Pure & Appl. Chem., Vol. 57, No. 5, pp. 709–716, 1985.
Printed in Great Britain.

Retinoids: an overview of some natural carotenoid metabolites and their synthetic analogs

FRITZ FRICKEL

BASF AG, D-6700 Ludwigshafen, Federal Republic of Germany

Abstract - Retinoids are very effective compounds for the control of both cellular differentiation and cellular proliferation. New synthetic analogs show pronounced activity in suppressing experimental carcinogenesis in vivo by inhibiting the development of the malignant phenotype in a non-cytotoxic manner. Beside the extensive use of retinoids in dermatology they are currently being tested for the prevention and therapy of cancerous diseases in man. Whether increased consumption of ß-carotene, the precursor of natural retinoids, can prevent cancer is being evaluated in clinical intervention trials.

INTRODUCTION

The naturally occurring retinoids (the term retinoid is applied to retinol and its natural and synthetic congeners designed to exhibit at least one of the biological properties of retinol and its metabolites) ultimately all have their origin in a small number of plant carotenoids - predominantly ß-carotene - since animals are incapable of de novo synthesis of vitamin A-active substances having an isoprenoidal substructure. ß-Carotene is metabolized mainly in the intestinal mucosa by two soluble enzyme systems, to give retinol. First, ß-carotene 15,15'-dioxygenase catalyzes the oxidative cleavage of ß-carotene at the central double bond, to give two molecules of retinal, and the latter is then reduced by retinaldehyde reductase to retinol. Retinol furthermore undergoes esterification with saturated fatty acids in the mucosal cell. Retinyl esters, mainly retinyl hexadecanoate, are stored in the liver and retinol is transported from there to a retinoid-requiring tissue by a highly regulated process (Ref. 1).

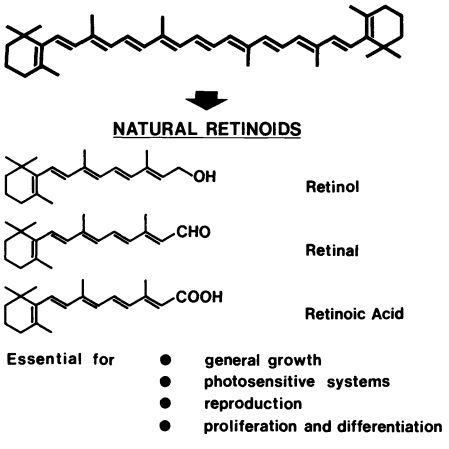

Fig. 1. Natural retinoids are oxidative metabolites of ß-carotene.

A wide range of photosensitive organisms utilize a retinal, coupled to a membrane protein via a protonated aldimine structure, as a light antenna. The reactions taking place after the absorption of light always include cis⟷trans isomerization of the chromophore. This sector of retinoid research has seen intensive work over the past forty years, and excellent review articles have been published (Ref. 2).

Other important physiological functions of retinol and of its naturally occurring congeners were found while studying general growth, and reproduction, of organisms. The results of this retinoid research are of great importance to the work of nutritionists in those parts of the world where inadequate intake of vitamin A-active substances is responsible for many diseases (Ref. 3).

EFFECTS OF RETINOIDS ON CELLULAR PROLIFERATION AND DIFFERENTIATION

As long ago as 1925 Wolbach and Howe documented the fundamental role of retinoids both in cellular proliferation and in cellular differentiation. This capability, originally found with natural retinoids, is now also well documented for synthetic analogs. In addition to the established therapy of dermatological disorders numerous clinical studies are being carried out for the prevention and therapy of cancerous diseases and it has been proposed that retinoids may be useful agents for disease whose cause resides in excessive proliferation of cells or extracellular matrix (Ref. 4).

The greatest advances with retinoids have hitherto undoubtedly been achieved in the therapy of severe dermatological disorders, such as cystic acne, psoriasis and related cutaneous keratinization disorders. Clinical investigations with 13-cis-retinoic acid have shown that the reduction in sebum production which commences before actual clinical improvement is one of the possible mechanisms of action of this retinoid (Ref. 5). For the development of new synthetic retinoids with reduced adverse effects - a development for which the need still remains - new test models have been developed, for example the rhino mouse model, in which the dermal differentiation activity of new retinoids is determined in terms of the reduction of horn-filled utriculi of the hairless rhino mouse ($hr^{rh}hr^{rh}$) (Ref. 6). Clinical testing of the therapeutic spectrum of some synthetic retinoids has also provided us with a better understanding of the toxicological properties of orally administered retinoids. On comparing the frequency and severity of adverse reaction in groups of patients which had been treated with retinol, all-trans-retinoic acid, 13-cis-retinoic acid or etretinate, qualitative differences in action were found. In general, the toxic symptoms of these retinoids are very similar to the long-established toxicological symptoms shown after excessive consumption of materials rich in retinyl esters and retinol, such as polar bear liver or halibut liver. The reader may wish to refer to a review on this topic concerning orally administered retinoids (Ref. 7). Although it is not possible to interpret these observations in molecular terms, work with synthetic retinoids suggests that there is a relationship between structure and activity; it is the task of organic chemists to design new structures in which there is a sufficient margin of safety between the therapeutically required dose and the dose which produces unacceptable side effects.

Research on the fundamental role of retinoids in controlling the state of cellular differentiation and in reversing rapid proliferation is likely to produce suitable candidates,

with which new modes of cancer treatment and, in particular, cancer prevention should be possible. Many studies document the fact that retinoids suppress experimental carcinogenesis in animals. These results led M. B. Sporn (Ref. 8) and W. Bollag (Ref. 9) to develop the concept of "chemoprevention of cancer with retinoids". The present data, utilizing for example the chemically induced mouse skin carcinogenesis assay, demonstrate that the retinoids available at the present time inhibit the promotion phase of skin tumor formation. Retinoids are substantially less effective in influencing the late stage of carcinogenesis, as may be seen in the treatment of malignant melanoma patients with 13-*cis*-retinoic acid. Retinoids are not conventional anticancer agents which act by a cytotoxic mechanism, although G. Peck *et al.* (5) observed complete clinical regression of several lesions in patients with multiple basal cell carcinoma.

In addition, there are a large number of investigations which substantiate the view that retinoids are potent agents for suppressing the development of the malignant phenotype, brought about *in vitro* by a carcinogen, and the action does not result from the killing of the premalignant or malignant cells. Lasnitzki (11) showed that, in an organ culture, retinol and retinyl esters suppressed the development of squamous metaplasia and the incidence of hyperplasia of mouse prostate glands in which carcinogenesis was initiated by the carcinogen 3-methyl-cholanthrene. Working with cell cultures of non-neoplastic mouse fibroblastic cells, Merriman and Bertram (12) succeeded, using retinyl acetate, in suppressing the malignant transformation after these cells had been treated with a carcinogen. In this experimental method, retinoids suppress the transformation of the cells even if the retinoid is only brought into contact with the cells a week after the initiation by the carcinogen. This last observation is of great importance in designing protocols for cancer prevention in man by means of retinoids. Numerous *in vivo* investigations have also confirmed that retinoids are able to inhibit the development of an invasive tumor even after there had been initiation of carcinogenesis. Moon *et al.* (13) carried out many investigations of this type on the chemoprevention of cancer of the mammary glands. In rats, the mammary carcinogenesis initiated by the carcinogen 7,12-dimethylbenz[a]anthracene was reduced by 52% by daily administration of 2.5 mg of retinyl acetate. Using the direct-acting carcinogen N-methyl-N-nitrosourea (MNU), this well-defined animal model was employed to screen about 20 further synthetic retinoids for their chemopreventive activity against mammary cancer. Five of these retinoids were found to have a substantial activity. Additional studies have shown that, evidently, retinoids act synergistically with other modifiers of mammary carcinogenesis. Mammary cancer induced by MNU was suppressed more effectively by retinyl acetate and selenium than by the treatment regimen alone; accordingly, it is vital that the possibility of combination chemoprevention studies with retinoids and other agents should receive consideration in clinical trials with human subjects (Ref. 13). Although the mechanism of how retinoids inhibit mammary carcinogenesis is at present unclear, Moon *et al.* (13) have demonstrated the inhibition of ductal branching and endbud proliferation in virgin female rats chronically treated with a diet supplemented with retinyl acetate. There is also substantial evidence that retinoids inhibit the development of chemically induced neoplasia of the urothelium in experimental animals. For some twelve retinoid structures it has been possible to demonstrate the chemopreventive activity against urinary bladder cancer in rats or mice. On the other hand a roughly equally large number of retinoids has been tested which were incapable of reducing the formation of bladder carcinomas.

While these animal studies provide good documentation of the potential for using retinoids in cancer prevention and therapy, there are comparatively few clinical data and these barely permit well-founded conclusions concerning the activity spectrum of retinoids in man. Thus, older clinical studies were carried out on only a small number of test subjects, without control groups. Moreover, the retinoids available at that time had a marked hypervitaminosis A toxicity which did not permit the use of the effective doses required for prevention or therapy. Very recently, a series of comprehensive studies with synthetic retinoids has been started but it will be some years before these permit unambiguous conclusions concerning the potential clinical use of retinoids in cancer prevention and in the therapy of preneoplastic and neoplastic lesions. As an example we may mention intervention trials with retinyl acetate and (all-E)-retinoic acid carried out on female subjects with cervical dysplasia (Ref. 13). In these double-blind studies, the retinoid is applied topically directly to the cervix. New clinical studies are handicapped by the fact that only a few retinoids are permitted for use in man and these few permitted retinoids have considerable toxic side effects. Intervention trials carried out with β-carotene - a remarkably safe drug even at high doses given over years - do not suffer from this handicap.

Epidemiological studies suggest that a low blood level of β-carotene may be associated with increased cancer risk. This observation is currently being investigated in a randomized clinical trial, lasting 5 years, using LUROTIN™ on a group of some 25,000 U.S. male physicians; the trial is supported by the National Cancer Institute and the National Heart, Lung and Blood Institute. Later research will have to answer the important question of whether the protective effect of β-carotene in reducing the risk of cancer is due to its enzymatic transformation to natural retinoids, so that ultimately it is the retinoids which are the active components, or whether β-carotene possesses cancer-preventive properties. There are in particular physicochemical arguments in favor of this last assumption, since β-carotene deactivates reactive chemical intermediates such as singlet oxygen and free radicals (Ref. 14). The large number of animal data, case reports in humans and the fact that retinoid deficiency leads to increased susceptibility to attack by carcinogens, tend to confirm that the importance of retinoids in clinical medicine lies less in the treatment of tumors than in that the retinoids may possibly be useful in cancer prevention.

The cellular and molecular mechanisms of action by which retinoids modify cell differentiation and cell proliferation are still unknown and it is presently not possible to offer an all-embracing and non-contradictory explanation of the extensive experimental data (Ref. 8).

One hypothesis maintains a cofactor role for retinol in glycoprotein synthesis through the formation of retinyl mannopyranosyl hydrogen phosphate, which may transfer its glycosyl moiety to a protein (Ref. 15). This monosaccharide transfer mechanism stresses the probability that the three oxidation states of the natural retinoids shown in Fig. 1 play unique roles in various areas. Further arguments in this direction are the long known fact that only retinal can enable the process of vision, that only retinol is able to support reproduction and that retinoic acid is more active in most retinoid assays than retinol or retinal.

However before any structure-specifity relationship is proposed in this way one should keep in mind that areas are difficult to demarcate in which only retinoic acid can effect a biological response since retinol and retinal are oxidized in vivo to retinoic acid.

Though retinoids do not appear to be agents for the treatment of transplantable tumors in vivo, they do exhibit substantial effects on the growth and differentiation of a great variety of neoplastic cells under cell culture conditions. Typical effects of retinoids on the growth of neoplastic cells are exemplified by the extensive investigations of Lotan et al. (10) on the influence of retinoids on murine melanoma cell lines and some human melanoma cell lines. After 48 to 72 hours' contact of the cells with an active retinoid, the growth rate decreases in a dose-dependent manner. This reduction in cell growth rate is independent of cell density and it has been demonstrated that both anchorage-dependent and anchorage-independent growth can be influenced. In these experiments, continuous exposure to retinoic acid is necessary, otherwise the cells revert to their original growth rate after a further 48 to 72 hours. It is important to mention that (all-E)-retinoic acid is not cytotoxic to these neoplastic cells at concentrations demonstrable in plasma under physiological conditions. A great number of very diverse retinoid structures have been studied for their antiproliferative activity in the murine melanoma cell line S 91, and structure-activity relationships can be worked out by comparing the ED_{50} values of the corresponding dose-response curves (Ref. 10). This is one of the numerous test systems which we have available for assessing how effectively a new retinoid structure can modify a standardized biological system. Such correlations between chemical structure and biological response are equally important for discovering new retinoid structures with potential application in man and for studying mechanistic details of the mode of action of retinoids.

The ability of retinoids to promote differentiation in various neoplastic cell types can also be demonstrated, only by way of example, from the induction of terminal differentiation of the human promyelocytic leukemia cell line HL-60. Some retinoids functionalized with a carboxylic acid group have proved to be very potent agents in stimulating the normal maturation sequence, which seems to be blocked in acute myelogenous leukemia leading to functionally and morphologically mature granulocytes. The degree of differentiation of this human leukemia cell line can be quantified by measuring the percentage of cells able to reduce nitroblue tetrazolium chloride i.e. NBT positive cells to the blue dye formazan, as well as by morphological criteria.

Fig. 2 shows the dose-response curve of (all-E)-retinoic acid and of two retinoidal benzoic acid derivatives (Ref. 16) of which the compound substituted by methyl in position 3 of the tetrahydronaphthalene moiety in this particular assay system is the most potent retinoid currently known (Ref. 17). In human myeloid leukemia cells it proved possible for the first time to confirm the supposition - already voiced at an earlier date - that retinoids can control oncogenes (Ref. 18). The expression of the oncogene c-myc is suppressed in HL-60 cells by physiological amounts of (all-E)-retinoic acid. This leads to the assumption that the ultimate cause of the arrest of differentiation and of excessive proliferation of HL-60 cells is to be sought at the gene level.

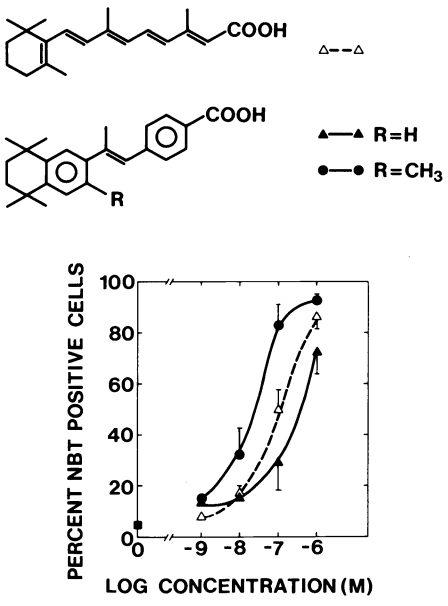

Fig. 2. Dose-response curves for three retinoids inducing differentiation in human HL-60 promyelocytic leukemia cells (with permission of Ref. 17)

Fig. 3 shows the close spatial similarity of (all-E)-retinoic acid and a novel retinoidal benzoic acid derivative (R = H) (Ref. 16) based on data from X-ray structure analyses.

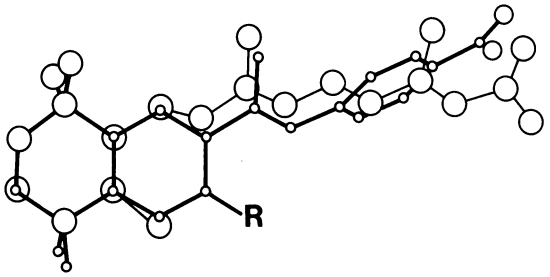

Fig. 3. Jointly projected structures based on X-ray crystallographic data of (all-E)-retinoic acid (○——○) and a retinoidal benzoic acid derivative (R = H, o——o) showing the close spatial similarity of these two types of retinoid (with permission of Ref. 19).

Since the discovery of two different intracellular retinoid-binding proteins (CRBP and CRABP) it has been assumed that retinoids are translocated to the nucleus as a specific retinoid-protein complex and this leads to an altered genemoic expression. Numerous details of this working hypothesis remain to be clarified in future investigations - for example, the question as to why neither CRBP nor CRABP are detectable in some cells which react very sensitively to the presence of a retinoid.

Retinol is transported from the liver to target tissues in a protein-protein complex consisting of molar amounts of thyroxine, transthyretin and retinol-binding protein (RBP). Human RBP is a single polypeptide chain with 182 aminoacid residues, whose sequence has already been determined; refined X-ray analyses may be expected in the near future. It has proved possible to isolate RBP from serum of various other species and the characteristics of these proteins show great similarities to one another. It is remarkable that this principle has been so strictly preserved in the course of evolution; this once again demonstrates the importance of plasma-circulating retinol-binding protein for adequate supply of retinoids to a specific organ site (Ref. 19).

The introduction of the Wittig reacion, shortly after its discovery, into retinoid and carotenoid chemistry by H. Pommer et al. has ultimately led to a great diversity of structures, in particular in the last decade (Ref. 20). We must not fail to mention that the combination of high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy enables us to determine the purity and configuration of a novel retinoid or carotenoid structure rapidly and reliably. This creates an excellent situation in which novel retinoids can be developed for the therapy of dermatological disorders and for further use in medicine (Ref. 4). These circumstances are also helpful in using derivatives of retinal as probes for studying mechanistic details of the process of vision, and in bacteriorhodopsin research. A number of screening systems with greater or lesser capability for predicting the practical use of a new retinoid structure in man are available.

On reading very comprehensive review articles on how to test the biological properties of a given new retinoidal structure one gains the impression that in reality we have no lack of standardized systems for determining the activity of a novel structure. The situation is less favorable when it comes to rapidly performable and reliable assay systems to give information on the specific toxicology of a retinoid. In this area, development work is essential, since chemoprevention is achievable only with those retinoids which can be administered over a long period, with a high degree of safety. For this reason, the intervention trials currently being carried out with β-carotene deserve particular attention, since no adverse effects at all would be expected with this preretinoid. If visible hypercarotenemia occurs, then this is an undoubted and non-dangerous symptom of too high a dosage. In addition, these investigations may possibly augur a new use of carotenoids in medicine, based on the ability of β-carotene to quench singlet oxygen and to trap radicals, leading to highly conjugated, fairly unreactive species. This ability of β-carotene is already being used in the protection of light-sensitive patients. These subjects have to be treated with large doses of β-carotene, and no remarkable adverse effect has been reported.

REFERENCES

1. D. S. Goodman and W. S. Blaner, in M. B. Sporn, A. B. Roberts and D. S. Goodman (eds.) The Retinoids, Vol. 2 , Chap. 7, Academic Press, New York (1984).

2. V. Balogh-Nair and K. Nakanishi, in C. Tamm (ed.) Stereochemistry, pp. 283 - 334, Elsevier Biomedical Press, Amsterdam (1982); T. G. Ebrey and T. Yoshizawa, Photochem. Photobiol. 33, 417 - 608 (1981); Vision Symposium, Photochem. Photobiol. 29, 655 - 753 (1979).

3. B. A. Underwood, in M. B. Sporn, A. B. Roberts, and D. S. Goodman (eds.) The Retinoids, Vol. 1, Chap. 6, Academic Press, New York (1984).

4. M. B. Sporn and E. D. Harris, Jr., Amer. J. Med. 70, 1231 - 1236 (1981).

5. G. L. Peck, in M. B. Sporn, A. B. Roberts and D. S. Goodman (eds.) The Retinoids, Vol. 2, Chap. 16, Academic Press, New Yok (1984).

6. J. A. Mezick, M. C. Bhatia and R. J. Capetola, J. Invest. Dermatol., in press.

7. J. J. Kamm, J. Am. Acad. Dermatol. 6, 652 - 659 (1982).

8. M. B. Sporn and A. B. Roberts, Cancer Res. 43, 3034 - 3040 (1983).

9. W. Bollag, Cancer Chemother. Pharmacol. 3, 207 - 215 (1979).

10. R. Lotan, Biochim. Biophys. Acta 605, 33 - 91 (1980).

11. I. Lasnitzki, Br. J. Cancer 9, 434 - 441 (1955).

12. R. L. Merriman and J. S. Bertram, Cancer Res. 39, 1661 - 1666 (1979).

13. R. C. Moon and L. M. Itri, in M. B. Sporn, A. B. Roberts and D. S. Goodman (eds.) The Retinoids, Vol. 2, Chap. 14, Academic Press, New York (1984).

14. G. W. Burton and K. U. Ingold, Science 224, 569 - 573 (1984).

15. L. M. De Luca, M. R. Brugh, C. S. Silverman-Jones and Y. Shidoji, Biochem. J. 208, 159 - 170 (1982).

16. P. Loeliger, W. Bollag and H. Mayer, Eur. J. Med. Chem.-Chim. Ther. 15, 9 - 15 (1980).

17. S. Strickland, T. R. Breitman, F. Frickel, A. Nürrenbach, E. Hädicke and M. B. Sporn, Cancer Res. 43, 5268 - 5272 (1983).

18. E. H. Westin, F. Wong-Staal, E. P. Gelman, R. D. Favera, T. S. Papas, J. A. Lautenberger, A. Eva, E. P. Reddy, S. R. Tronick, S. R. Aaronson and R. C. Gallo, Proc. Natl. Acad. Sci. U.S.A. 79, 2490 - 2494 (1982).

19. D. S. Goodman, in M. B. Sporn, A. B. Roberts and D. S. Goodman (eds.) The Retinoids, Vol. 2, Chap. 8, Academic Press, New York (1984).

20. F. Frickel, in M. B. Sporn, A. B. Roberts and D. S. Goodman (eds.) The Retinoids, Vol. 1, Chap. 2, Academic Press, New York (1984).