [7] have reviewed the extensive literature describing the protective properties of retinoids against carcinogen-induced lung, mammary, urinary, bladder, skin and digestive tract carcinomas. The molecular mechanisms by which carcinogens trigger tumor formation are beginning to be unraveled but are not yet fully elucidated. Thus it is premature to speculate on the way in which retinoids counteract carcinogenesis. However, studies have indicated that retinoids interfere with the promotion (rather than the initiation) phase of carcinogenesis (see Ref. [8]). Both natural and synthetic retinoids are active as chemopreventive agents. Among the most potent of the latter is *N*-(4 hydroxyphenyl)retinamide, but even this analog is not uniformly effective: it has superior potency in the prevention of carcinogen-induced mammary and bladder carcinomas, but has little or no activity against methylnitrosourea-induced tracheal, colon and pancreatic carcinoma [7]. Hartmann and Bollag [9] have demonstrated the effectiveness of two arotinoids in the rat mammary carcinogenesis model, but unlike the arotinoid Ro 15-0778, there was only a narrow dose range in which these compounds suppressed tumor growth without eliciting hypervitaminosis A symptoms.

Some promising clinical indications have been reported in head and neck, as well as skin, cancers [7], but overall, retinoids have shown little evidence of efficacy as chemotherapeutics against solid tumors, alone or in combination with other agents. On the other hand, retinoids have demonstrated considerable potency in correcting preneoplastic lesions and preventing their progression to malignancy. Evidence for this has derived from studies *in vitro*, in animals and in the clinic. Representative examples, reviewed by Moon and Itri [7], are alteration *in vitro* of the premalignant phenotype of carcinogen-treated mouse prostate glands, regression of carcinogen-induced skin papillomas in mice, and suppression of actinic keratoses and oral leukoplakia in patients. Several arotinoids are extremely potent in reversing preneoplastic lesions *in vitro* and in mice [2, 5]. Therefore, it would not be surprising if the mechanisms involved in retinoid and arotinoid chemoprevention were found to be related to their ability to counteract progression of preneoplastic lesions.

HEMATOLOGIC MALIGNANCIES

The evidence demonstrating the chemotherapeutic efficacy of retinoids is more convincing in hematologic malignancies than for solid tumors. Even so, additional data are required to define their therapeutic value with this class of tumors. Retinoids inhibit the proliferation of numerous hematologic tumor cell lines of either murine or human origin *in vitro*; at least to some extent, they achieve this by promoting terminal cell differentiation [10]. More relevant, certainly, are studies which establish the clinical efficacy of retinoids, particularly in myelodysplastic syndrome [7, 10]. Since myelodysplastic syndrome consists of diverse disorders, including patients who show a predisposition toward the development of acute myelogenous leukemia, it may be appropriately regarded as a premalignant disease. There is, therefore, an obvious parallel to the efficacy of retinoids against solid premalignancies, as discussed above.

Although several retinoids have been used in clinical studies, only preclinical data currently provide information about retinoid structure–activity relationships. One of the key structural requirements for retinoids, including arotinoids, is the apparent obligatory requirement for a carboxyl or other acidic group at carbon 15 [3, 4, 11]. Not surprisingly, then, Ro 15-0778 neither inhibits proliferation nor induces differentiation of cancer cells, whereas its carboxylated derivative has both activities [4, 11]. Furthermore, structural alterations in other parts of the retinoid or arotinoid molecule can have a marked quantitative effect upon activity levels (e.g. Refs [4, 11]). Finally, structure–activity profiles have been found to vary quantitatively in hematologic vs. solid tumor cell lines [4].

LIABILITIES

Retinoids and arotinoids which are biologically active can exert unwanted side effects at sufficiently high doses. The effects, which can be quite varied in animals and humans, are collectively referred to as hypervitaminosis A and include increased cerebrospinal fluid pressure, anorexia, nausea, vomiting, dryness and scaling of the skin, dryness and irritation of the mucous membranes, tenderness and weakening of the long bones, hyperostosis, hepatomegaly, splenomegaly, teratogenicity and night blindness [2, 12, 13]. As mentioned above, some acidic arotinoids show antitumor effects in animals and antiproliferative effects on malignant cells *in vitro* at very low concentrations; however, they also elicit typical hypervitaminosis A symptoms at much lower doses than many conventional retinoids [2, 5].

PERSPECTIVES AND PROSPECTS

There is ample evidence that arotinoids and other retinoid analogs have chemopreventive and/or chemotherapeutic properties in a number of *in vitro* and animal tumor model systems. It also appears from activity profiles that no single retinoid will be effective as a broad-spectrum agent; different retinoids might have applications in specific malignancies or premalignant lesions. The critical issue concerning the clinical efficacy of retinoids at present, however, is whether there exists for any retinoid an adequate window between therapeutic and toxic doses, particularly in chemoprevention, since long-term administration might be an important requisite for successful treatment. As a therapeutic modality against established malignant diseases or selected premalignant lesions which might in themselves be life-threatening, some of the toxic manifestations of the hypervitaminosis A syndrome might fall within acceptable limits, in view of the consequences. However, the use of arotinoids or other retinoids as cancer chemopreventive agents requires that they be virtually devoid of toxicity in order to be of value to people who, although at increased risk to develop malignant disease, are clinically normal. Based on the data of Loeliger *et al.* [2] and Bollag and Hartmann [1], the arotinoid Ro 15-0778 is a promising candidate since it has demonstrated efficacy as a chemopreventive agent, at least for mammary cancer in a rodent model system, yet appears to be devoid of several of the liabilities observed with other retinoids and arotinoids that might preclude its long-term use. Whether or not this arotinoid proves to be clinically useful, it provides the impetus to continue efforts to design retinoids which retain their therapeutic value but are devoid of liabilities.

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