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General Review

Retinoids and Cancer

W. Bollag

Pharmaceutical Research Department, F. Hoffmann-La Roche & Co. Ltd., CH-4002 Basle, Switzerland

Summary. *The early and recent investigations in the field of retinoids and cancer are reviewed. The retinoids, including natural vitamin A compounds and their synthetic analogs, present a new class of substances exerting a prophylactic and a therapeutic effect both in certain experimental tumor models and in certain clinical conditions of preneoplastic and neoplastic lesions. Because of a particular physiological mechanism of action, the retinoids offer a new approach to the cancer problem, which is different from those of surgery, X-ray therapy, conventional chemotherapy, and immunotherapy.*

Introduction

We are all aware that prevention and therapy of cancer are far from being a solved problem in medicine. Beyond any doubt, surgery and X-rays are definitely able to cure a certain percentage of cancer patients. Cancer chemotherapy with the conventional cytostatic agents is a further means by which oncologists can help patients. However, this therapy is still unsatisfactory in a high percentage of cases. Immunotherapy is still at the beginning of its development, but we are justified in expecting further progress. New approaches to the cancer problem are badly needed. In this article we would like to present the fairly new development of retinoids in relation to cancer. We use the term retinoids to mean the natural vitamin A compounds and their synthetic analogs.

History of the Relationship Between Vitamin A and Cancer

Vitamin A is well known for its importance in general growth, differentiation of epithelial tissues, visual function, and reproduction. The relationship of vitamin A to cancer, however, also attracted the interest of research workers as long ago as 1926, when Fujimaki [33] found the development of carcinomas in the stomach of rats fed a vitamin A-deficient diet. Furthermore, as early as the 1920s it had been noticed in animal experiments that a deficiency of vitamin A leads to metaplastic changes in the epithelia of the respiratory, the gastrointestinal and the urogenital tracts [65, 66, 95, 96]. These metaplasias may be considered the first step in the transformation process from normal to neoplastic tissue. The similarity between the histological changes in epithelial tissues of vitamin A-deficient animals and certain precancerous lesions was the starting point for interesting new investigations. It was assumed that vitamin A might be able to prevent the development of benign epithelial tumors and squamous metaplasias and hence also of carcinomas arising later from these first precancerous changes. Indeed, in organ culture work with prostate epithelium it was possible to confirm this hypothesis [43]. In a vitamin A-deficient medium the secretory cylindrical epithelium of the prostate is transformed into a keratinized squamous epithelium. Very similar changes, consisting of hyperplasia, dysplasia and metaplasia, could be produced with carcinogenic hydrocarbons. By the addition of high doses of vitamin A these changes could be prevented and even reverted. Several authors [17, 20, 78] demonstrated the prophylactic effect of vitamin A substances in vivo on the induction of such precancerous conditions as benign epithelial tumors and metaplasias as well as of carcinomas. In a carefully planned study, Saffiotti et al. [80] reported the preventive effect of high doses of vitamin A on squamous metaplasias of trachea and bronchus induced in hamsters by intratracheal instillations of benzpyrene, a carcinogenic hydrocarbon. By prevention of the metaplasias the development of carcinomas was actually delayed or avoided. Whereas these first experiments were all carried out with natural vitamin A compounds such as retinol and retinylesters, and only in prevention tests, more recent investigations have involved the use of retinoic acid in chemoprevention and chemotherapy, in the hope of better antitumor activity and less side effects [4].

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Retinoic Acid

Animal Experiments

The first successful therapeutic experiments with retinoic acid were carried out with chemically induced papillomas and carcinomas [5–7]. The initiator used was 7,12-dimethyl-benzanthracene, and on days 1 and 15 this was painted on the back skin of female Swiss albino mice (2 × 150 μg); croton oil (500 μg, 2 × weekly) was then applied as promoter. Papillomas usually appeared after 3–8 months, whereas carcinomas were not induced until after 5–12 months. In a typical therapeutic papilloma experiment carried out with this model, the control animals showed an increase in the mean papilloma diameter per animal of 22.7% within 14 days, whereas in the animals treated with retinoic acid a regression of up to 51.4% was observed. Furthermore, even chemically induced skin carcinomas responded with regressions to some degree. Retinoic acid given during the promotion phase was also effective in preventing the appearance of DMBA-induced papillomas and carcinomas in mice [8, 9].

Clinical Results

After these positive animal experiments, an attempt was made to transfer the results obtained to clinical therapy. The following results were obtained with retinoic acid in topical treatment of actinic keratoses, a precancerous condition, and of basal cell carcinomas [14, 15]. Of the patients with actinic keratoses, 40% responded with a complete regression and 45% with a partial regression. In patients with basal cell carcinomas, the corresponding figures amounted to 31% and 63%, respectively. Positive clinical results have also been obtained in oral treatment with retinoic acid. In this case, papillomas of the urinary bladder have been favorably influenced. Of 33 cases, 10 showed complete, and 12 partial regression [28; A. Sulmoni, unpublished results]. Furthermore, oral treatment with retinoic acid led to regressions of leukoplakias of the mouth, tongue, and larynx [79]. However, carcinomas – except for basal cell carcinomas – did not respond to retinoic acid.

Hypervitaminosis A and the Therapeutic Ratio

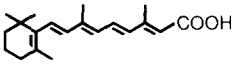
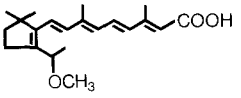
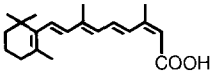
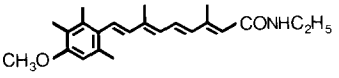
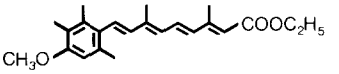
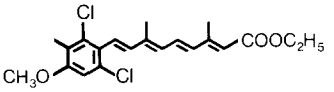
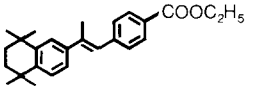
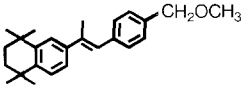
Although from a scientific point of view the reported results appeared encouraging, this treatment could not be recommended for practical purposes because retinoic acid induces the toxic effects of the hypervitaminosis A syndrome both in animals and in man. In man, the main symptoms are changes in the skin (e.g., erythema, desquamation, hair loss) and mucous membranes (e.g., cheilitis, stomatitis, conjunctivitis), hepatic dysfunction, and headache. All these symptoms prohibited the use of higher doses, which may be required for the successful treatment of precancerous conditions and particularly of carcinomas. Therefore, the synthesis of retinoic acid analogs was initiated with the aim of producing compounds which would, it was hoped, possess high activity and high tumor specificity together with low toxicity. To this end, a new screening system has been developed and applied, which has now made it possible to detect a dissociation in vivo between the antitumor effect and the hypervitaminosis A syndrome. The therapeutic ratio was defined [10, 11] as the ratio between the dose that when given IP once a week for 2 weeks caused a 50% regression of papillomas and the lowest daily IP dose (14 days' study) causing a defined degree of hypervitaminosis A. In mice, hypervitaminosis A becomes manifest in the form of weight loss, desquamation of the skin, hair loss, and bone fractures. A grading system of 0–4 (none to very marked) for each of the above-mentioned symptoms was used. Hypervitaminosis A was defined as the condition of the animals when the addition of all the symptom grades yielded at least 3. Thus, the therapeutic ratio enables us to compare retinoids with each other (Table 1).

The Search for New Retinoids

All previous experimental and clinical trials have demonstrated that the toxic symptoms of the so-called hypervitaminosis A syndrome are a serious handicap to the administration of the rather high doses of retinoids that are probably necessary for more successful prevention and therapy of cancer. The aim of a chemical program is therefore directed at finding compounds with a better therapeutic margin. In the Roche laboratories alone, about 1000 retinoids have been synthesized and tested biologically. Molecular modifications have been made to all three building units of the vitamin A molecule: the cyclic end group, the polyene chain, and the polar end group [59]. Table 1 lists a series of compounds that are active in very different doses and possess different therapeutic ratios. The three aromatic compounds Ro 11-1430, Ro 10-9359, and Ro 12-7554 and the two new analogs with a second aromatic ring in the side chain, Ro 13-6298 and Ro 13-7412 [P. Loeliger et al., to be published], each possess a therapeutic ratio of 0.5, which is 10 times more favorable than that of retinoic acid, which is 5.0.

It can be seen that Ro 13-6298 is 8000 times more active than Ro 1-5488 (all-*trans*-retinoic acid) in the antipapilloma test. The tiny dose of 0.05 mg/kg, given once a week, leads in a 2-week experiment to 50% re-

Table 1. Therapeutic ratios of some Retinoids

Ro no.	Chemical structure	Hypervitaminosis A (mg/kg)	Antipapilloma effect (mg/kg)	Therapeutic ratio
1-5488		80	400	$\frac{400}{80}=5$
10-1770		6	24	$\frac{24}{6}=4$
4-3780		400	800	$\frac{800}{400}=2$
11-1430		100	50	$\frac{50}{100}=0.5$
10-9359		50	25	$\frac{25}{50}=0.5$
12-7554		12	6	$\frac{6}{12}=0.5$
13-6298		0.1	0.05	$\frac{0.05}{0.1}=0.5$
13-7412		0.1	0.05	$\frac{0.05}{0.1}=0.5$

gression of established papillomas. Very many compounds have been tested in the experiments described below. The most frequently investigated retinoids include retinylesters such as retinylacetate and retinylpalmitate, retinyl methyl ether, all-*trans*-retinoic acid (Ro 1-5488), 13-*cis*-retinoic acid (Ro 4-3780), the aromatic retinoid Ro 10-9359, and the corresponding ethylamide Ro 11-1430.

Results of the Effect of Retinoids in Different Model Systems

Cell Cultures

Several authors have reported on the inhibition of growth of several cell lines by retinoids. Untransformed, transformed, and tumor cells were investigated. Growth of a considerable number of transformed and tumor cells could be inhibited, probably because of a direct inhibition of cell proliferation. In particular, melanoma cells and preneoplastic and neoplastic mammary cells responded with a marked diminution of growth [25, 29, 48, 49, 51, 52]. It must be emphasized, however, that not all cell lines tested were inhibited in their proliferation.

Organ Cultures

Organ cultures of prostate and trachea, when cultivated in a medium deficient in vitamin A or treated with a carcinogen, develop hyperplasia and squamous metaplasia, phenomena attributed to a preneoplastic state. It has been shown that hyperplasia and metaplasia do not develop, when retinoids are added prophylactically to the medium [16, 44, 45, 68, 85, 86]. Even when these changes have already developed, they can be reverted by retinoids. Recently it has been demonstrated that the mammary gland in organ culture is also a useful tool to test the effect of retinoids. Carcinogen-induced transformation of mammary gland epithelium was markedly inhibited by retinoids [23]. The destruction of cartilage, which is one of the toxic symptoms of retinoids, can also be demonstrated in organ culture [2, 39].

Transplantable Tumors

Almost all the agents used in present-day cancer chemotherapy have been detected in screening programs with transplantable tumors. This applies to alkylating agents, antimetabolites, plant alkaloids, antitumor antibiotics, nitrosoureas, and various other anticancer drugs. With retinoids the situation is quite different. Most of the conventionally used transplantable tumors do not respond to retinoids [6, 7]. However, a few exceptions have to be mentioned. It is well established that retinoids have both a prophylactic and a therapeutic effect on transplantable chondrosarcomas in rats. The therapeutically induced regressions with aromatic retinoids are very striking [91]. Furthermore, an inhibiting effect on a transplantable mammary adenocarcinoma in mice [77] and on the transplantable Cloudman melanoma S-91 [30] has been reported. Recently an inhibitory effect of a retinoid on the growth of a human bronchial carcinoma serially transplanted in athymic nude mice has been found [40].

Chemically Induced Tumors

Most of the experimental work on the influence of retinoids on in vivo model systems has been carried out with chemically induced tumors. The work mentioned earlier [17, 20, 78, 80] that was carried out exclusively with natural vitamin A compounds had already demonstrated a marked preventive effect on chemically induced tumors. In these, as in later experiments, dimethylbenzanthracene (DMBA), benzpyrene (BP), methylcholanthrene (MCA) or nitroso compounds have been used as carcinogenic agents. A large number of investigations followed the earlier ones, various carcinogens being used to induce all sorts of tumors at different organ sites of the body. In some experiments, not only initiating agents but also promoting agents were administered. The majority of investigations concern the *preventive* property of retinoids on chemically induced tumors, and in these the retinoid was administered during the induction phase or more specifically during the promotion phase. Thus, the induction of skin papillomas and carcinomas in mice by DMBA and croton oil or 12-0-tetradecanoyl-phorbol-13-acetate (TPA) was retarded or prevented by systemic application of retinoic acid or other retinoids during the promotion phase [8, 9, 12]. The DMBA-induced keratoacanthoma on the skin of the rabbit's ear could be completely prevented by administering an aromatic retinoid during the induction phase [57]. In mammary carcinomas induced by feeding either DMBA or *N*-methyl-*N*-nitrosourea (NMU), the administration of retinoids such as retinylacetate, retinyl methyl ether, and a retinamide reduced the incidence of benign and malignant mammary tumors and prolonged the latent period before their appearance [34, 62–64].

A large number of experiments done with chemically induced bladder tumors showed the striking influence of 13-*cis*-retinoic acid. This compound inhibited the incidence and extent of preneoplastic and neoplastic lesions of the urinary bladder [3, 87, 88]. Retinoids were also shown to have a preventive role in the carcinogenesis of chemically induced colon carcinoma [67] and carcinomas of the respiratory tract [19, 75]. In some special conditions, however, a promoting effect has also been observed following topical application of the retinoid [46, 76]. In various other experiments, retinoids had neither an inhibitory nor an enhancing effect on carcinogenesis.

Chemically induced skin tumors could be influenced *therapeutically* particularly by the aromatic retinoids. Well-established papillomas and carcinomas could be brought to regression. Whereas only small doses of the retinoids were needed for regression of papillomas, the regression of carcinomas was only achieved by giving doses that caused a certain degree of hypervitaminosis A toxicity [5–7, 10, 11, 13].

Virus-induced Tumors

Virus-induced tumors also respond to retinoids. Retinylpalmitate retards the growth and delays the initial appearance of the Shope rabbit papilloma [56]. In rabbits inoculated with Shope papilloma virus, the appearance of papillomas could be completely prevented by treatment of the animals with the aromatic retinoid Ro 10-9359 during the induction phase. In established papillomas, this retinoid produced a therapeutic regression, and moreover, in squamous cell carcinomas arising from Shope papillomas the growth of the primary tumor and of metastases was inhibited [Y. Itoh, personal communication]. High doses of vitamin A also decreased the incidence and severity of tumor development in mice inoculated with a murine sarcoma virus of the Moloney strain [84]. In this context, the results of the inhibition of the Epstein-Barr virus induction by the tumor promoter TPA seem to be important [97].

Biochemical Reactions

One of the most interesting phenomena observed in skin carcinogenesis is the observation that the ornithine decarboxylase activity induced in mouse epidermis by the tumor promoter TPA is inhibited by retinoic acid [92]. This biochemical reaction has been used to test various

retinoids [93], and a positive correlation between this biochemical phenomenon and skin tumor promotion has been established [94].

Clinical Trials with Retinoids for Prevention and Therapy of Cancer

The intention in providing the above-mentioned data from in vitro and in vivo experiments is to offer the clinician the possibility of choosing one retinoid or another for clinical trials either in prevention or in therapy of cancer. Everybody is aware that this is always a difficult task, in view of the fact that it is usually impossible to foresee whether the results of a certain animal experiment can be transferred to clinical practice. So many factors are involved, such as toxicology, pharmacokinetics, tissue distribution, metabolism, etc., thus rendering the outcome of every new clinical trial unpredictable. Furthermore, whereas a trial dealing with the therapy of precancerous or cancerous lesions can be planned and carried out quite accurately, the realization of a well-designed prophylactic study is an overwhelmingly difficult task, with many obstacles. Therefore, most clinical trials so far have been therapeutic ones. The successful therapy of actinic keratoses and basal cell carcinomas of the skin with topical application of retinoic acid was a first step in this direction [14, 15]. Further positive results have been obtained from the oral application of retinoids in leukoplakias [41, 42, 79], bladder papillomas [28], and basal cell carcinomas [74].

Besides these published results, many more clinical trials have been done in Europe, in which positive but not optimal therapeutic results have been achieved in leukoplakias, actinic keratoses, basal cell carcinomas, and urinary bladder papillomas with various retinoids, but mostly with Ro 4-3780 (13-*cis*-retinoic acid) and Ro 10-9359 (aromatic retinoid). The results of clinical trials with Ro 10-9359 in monotherapy of squamous cell carcinomas of different organs were unsatisfactory, since partial remissions could only be achieved with doses that induced intolerable side effects. Prophylactic trials in high-risk groups for cancer of the bladder and the bronchus are planned or under way. The great difficulties of such trials have been mentioned. There is no doubt that the retinoids presently available are still not effective enough or are too toxic, and our hope is based on finding retinoids with a better therapeutic margin.

Mechanism of Action

There is still much speculation about the mechanism of action of retinoids on normal and neoplastic cells. The situation is further complicated by the fact that different cells, tissues, and organs vary in their reaction to retinoids. Experimental facts and hypotheses will be enumerated, without offering a simple explanation for the efficacy of retinoids.

Proliferation

There may be a direct effect of retinoids on proliferation and DNA synthesis. The growth rates of certain transformed and tumor cells are inhibited [29, 48, 49, 51, 52], possibly by restoration of the density-dependent growth inhibition [25, 73] or of anchorage-dependent growth control [26]. The hyperplasia of several tissues in organ culture can be prevented or abolished by adding retinoids. It can be explained by an antiproliferative effect caused by a reduction of DNA synthesis [45].

Differentiation

It is known that the development of cancer is accompanied by a loss of cellular differentiation. Vitamin A guarantees the normal differentiation of epithelial tissues. Therefore, retinoids represent a physiological approach to the problem of chemoprevention and chemotherapy. Recently the induction of differentiation of embryonal carcinoma cells by retinoids has been discovered [37, 89].

Malignant Transformation

Several recent investigations have dealt with the inhibition or reversion of transformation and with the restoration of the properties of nontransformed cells. Thus the application of retinoids in vitro to mouse fibroblasts in cell cultures was shown to inhibit MCA-induced malignant transformation [60]. Another example is the inhibition of a radiation-induced oncogenic transformation by an aromatic retinoid [35]. In organ culture, the DMBA-induced mammary gland epithelial transformation and carcinogenesis was prevented by retinoids [23]. The vitamin A deficiency or MCA-induced hyperplasia and metaplasia of prostate and trachea in organ culture and their prevention and reversion may, in the broader sense, also be called an inhibition of the transformation process [43, 44, 85]. During the transformation process, the cells change the pattern of various properties. Under the influence of retinoids, some of the properties of a transformed cell revert to those of a normal cell. Thus the restoration of contact inhibition or density-dependent growth inhibition [25, 73], saturation density [1], anchorage-dependent growth [26], cell adhesiveness [1, 54, 55], and composition of glycoproteins and glyco-

lipids in membranes [73] was found. All these phenomena suggest that retinoids play a role in the prevention and reversion of the transformation process and that their point of attack may be the membranes.

Biochemistry

The series of biological alterations mentioned above must be based on biochemical reactions taking place during carcinogenesis under the influence of retinoids. Stimulation of glycoprotein synthesis by glycosyl transfer reactions in membranes and other organelles of the cell [47, 53, 54, 76] and possibly of glycolipid synthesis [73] may perhaps play an important role in the mechanism of action of retinoids. In the papilloma model, retinoids lead to necroses of cells [32]. An ultrastructural and histochemical analysis revealed that stimulation of glycoprotein synthesis takes place intracellularly in the Golgi apparatus and that glycosaminoglucans are secreted into the intercellular space in large amounts, thus detaching cells from each other while loosening their anchorage. These cells may then either be eliminated and lost, or necrotize, perhaps partly because the enlarged intercellular space prevents the adequate exchange of nutrients. The observed cell membrane modifications are probably also of importance [58]. Furthermore, the release of lysosomal enzymes by labilization of the lysosomal membrane may play a role in the destruction of tumor cells [24, 40].

It is not yet known by what chemical reactions retinoids inhibit the TPA-induced ornithine decarboxylase activity. An increase in cyclic AMP-dependent protein kinase under the influence of retinoids has also been reported [69]. Furthermore, retinoids block the growth-stimulating effect of a sarcoma growth factor [90].

Cellular Retinoid-binding Proteins

Several investigations suggest that the activity of retinoids is mediated by specific cellular binding proteins, perhaps in a manner similar to that known for steroid hormones. Two binding proteins have been described, a cellular retinol-binding protein and a cellular retinoic acid-binding protein. There is a partial but not an absolute positive correlation between the biological activity of retinoids and their binding capacity to cellular retinoid binding proteins. A relationship also exists between normal, preneoplastic, and neoplastic tissues and a correspondingly increasing content of cellular retinoid-binding proteins. Complexes of retinoids and binding protein may be transported into the nucleus where they modify gene transcription [18, 36, 37, 70–72, 81–83].

Immunological Effects

A further mechanism by which an antitumor effect may be exerted or be supported involves immunological reactions. Vitamin A has long been known to possess an immune adjuvanticity [27]; the acceleration of the rejection of allogeneic skin grafts indicated this [31, 38]. Recently, research work in the field of retinoids and immunological reactions has been extended. Thus it has been found that retinoic acid and other retinoids exert an effect on the immune system by stimulation of T killer cell induction in vitro [21], of T-cell cytotoxicity in vivo [50], and of mouse killer T cells in allogeneic and syngeneic systems [22]. Macrophages are stimulated and antibody-dependent cell cytotoxicity is increased by an aromatic retinoid [I. Florentin, personal communication]. Clinically it has been claimed that retinoids have an immune-potentiating effect [61]. However, it has not yet been clearly established whether the immunological effects of retinoids play a role in prevention and therapy of tumors.

Concluding Remarks

In this article we have reviewed early and recent investigations in the field of retinoids. The retinoids, including natural vitamin A compounds and synthetic structural analogs, represent a new class of compounds with remarkable prophylactic and therapeutic activities in oncology. In various in vitro and in vivo models, retinoids have proved to be capable of retarding or preventing the transformation of a normal to a neoplastic cell. Moreover, the reversion of transformed cells and the regression of certain tumors have even been observed. The activities of retinoids depend on their chemical structure. Different cells, tissues, or organs react to retinoids in different ways. Whereas epithelial tissues are the most prominent target of this class of compounds, other tissues also show profound alterations under the influence of retinoids.

The main handicap to the practical use of retinoids is the so-called hypervitaminosis A syndrome. New synthetic retinoids have already been shown to possess a better therapeutic ratio than natural vitamin A compounds. Only if it is possible to find analogs with a marked dissociation of the antitumor effect and the toxic symptoms, i.e., with a broad therapeutic margin, will practical success in the clinical chemoprevention and chemotherapy of cancer be achieved. The use of retinoids with their particular physiological mechanism of action represents a new approach to cancer prevention and therapy, differing from the conventional chemotherapy with cytotoxic agents. The usefulness of retinoids in the therapy of nonneoplastic dermatological diseases (acne, psoriasis, and other keratinizing dermatoses) has not been discussed in this article.

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