Refining Retinoids with Heteroatoms

D.M. Benbrook*

Department of Obstetrics and Gynecology, The University of Oklahoma Health Science Center, P.O. Box 26901, Room WP2470, Oklahoma City, OK 73190, USA

Abstract: Retinoids are a group of synthetic compounds designed to refine the numerous biological activities of retinoic acid into pharmaceuticals for several diseases, including cancer. Designs that conformationally-restricted the rotation of the structures resulted in arotinoids that were biologically active, but with increased toxicity. Incorporation of a heteroatom in one cyclic ring of the arotinoid structures drastically reduced the toxicity, while retaining biological activity. Clinical trials of a heteroarotinoid, Tazarotene, confirmed the improved chemotherapeutic ratio (efficacy/toxicity).

TARGETING BIOLOGICAL ACTIVITIES

Medicinal chemists have exploited the wide range of biological activities of retinoic acid (RA) isomers, such as all-*trans*-RA [*trans*-RA, **1**] and 9-*cis*-RA [**2**], in their efforts to develop retinoid pharmaceuticals for skin diseases, cancer, cardiovascular diseases and diabetes [all numbered chemical structures are depicted in Fig. (**1**)]. These activities include regulation of growth, differentiation, apoptosis, embryo development, angiogenesis, metastasis and immune function. Several reviews have previously discussed the synthesis, clinical use and biological activities of retinoids [1]. This review will focus on the use of the heteroatom in the design of retinoids. Retinoids consisting of dramatic modifications of the naturally occurring *trans*-RA [**1**] structure, retain the biological activities, but also exhibit the toxicities of the natural compound. The toxicities associated with chronic retinoid treatment affect the skin, mucus membranes, hair, eyes, gastrointestinal system, liver, neuromuscular system, endocrine system, kidneys and bone, and are collectively termed hypervitaminosis A [2].

The ultimate goal in the design of retinoids is to retain the activities specifically associated with potential therapeutic effects and to eliminate the activities associated with toxicity and teratogenicity. Early efforts to develop retinoids selective for the wide range of biological activities of RA were based on the premise that the flexibility of the tetraene side chain of *trans*-RA [**1**] allowed several conformations that were responsible for the individual activities and, therefore, synthetic compounds designed to be restricted in flexibility would be capable of only a subset of these activities. In the 1980's, conformationally restricted retinoids were designed by constraining the retinoid double-bonds that correspond to the tetraene side chain of trans-RA, by inclusion in an aromatic ring. *In vitro* testing of the anti-cancer activity of these early compounds was performed by three standard bioassays, namely reversal of abnormal differentiation in vitamin-A deficient hamster tracheal organ cultures (TOC assay), induction of differentiation in HL60 leukemia cells measured with nitroblue tetrazolium (NBT assay), and repression of an enzyme involved in cancer cell proliferation, ornithine decarboxylase, (ODC assay) (reviewed in [1]).

One of the first conformationally restricted retinoids, (TTNPB) [**3a**], incorporated the bonds corresponding to the 5,7-double bonds and the 11,13-double bonds of trans-RA [**1**] into a tetrahydronaphthalene ring and phenyl ring systems, respectively, to restrict such bonds to planar cisoid conformations. In addition, a *gem*-dimethyl group was incorporated into the tetrahydronaphthalene ring to block oxidation of the ring at the location that corresponded to the 4-position of the β-cyclogeranylidene ring of trans-RA [**1**]. TTNPB [**3a**] was10-fold more potent than *trans*-RA [**1**] in the TOC assay and was equally effective in the ODC assay [3]. The clinical utility of this compound however, was prevented by a 10,000- fold greater toxicity in comparison to *trans*-RA [**1**] [4-6]. A subsequent strategy to reducing the toxicity of arotinoids was to block oxidation by incorporation of oxygen or sulfur heteroatoms to replace one of the *gem*-dimethyl groups in the tetrahydronaphthalene ring (heteroarotinoids [**3b** and **3c**, respectively]). Both of these heteroarotinoids exhibited significant activity in the TOC and ODC assays, but with reduced potencies in comparison to TTNPB [3,7]. Monaryl oxygen and sulfur heteroarotinoids [**4a, 4b**] that did not incorporate the 11,13 double bonds of *trans*-RA [**1**] into a phenyl ring were equally effective at inhibiting ODC as the corresponding diaryl compounds **3b** and **3c** [8]. Based on the NBT assay, the **4a** and **4b** monoaryls appeared to be better inducers of differentiation, with nanomolar ED_{50} values similar to *trans*-RA [**1**], compared to micromolar ED_{50} values for the diaryls [8]. In the TOC differentiation assay however, the diaryl compounds exhibited ED_{50} values in the nanomolar range similar to *trans*-RA [**1**], but unfortunately the monoaryl heteroarotinoids were not evaluated [3].

THERAPETIC RATIOS IN ANIMAL MODELS

The therapeutic ratios of TTNPB [**3a**] and the oxygen and sulfur heteroarotinoid derivatives [**3b** and **3c**, respectively] were compared in animal models of

*Address correspondence to this author at the Department of Obstetrics and Gynecology, The University of Oklahoma Health Science Center, P.O. Box 26901, Room WP2470, Oklahoma City, OK 73190, USA; Ph.: +1-405-271-5523; Fax:+1-405-271-2968; e-mail: Doris-Benbrook@ouhsc.edu

1389-5575/02 $35.00+.00

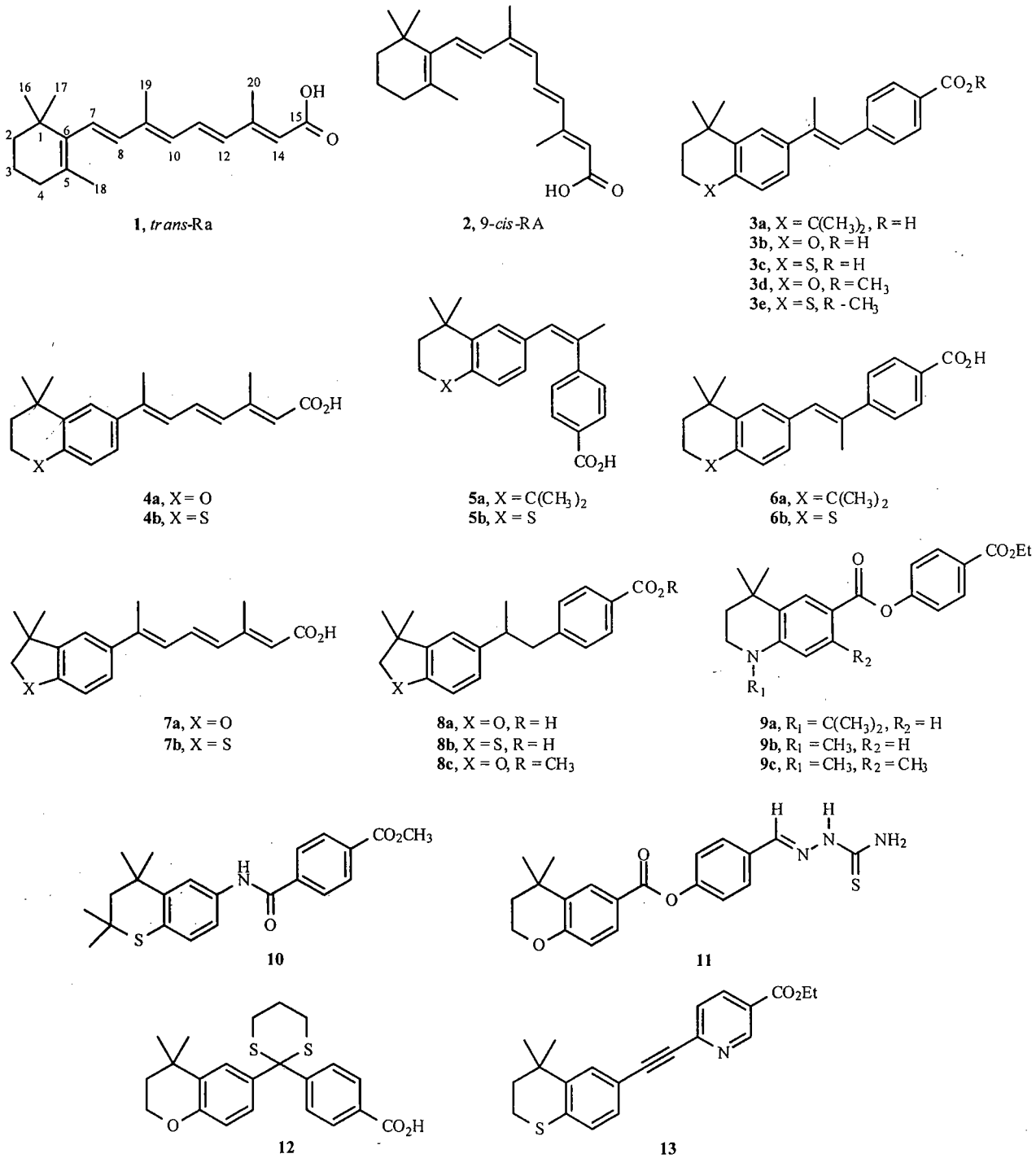

Fig. (1). Structures of retinoids and heteroarotinoids.

chemoprevention and toxicity [3]. In the chemoprevention model, CD-1 mice were treated with DMBA and TPA to initiate and promote tumor formation, respectively. At concentrations of 17 and 170 nanomolar, the heteroarotinoids [**3b**, **3c**] significantly inhibited tumor formation without toxic symptoms, while TTNPB [**3a**] caused animal death due to hypervitaminosis A. At the lower dose of 1.7 nanomoles of TTNPB [**3a**], hypervitaminosis A was observed without animal death, and tumor formation was significantly inhibited. In a toxicity animal model, female Swiss mice were given intraperitoneal injections of a range of concentrations of these compounds over a period of two weeks, and the number of animal deaths was monitored. The results of this model demonstrated that, TTNPB [**3a**] was far more toxic than *trans*-RA [**1**], and killed all of the animals at concentrations as low as 3.3 micromoles per kilogram per day (μM/kg/day), while *trans*-RA [**1**] did not kill the animals at concentrations as high as 100 μM/kg/day. In contrast, the heteroarotinoids [**3b** and **3c**] were actually less toxic than *trans*-RA [**1**]. At a concentration of 200 μM/kg/day, *trans*-RA [**1**], the oxygen heteroarotinoid [**3b**] and the sulfur heteroarotinoid [**3c**] killed 37%, 10% and 20% of the mice, respectively.

In a detailed study of toxicity, **3a, 3b, 4a** and **4b** were administered to groups of 16 animals each by gavage over a range of concentrations for 65 days [6]. Mortalities, body weight, bone fracture incidence, signs of hypervitaminosis, hematologic parameters and pathology were documented. The maximum tolerated doses (MTD's) for each compound were calculated as the doses required to induce 10% weight loss in the treated animals. The MTD for TTNPB [**3a**] was 0.001 mg/kg/day, which is 10,000-fold more toxic than the 10 mg/kg/day MTD of *trans*-RA [**1**]. In contrast, the MTD for the diaryl oxygen heteroarotinoid [**3b**] was 9.4 mg/kg/day, which is comparable to *trans*-RA [**1**]. The 10,000-fold difference in the MTD's for **3a** and **3b** demonstrated that the heteroatom greatly and significantly decreases the toxicity of the arotinoid structure. The MTDs of 32 and 34 mg/kg/day of the monoaryl heteroarotinoids with oxygen **4a** and sulfur **4b** heteroatoms, respectively, revealed a 3-fold decrease in the toxicity of the monoaryl compounds in comparison to *trans*-RA [**1**] and the diaryl heteroarotinoid [**3b**]. One heteroarotinoid, **6b**, was evaluated for teratogenicity in a fetal hamster model [9]. The ED_{50} of **6b** in this model was 5.0 μmol/kg, which is within one order of magnitude of the ED_{50} of 35 μmol/kg for *trans*-RA [**1**] and two orders of magnitude less teratogenic than the ED_{50} of 0.085 μmol/kg for TTNPB [**3a**] [9]. Thus, inclusion of the heteroatom in the arotinoid structure was shown to greatly improve the therapeutic ratio (efficacy/toxicity) in animal models.

TARGETING INDIVIDUAL RETINOID RECEPTORS

A significant breakthrough in the elucidation of the molecular mechanism of action of retinoids came with the discovery and cloning of retinoid receptors in the nuclei of cells. Extensive reviews on these receptors have been published [10,11]. These receptors are transcription factors that mediate the biological effects of retinoids by binding to specific DNA sequences found in the promoters of retinoid regulated genes, called RA response elements (RAREs). Dimerization of two retinoid receptor molecules is required for DNA binding, which can occur in the absence of retinoid binding. There are two classes of retinoid receptors, the RARs and the RXRs, each of which has three receptor subtypes, α, β and γ. These two classes differ, in that the RARs are bound by *trans*-RA [**1**] and 9-*cis*-RA [**2**], and only activate transcription when heterodimerized with RXRs, while RXRs are bound by 9-*cis*-RA [**2**] only and can function as homodimers with self and as heterodimers with RARs and a large number of other nuclear ligand-receptor and orphan-receptor partners. Since each receptor exhibits a unique profile of expression and activities, it was hypothesized that targeting individual receptors would be a reasonable approach to further improving the therapeutic ratio of retinoids.

Efforts to develop compounds selective for individual receptors also utilized conformational restriction in the drug design. Since, the flexibility of the tetraene side chain of *trans*-RA [**1**] allows it to adopt the different conformations required for binding to the ligand-binding pockets of both the RARs and RXRs, it was hypothesized that restricting the rotation of synthetic retinoids using conformational barriers will cause selectivity for individual retinoid receptors. A method called the co-transfection assay, described in Fig. (**2**), was used to test the ability of retinoids to activate individual receptors. Retinoids that exclusively activate one or a subset of the receptors are termed "specific" for that receptor or subset of receptors. Retinoids that exhibit a much greater potency for one receptor or subset of receptors are termed "selective" for that receptor or subset of receptors. Using this assay, TTNPB [**3a**] and the diaryl heteroarotinoids **3b** and **3c** were found to be selective for RAR receptors [12,13]. Since 9-*cis*-RA [**2**] is capable of binding the RXR receptors, it was hypothesized that TTNPB-derivatives restricted in the *cis* (Z) conformation would activate RXR receptors [12]. To test this theory, the structure of TTNPB [**3a**] and its sulfur heteroarotinoid derivative **3c** were modified by moving the methyl group on the double bond corresponding to the 9 position of *trans*-RA [**1**] to the double bond corresponding to the 10 position, and then *cis* [**5a, 5b**] and *trans* [**6a, 6b**] isomers of each of these compounds were separated. The co-transfection assay was then used to demonstrate that only the *cis* compounds **5a** and **5b** were capable of RXR activation. ^1H NMR studies indicated that interactions between the aromatic rings of the *cis* and *trans* isomers were quite different [12]. X-ray crystallographic and computational analysis demonstrated two distinct conformations for the *cis* and *trans* isomers, with the aliphatic rings of the *cis* isomers occupying spatial positions similar to 9-*cis*-RA [**2**] and other known RXR-specific retinoids (rexinoids) [14]. The RXR active compounds exhibited greater C5'-CO_2H interatomic distances than the compounds incapable of RXR activation [12,14]. Monaryl heteroarotinoids **3a** and **3b** that do not restrict the bonds corresponding to the 11,13 double bonds of *trans*-RA [**1**] were also found to be capable of RXR activation, but with very weak potency [13].

Comparison of a series of 10 heteroarotinoids that differed by single structural alterations revealed that single substitutions could dramatically alter the receptor selectivity of the compounds [13]. In addition to the diaryl **3b, 3d** and **3e**, monoaryl compounds, **4a** and **4b**, and related structures containing 5-membered instead of 6-membered rings [**7a, 7b, 8a, 8b** and **8c**] were evaluated. Two dominant associations between structure and specificity for RAR receptors were consistent across all compounds. The six-membered ring systems conferred increased RARβ specificity over the five-membered ring systems. The sulfur atom conferred greater specificity for RARγ than the oxygen atom. RARα specificity was attenuated by a combination of influences from the heteroatom and aryl groups.

The strong affect of individual substitutions on receptor specificity was also observed in a study of structurally related nitrogen heteroarotinoids that differed by single substitutions at positions corresponding to the C-4 and C-5 positions of *trans*-RA [**1**], namely compounds **9a, 9b** and **9c** [15]. These compounds contained ester linkers between the two aryl rings, which increased the flexibility of the compounds in comparison to their counterparts containing alkenyl linkers [6]. Maximal activation of RAR receptors appears to require the presence of a lipophilic group that extends outward and away from the plane of the ring at the

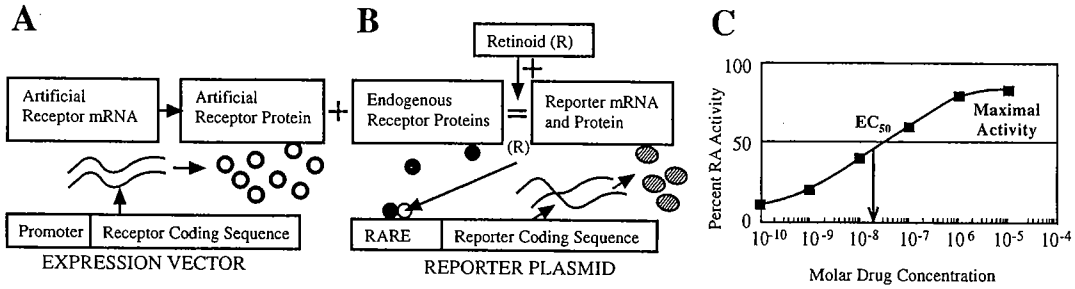

Fig. 2. Retinoid receptor activation assay.

The co-transfection assay requires steps A, B and C, while the Reporter Cell Line Assay only requires steps B and C.

A. A method, called transfection, is used to place a DNA plasmid containing the coding sequence of the specific receptor inside living cells. The DNA plasmid, called an expression vector, also contains another DNA sequence element, called a promoter, which causes the transcription of the receptor's coding sequence into RNA (curving lines), which is then translated into protein (open circles). The amount of receptor protein expressed from the artificial DNA plasmid is much greater than the amounts of endogenous retinoid receptor proteins (closed circles) in the cells. Although these artificially expressed receptors dimerize with endogenous receptors, their high levels are assumed to be the dominating influence on the retinoid effects measured in steps B and C.

B. Another DNA plasmid, called a reporter plasmid, is transfected into the cells. The reporter plasmid contains the RARE DNA sequence next to a coding sequence for a protein (reporter protein) that can be measured. Activated receptor dimers (coupled open and closed circles) bound at the RARE sequence will increase expression of the reporter mRNA (curving lines) and protein (striped circles). The ability of a retinoid to activate the receptors is then measured by treating transfected cell cultures with a range of retinoid concentrations (usually 10^{-12} to 10^{-5} Molar). If the retinoid being tested activates the receptor proteins, the activated receptor dimers will increase expression of the reporter protein. After 48 hours of treatment, amounts of reporter protein in the cultures are measured.

C. The amount of reporter protein in the culture treated with the test retinoid is divided by the amount in the control culture treated with *trans*-RA [**1**] or 9-*cis*-RA [**2**] and multiplied by 100 to derive the percent of RA activity exhibited by the test compound. This value is plotted against the concentration of test retinoid administered. The efficacy of the retinoid is defined as the maximal percent of RA activity achieved. The concentration required to induce half-maximal activity, called an EC_{50} value, is derived from the graph and used as a measure of potency.

position of the retinoid corresponding to C-4 of *trans*-RA [**1**] [1]. Consistent with this observation, it was found that screening of the polarity of a nitrogen heteroatom with an isopropyl group [**9a**] resulted in greater receptor activation than screening with a methyl group [**9a**]. Although these compounds activated RARα, RARβ and all three RXR receptors, neither compound was capable of activating the RARγ receptor. Substitution of a methyl group at the position corresponding to C-5 of *trans*-RA [**1**] in **9c** decreased the flexibility around the ester linker and conferred the ability to activate the RARγ receptor. Molecular modeling using SYBYL 6.5 software and employing the licensed "Biopolymer", which possesses a program designated "FlexiDock", demonstrated that **9c** occupied the RARγ ligand binding pocket with a similar conformation and energies of interaction as RA isomers, while **9a** and **9b** fit with energies of interaction that were significantly higher. The amino acid Phe230 of RARγ assumed a different orientation when bound by the RARγ inactive verses RARγ active compounds. Other amino acids of RARγ, such as Ala234, Met272, and Leu271, appeared to be involved in the selectivity for different ligands through van der Waal interactions [15].

To determine if receptor specific/selective compounds have therapeutic potential, biological assays that had endpoints predictive of therapeutic responses were utilized. Therapeutic endpoints for cancer commonly evaluated for the receptor selective compounds were inhibition of growth and induction of differentiation or apoptosis in cancer cell cultures, as well as the regulation of enzymes involved in these processes, such as transglutaminase (TGase). In these assays, compounds containing sulfur or nitrogen heteroatoms consistently exhibited greater biological activities than compounds with oxygen heteroatoms [3,6,15,16]. This trend was not due to bioavailability, since the sulfur heteroarotinoids were less toxic than their oxygen counterparts [3,6]. The biological activities of receptor selective compounds appeared to be weaker than the RAR panagonist *trans*-RA [**1**] and the RAR and RXR panagonist 9-*cis*-RA [**2**]. Several studies have demonstrated that simultaneous treatment with retinoids that have complementary receptor profiles exhibited greater biological activity than single treatments with the individual receptor selective compounds [17-22]. The receptor panagonist nitrogen heteroarotinoid **9c** exhibited much greater activity against cancer cell lines derived from the vulvar skin than the related **9a** and **9b** devoid of RARγ activation and also greater than *trans*-RA [**1**] and 9-*cis*-RA [**2**] [15]. RARγ is selectively expressed at high levels in skin and has been shown to be the mediator of retinoid pharmacological action in skin [23-25]. Therefore, the approach of designing retinoids that are specific for individual receptors produced weak compounds, while compounds that activate more than one receptor were more efficient. An alternative approach of screening for biologically relevant effectiveness before

determining receptor specificity was used by a research group in Oklahoma, USA, to develop heteroarotinoids **3a, 3b, 3c, 4a, 4b, 7a, 7b, 8a, 8b, 8c, 9a, 9b, 9c, 10** and **11** and study their mechanism of action as described below.

TARGETING ENDOGENOUS RECEPTORS

The Oklahoma research group took an alternative approach to the targeting of individual retinoid receptors. This approach is based on the reality that the complex mixture of retinoid receptors, along with other cellular proteins, determines the ultimate outcome of retinoid treatment in target cells. The activities of the endogenous retinoid receptor proteins are modulated by direct binding of endogenous co-activator and co-repressor proteins and by phosphorylation of the retinoid receptor protein by endogenous enzymes called kinases and phosphatases [26-29]. In addition, other transcription factors that are bound by the same co-factors and co-repressors can either sequester these factors or form inactive tertiary complexes with the retinoid receptors and co-factors. Therefore, efforts to control the specific activities of retinoids by targeting individual receptors may be hampered by the intracellular environment of the target cells. To allow for this complexity, the Oklahoma group tested the activity of their synthetic retinoids in target cells without artificial expression of retinoid receptors.

A cancer cell line, derived from a low grade cervical tumor, CC-1, that was growth inhibited by retinoids was chosen to be used in the development of a biologically relevant assay for retinoid action. The CC-1 cell line was characterized for retinoid receptor expression, and like many epithelial tumors, that have decreased or lost expression of the RARβ retinoid receptor; CC-1 expressed all of the receptors except for RARβ [30]. The next step was to demonstrate that the endogenous receptors in CC-1 were activated by RA isomers in a dose-responsive manner. As described in Fig. (**2**), a reporter plasmid was permanently transfected into CC-1, and the abilities of retinoids to activate transcription from that plasmid were measured. The CC-1 reporter cell line demonstrated a dose-responsive induction of CAT activity when treated with *trans*-RA [**1**], 9-*cis*-RA [**2**] and 13-*cis*-RA, thus providing a biologically relevant assay of retinoid activity [30]. Next, a series of 14 heteroarotinoids was screened for inhibition of growth and activation of endogenous receptors using the CC-1 reporter cell line. A statistically significant correlation was noted between the ability of the individual compounds to inhibit the growth and activate the endogenous receptors of the reporter cell line.

In a similar study of 15 compounds using two Head and Neck cancer cell lines, called SCC-2 and SCC-38, a sulfur and an oxygen heteroarotinoid [**10** and **11**, respectively] were identified as the most active compounds against both cell lines [16]. Using the co-transfection assay, **10** was found to be a potent receptor panagonist, while **11** was noted to be specific for RXR receptors. Both compounds significantly inhibited growth of SCC-38 xenograft tumors in a mouse model without any evidence of toxicity. The efficacy of the RXR-specific **11** indicated that RXR activation was sufficient to inhibit tumor growth, while the greater activity of **10** indicated that both RAR and RXR activation were required for maximal activity. RXR-specific compounds, such as **11**, have tremendous potential for pharmacological manipulation of a wide variety of diseases due to the large number of ligand-activated and orphan receptors that dimerize with RXR receptors.

TARGETING ANTI-AP-1 ACTIVITY

Retinoids possess another transcriptional regulatory activity that is distinct from the RARE transactivation. Activated retinoid receptors can inhibit the induction of gene expression by the AP-1 family of transcription factors [31]. Induction of these two different activities in the same receptor protein was hypothesized to require different receptor protein conformations that are induced or stabilized by retinoid binding. To test this hypothesis, a series of conformationally restricted receptor selective retinoids were screened for their effects on RARE and AP-1 driven transcription [32]. For each retinoid, the set of receptors responsible for RARE transactivation was distinct from the set of receptors responsible for AP-1 repression. The receptors that mediated the anti-AP-1 activity of a certain compound were slightly antagonized for RARE transactivation by that compound, thus supporting the hypothesis that different receptor conformations are required for the two activities. One of the compounds tested in this study was an oxygen heteroarotinoid, called SR11238 [**12**], which was only 10% as active as 9-*cis*-RA [**2**] in inducing RARE transactivation, but was greater than 81% as active as 9-*cis*-RA [**2**] in repressing AP-1 driven transcription. While the RARE transactivation activity of this compound was mediated through RARγ and RXRα, the anti-AP-1 activity exhibited a different receptor profile (RARβ > RARγ > RXRα ≥ RARα).

The profiles of biological activities of SR11238 [**12**] and other selective anti-AP-1 retinoids were different from the activity profiles of compounds *trans*-RA [**1**] and 9-*cis*-RA [**2**] [32]. The anti-AP-1-selective compounds did not induce differentiation or inhibit proliferation in F9 mouse teratocarcinoma cells, but they were potent inhibitors of growth of two cancer cell lines, namely the Calu-6 lung and the T47D breast carcinoma cell lines. These results suggest that anti-AP-1 selective compounds might exhibit better therapeutic ratios in cancer chemoprevention, because a lack of RARE transactivation might reduce toxicity. Not all tumors are growth inhibited by anti-AP-1 selective retinoids however, and retinoids have been shown to be capable of inhibiting tumor cell growth through anti-AP-1 independent mechanisms [19,33]. Therefore, anti-AP-1 selective compounds may be weaker than agonists possessing both anti-AP-1 and RARE transactivation capabilities and may not be effective against some tumors.

CLINICAL USE

The clinical application of a heteroarotinoid Tazarotene [**13**] produced by Allergan, has confirmed the improved therapeutic ratio predicted for compounds with heteroatoms

[34]. The ethyl ester form of the drug, which does not bind retinoid receptors, is used as prodrug that is readily metabolized to the receptor-active tazarotenic acid. A nicotinic acid moiety is utilized as the terminal aryl group to ensure the rapid metabolism to the acid form. The sulfur heteroatom of circulating tazarotenic acid is metabolically deactivated by oxidation, thereby producing the inactive sulfoxide and sulfone metabolites that are excreted in the urine [35]. In addition, a triple bond between the two aromatic rings conformationally restricts this compound. Tazarotene is being investigated as a single agent for the treatment of acne vulgaris and in combination with corticosteroids for the treatment of psoriasis [36,37]. The mechanism of action in psoriasis is thought to occur through direct regulation of genes involved in differentiation and inflammation [38]. Tazarotene is administered topically, and its good safety profile is most likely due to the low penetration through the skin to the blood system, rapid metabolism and elimination from the body [39]. Tazarotene does not cause contact sensitization, and induces only mild to moderate reversible skin irritation. Since the toxic effects of retinoids in skin are associated with specific activation of RARγ, the RARβ/ RARγ selectivity of Tazarotene, could explain its decreased toxicity in contrast to the severe skin irritation caused by other RARγ-selective compounds. This finding supports the theory that targeting individual receptors in retinoid drug design may not be as effective as targeting subsets of receptors or biological activities in the natural environment of the target cells.

CONCLUSIONS

The heteroatom has redeemed the clinical potential of the arotinoid structure by dramatically decreasing the toxicity. The reduced toxicity is not directly related to decreased efficacy, thus improving the chemotherapeutic ratio. The receptor specificity and other biologic activities can be further refined by single structural alterations of the heteroarotinoid structure. Clinical trials of the heteroarotinoid, Tazarotene [**13**], have confirmed the increased effectiveness and reduced toxicity of retinoids containing heteroatoms. These results demonstrate the clinical promise of the use of heteroarotinoids in dermatological disorders, cancer and other diseases.

ACKNOWLEDGEMENTS

The author is very grateful for the critical review of this manuscript by her collaborator K. Darrell Berlin, PhD, at Oklahoma State University.

REFERENCES

[1] Gale, J.B.; In *Progress in Medicinal Chemistry*; G.P. Ellis and D.K. Luscombe, Ed.; Elsevier Science B.V. Amsterdam, **1993**; Vol. *30*, pp. 1-55.

[2] Silverman, A.K.; Ellis, C.N.; Voorhees, J.J. *J. Am. Acad. Dermatol.*, **1987**, *16*, 1027.

[3] Dawson, M.I.; Hobbs, P.D.; Derdzinski, K.; Chan, R.L.-S.; Gruber, J.; Chao, W.-R.; Smith, S.; Thies, R.W.; Schiff, L.J. *J. Med. Chem.*, **1984**, *27*, 1516.

[4] Lindamood, C., III; Cope, F.O.; Dillehay, D.L.; Everson, M.P.; Giles, H.D.; Lamon, E.W.; McCarthy, D.J.; Sartin, J.L.; Hill, D.L. *Fund. Appl. Toxic.*, **1990**, *14*, 15.

[5] Lindamood, C.I.; Dillehay, D.L.; Lamon, E.W.; Giles, H.D.; Shealy, Y.F.; Sani, B.P.; Hill, D.L. *Toxicol. Appl. Pharamcol.*, **1988**, *96*, 279.

[6] Benbrook, D.M.; Madler, M.M.; Spruce, L.W.; Birckbichler, P.J.; Nelson, E.C.; Subramanian, S.; Weerasekare, G.M.; Gale, J.B.; Patterson, Jr., M.K.; Wang, B.; Wang, W.; Lu, S.; Rowland, T.C.; DiSilvestro, P.; Lindamood, C.; Hill, D.L.; Berlin, K.D. *J. Med. Chem.*, **1997**, *40*, 3567.

[7] Waugh, K.M.; Berlin, K.D.; Ford, W.T.; Holt, E.M.; Carroll, J.P.; Schomber, P.R.; Schiff, L.J. *J. Med. Chem.*, **1985**, *27*, 116.

[8] Spruce, L.W.; Rajadhyaksha, S.N.; Berlin, K.D.; Gale, J.B.; Miranda, E.T.; Ford, W.T.; Blossey, E.C.; Verma, A.K.; Hossain, M.B.; van der Helm, D.; Breitman, T.R. *J. Med. Chem.*, **1987**, *30*, 1474.

[9] Willhite, C.C.; Jurek, A.; Sharma, R.P.; Dawson, M.I. *Tox. Appl. Pharm.*, **1992**, *112*, 144.

[10] Giguére, V. *Endocrin. Rev.*, **1994**, *15*, 61.

[11] Petkovich, M. *Annu. Rev. Nutr.*, **1992**, *12*, 433.

[12] Jong, I.; Lehmann, J.M.; Hobbs, P.D.; Harlev, E.; Huffman, J.C.; Pfahl, M.; Dawson, M.I. *J. Med. Chem.*, **1993**, *36*, 2605.

[13] Benbrook, D.M.; Subramanian, S.; Gale, J.B.; Liu, S.; Brown, C.W.; Boehm, M.F.; Berlin, K.D. *J. Med. Chem.*, **1998**, *41*, 3753.

[14] Lehmann, J.M.; Jong, L.; Fanjul, A.; Cameron, J.F.; Lu, X.P.; Haefner, P.; Dawson, M.I.; Pfahl, M. *Science*, **1992**, *258*, 1944.

[15] Dhar, A.; Liu, S.; Klucik, J.; Berlin, K.D.; Madler, M.M.; Lu, S.; Ivey, R.T.; Zacheis, D.; Brown, C.W.; Nelson, E.C.; Birckbichler, P.; Benbrook, D.M. *J. Med. Chem.*, **1999**, *42*, 3602.

[16] Zacheis, D.; Dhar, A.; Lu, S.; Madler, M.M.; Klucik, J.; Brown, C.W.; Liu, S.; Clement, F.; Subramanian, S.; Weerasekare, G.M.; Berlin, K.D.; Gold, M.; Houck, J.R.; Fountain, K.R.; Benbrook, D.M. *J. Med. Chem.*, **1999**, *42*, 4434.

[17] Lotan, R.; Dawson, M.I.; Zou, C.-C.; Jong, L.; Lotan, D.; Zou, C.-P. *Cancer Res.*, **1995**, *55*, 232.

[18] Roy, B.; Taneja, R.; Chambon, P. *Mol. Cell. Biol.*, **1995**, *15*, 6481.

[19] Giannini, G.; Dawson, M.I.; Zhang, X.-K.; Thiele, C.J. *J. Biol. Chem.*, **1997**, *272*, 26693.

[20] Joseph, B.; Lefebvre, O.; Mereau-Richard, C.; Danze, P.M.; Belin-Plancot, M.T.; Formstecher, P. *Blood*, **1998**, *91*, 2423.

[21] Brooks III, S.C.; Kazmer, S.; Levin, A.A.; Yen, A. *Blood,* **1996**, *87*, 227.

[22] Wu, S.; Zhang, D.; Donigan, A.; Dawson, M.I.; Soprano, D.R.; Soprano, K.J. *J. Cell. Biochem.*, **1998**, *68*, 378.

[23] Chen, S.; Ostrowski, J.; Whiting, G.; Roalsvig, T.; Hammer, L.; Currier, S.J.; Honeyman, J.; Kwasniewski, B.; K.-L., Y.; Sterzycki, R.; Kim, C.U.; Starrett, J.; Mansuri, M.; Reczek, P.R. *J. Invest. Dermatol.*, **1995**, *104*, 779.

[24] Look, J.; Landwehr, J.; Bauer, F.; Hoffman, A.S.; Bluethmann, H.; Lemotte, P. *Am. J. Physiol.*, **1995**, *269*, E91.

[25] Zelent, A.; Krust, A.; Petkovich, M.; Kastner, P.; Chambon, P. *Nature,* **1989**, *339*, 714.

[26] Dowhan, D.H.; Muscat, G.E.O. *Nucl. Acids Res.*, **1996**, *24*, 264.

[27] Lefebvre, P.; Gaub, M.-P.; Tahayato, A.; Rochette-Egly, C.; Formstecher, P. *J. Biol. Chem.*, **1995**, *270*, 10806.

[28] Bhat, M.K.; Ashizawa, K.; Cheng, S.-Y. *Proc. Natl. Acad. Sci.*, **1994**, *91*, 7927.

[29] Rochett-Egly, C.; Adam, S.; Rossignol, M.; Egly, J.-M.; Chambon, P. *Cell,* **1997**, *90*, 97.

[30] Benbrook, D.M.; Lu, S.; Flanagan, C.; Shen-Gunther, J.; Angros, L.H.; Lightfoot, S.A. *Gyn. Oncol.*, **1997**, *66*, 114.

[31] Pfahl, M. *Endocrin. Rev.*, **1993**, *14*, 651.

[32] Fanjul, A.; Dawson, M.I.; Hobbs, P D.; Jong, L.; Cameron, J.F.; Harlev, E.; Graupner, G.; Lu, X.-P.; Dawson, M.I.; Pfahl, M. *Nature,* **1994**, *372*, 107.

[33] Lu, S.; Dhar, A.; Berlin, K.D.; Benbrook, D.M. *J. Med. Chem.*, **2001**, submitted.

[34] Chandraranta, R.A.S. *Br. J. Dermatol.*, **1996**, *135*, 18.

[35] Chandraratna, R.A.S. *J. Am. Acad. Dermatol.*, **1997**, *37*, S12.

[36] Kakita, L. *J. Am. Acad. Dermatol.*, **2000**, *43*, S51.

[37] Lebwohl, M. *J. Am. Acad. Dermatol.*, **2000**, *43*, S43.

[38] Duvic, M.; Nagpal, S.; Asano, A. T.; Chandraratna, R.A.S. *J. Am. Acad. Dermatol.*, **1997**, *37*, S18.

[39] Menter, A. *J. Am. Acad. Dermatol.*, **2000**, *43*, S31.