Review Article

The role of vitamin A in cancer

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Summary

The differentiation and maintenance of epithelial tissues is a well-known function of vitamin A. The most dramatic expression of this is the antineoplastic effect. This biological activity of vitamin A is reviewed with regard to anticarcinogenesis, the reversal of transformation and a possible role in cancer therapy. A brief account is given of vitamin A absorption and transport in the body and the importance of varying levels of cellular binding proteins in normal and malignant tissues.

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The past decade has seen a rapid escalation of interest in vitamin A and its analogues (retinoids), based on the realization that vitamin A control of epithelial tissue differentiation may be connected with its inhibition of the development of epithelial cancer.

Vitamin A is vital for general growth, reproduction, visual function and differentiation of epithelial tissues. A connection between vitamin A and cancer was first noted in 1926 when vitamin A-deficient rats were found to develop stomach cancer.[1] Also at this time epithelial metaplasia resulting from the effects of vitamin A deficiency on respiratory, genito-urinary and gastro-intestinal mucosa was reported.

The histological similarity between vitamin A-induced metaplasia and certain precancerous skin lesions, coupled with the demonstration that vitamin A administration could inhibit carcinogenesis, has stimulated intensive investigations.

The initial investigators used naturally occurring vitamin A (retinol and retinyl esters) which are toxic to man and animals, inducing the hypervitaminosis A syndrome when used in high dosage. The limitations imposed by this effect have to some extent been overcome by the recent synthesis of less toxic and more potent analogues of vitamin A. The retinoids thus represent a new development in the cancer field, offering a new approach and differing markedly in their more physiological mode of action from existing methods of cancer therapy and prevention.

Reports in the lay press of the beneficial effects of carrots in cancer and the food fad explosion further justify a review of this most interesting aspect of nutritional therapy.

Extensive and detailed reviews of the subject already exist.[2-4] This paper deals with the more significant facts that have emerged and considers some of the possible future uses of retinoids as adjuncts in cancer management. However, first it is necessary to consider briefly some general aspects of the vitamin.

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History

A fat-soluble extract essential to life was first obtained from egg yolk in 1909 and termed 'fat-soluble A'.[5] Later this substance was also found in animal and fish oils and named vitamin A. In 1931 vitamin A was purified and called retinol and the formula was derived.

The history of the carotenoids predates that of vitamin A by almost 100 years. Carotene pigment was first isolated in 1831, although its high vitamin A activity was not recognized until 1928. In 1930 the formula of this provitamin ß-carotene and its metabolism and storage in the liver were described. Carotenoids are the ultimate source of vitamin A from plants.

Pharmacology

The term vitamin A refers to a group of compounds able to reverse the effects of vitamin A deficiency. The formula of vitamin A (Fig. 1) comprises a cyclic end-group, a polyene chain and a polar end-group. The natural retinoids (Fig. 2) differ only in the nature of the polar end-group, which may be an alcohol retinol or vitamin A), an aldehyde (retinal) or an acid group (retinoic acid). Beta-carotene is in effect two molecules joined at the polar end-group.

Each region of the retinoid molecule can be altered chemically in a number of ways, resulting in an almost unlimited number of synthetic analogues called retinoids. Some of these are of particular oncological interest because they are both less toxic and yet more potent than vitamin A in their action on tissue differentiation.

Metabolism

Some aspects of the absorption, metabolism and transport of retinoids in man need to be considered in order to understand their pharmacological dynamics better.

Dietary vitamin A exists as retinyl esters or as the provitamin ß-carotene. The latter can be absorbed from the gut lumen directly into the intestinal mucosa without modification. Thereafter the ß-carotene molecule is split into two retinol molecules. Retinyl esters must first be converted by specific esterases in the gut lumen into retinol before absorption can occur (Fig. 3).

Once within the mucosa the retinol must be re-esterified to allow transport through the intestinal lymphatics as chylomicrons to the liver, where storage takes place.

Mobilization and transport of vitamin A from the liver where it is stored (Fig. 4) requires hydrolysis of the retinyl esters followed by conjugation of free retinol with a specific transport protein (RBP) synthesized in the liver. This conjugated holoprotein is then released into the circulation, where further binding to pre-albumin occurs. The resulting complex is the form in which retinol reaches the target organ. Toxicity will occur when free retinol is allowed to circulate and indiscriminately react with cell membranes causing labilization. Protein binding prevents this surface action on membranes.

Not all cell types require vitamin A for their maintenance, but those that do have specific surface receptor sites for uptake of retinol. This is achieved with the splitting off of the transport complex and the rapid binding of retinol to the cell surface.

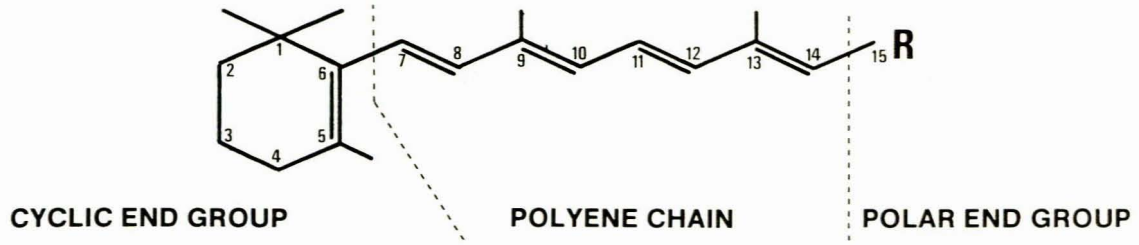

Fig. 1. The formula of vitamin A.

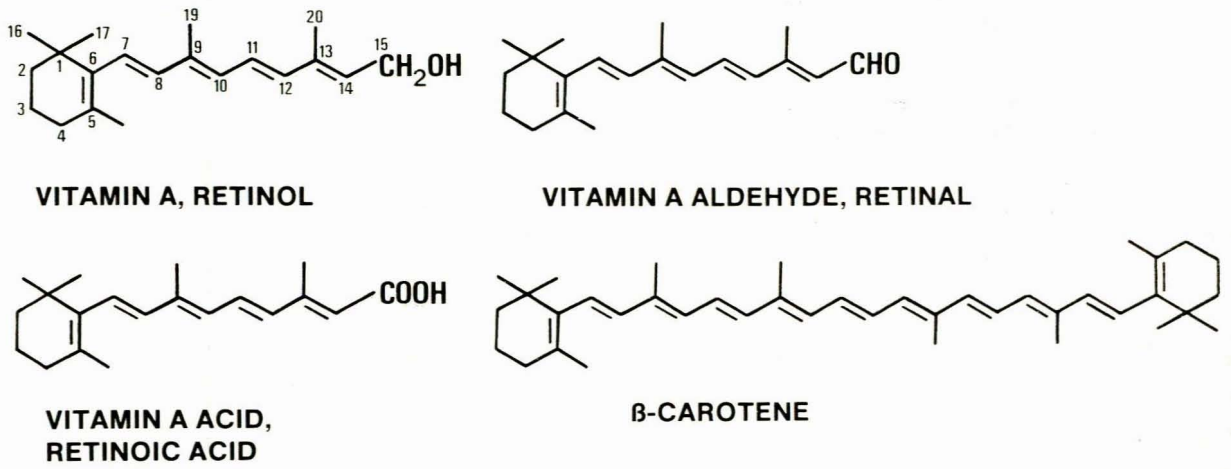

Fig. 2. The natural retinoids of vitamin A.

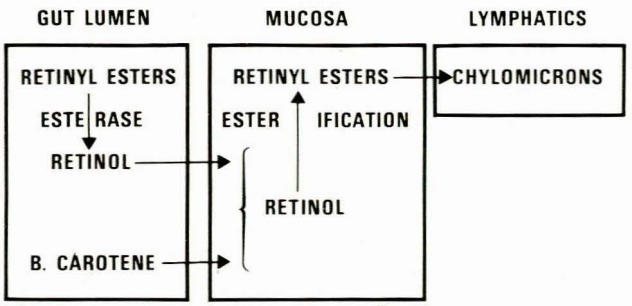

Fig. 3. Vitamin A absorption.

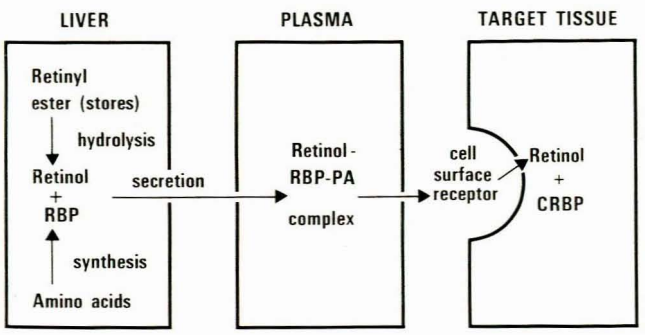

Fig. 4. Vitamin A transport.

Transport of retinol within the cell is now believed to be mediated by means of specific intracellular binding proteins analogous in many ways to steroid hormones. This finding is of immense interest when we consider the significance of the different concentrations of these intracellular binding proteins in both normal and malignant tissues.

The biological action of vitamin A

Fig. 5 illustrates the biological action of the three natural retinoids. Retinal, which is responsible for the visual cycle, is reversibly formed from retinol in the body. The conversion of retinol to retinoic acid is irreversible. Both the latter compounds are responsible for the differentiation effect and the maintenance of epithelial tissue, the most dramatic expression being the anti-neoplastic effect. This effect can be potently reproduced by the synthetic analogues or retinoids.

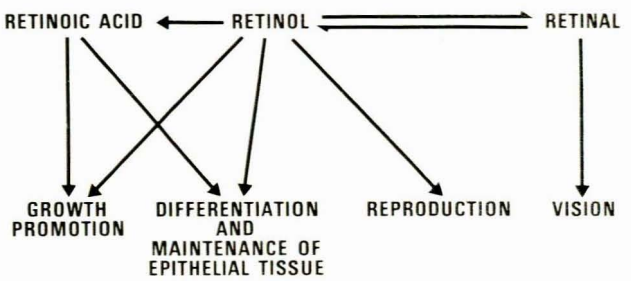

Fig. 5. The biological action of vitamin A.

The vitamin A effect on cellular differentiation has been studied under conditions of vitamin A deficiency and excess.

Deficiency results in squamous metaplasia in epithelia of the eye, respiratory tract, urogenital tract and salivary and prostate glands, and a reduction in the number of mucous glands in intestinal mucosa. These changes are associated with an increase in RNA and a decrease in DNA synthesis with diminished

mitotic activity. Vitamin A administration results in restoration of the normal state.

Excess of vitamin A suppresses keratinization of epithelial tissue, and mucous metaplasia of the skin of the chick embryo in the early stages of its development has been reported.

While the above observations have proved reproducible, other studies have given conflicting results.

Normal and malignant cells grown in culture in the presence of vitamin A may show growth enhancement, e.g. epidermal and some embryonic tissues, or growth inhibition, e.g. in some transformed and malignant cell lines.[2]

The basic mechanisms of these actions are complex and not fully understood. The identification of intracellular retinol-binding protein (IRBP) and intracellular retinoic acid-binding protein (IRABP) in target cells and their quantitative relationship to the retinoid effect suggest that this is an integral part of their biochemical action, which is mediated through translocation into the nucleus and a resultant alteration in the gene expression.

Either IRBP or IRABP or both may be present or absent in a variety of normal fetal and adult tissues tested, and the levels may fluctuate during development, indicating variations in the requirements of the target tissues and their response to vitamin A.

Retinoid-binding proteins in malignant tissue

An intriguing and perhaps significant finding is the demonstration that the level of binding protein is often lower in adult tissue than in its malignant counterpart. This has been demonstrated in both human and rat breast and lung tissue and in a variety of human tumours. A similar distinction occurs between transformed and untransformed cells and in some tumour cell lines. Some of the human tumour lines in which binding proteins have been identified include retinoblastoma, neuroblastoma, melanoma, breast and colon carcinoma, leukaemia, lymphoma, glioma, cervical carcinoma and embryonal carcinoma.

However, many systems studied which show retinoid sensitivity fail to demonstrate binding protein, and a number of other possible mechanisms exist to explain the vitamin A control over tissue growth and cell differentiation and the antineoplastic action:

1. Its effects on carbohydrate metabolism and the amounts of glycosaminoglycans and proteoglycans appearing on the cell surface, which affect cell adhesion and anchorage dependence and increase immunogenicity.
2. A nonspecific release of lysosomal hydrolases with labilization of the cell membrane.
3. Enhanced host immunity — retinoids have been shown to cause hyperplasia of lymphoid and thymic tissue in mice. This is associated with immunopotentiation and increased rejection of skin grafts which would not be inhibited by immunosuppressive drugs.[6] Retinoic acid administered with *Corynebacterium parvum* to mice with Lewis lung carcinoma resulted in a significant increase in lifespan, an effect believed to be due to T (killer)-cell stimulation.
4. Yet another possible explanation of the antineoplastic effect of vitamin A is the inhibition of tumour angiogenesis.[7]

Cancer prevention

The anticarcinogenic action of vitamin A has been extensively studied and unequivocably verified in many systems. This effect is shared by many of the recently synthesized analogues, which inhibit chemical carcinogenesis of skin, respiratory tract, urinary tract and breast. Protection is also achieved against viral carcinogenesis with rabbit papilloma and murine sarcoma virus. Other systems that have shown vitamin A inhibition of carcinogenesis include the testosterone-stimulated proliferation of prostatic organ culture, the carcinogenic effect of asbestos on tracheal epithelium and the radiation-induced oncogenic transformation in mouse fibroblasts.

The anticarcinogenic effect of retinoids has been shown to be due to an antagonism to tumour promoters. Retinoid administration at the same time as the promoter interferes with the induction of ornithine decarboxylase, which is the rate-limiting enzyme of polyamine biosynthesis. The accumulation of polyamines is believed to provide the tumour promoter effect.

While in some systems studied protection against tumour formation was not complete, tumour appearance was delayed. However, it should be emphasized that there are a number of sporadic reports of the complete absence of the anticarcinogenic effect of vitamin A, and even in some cases enhancement of tumour development. The reason for this is not yet known, but it may be due to the induction by retinoids of plasminogen activator and also the release of prostaglandins.

Epidemiological studies on the relationship of dietary vitamin A and cancer in man have shown an inverse association between vitamin A intake and cancer of the lung, bladder, stomach and colon and rectum. While some studies support the protective role of vitamin A in areas of high incidence of oesophageal carcinoma,[8,9] a recent study on oesophageal cancer in Transkei demonstrated the reverse.[10] The latter investigation showed serum vitamin A levels to be lower in the high-risk areas than in the low-risk areas. However, even in the latter areas serum vitamin A levels were lower than normal for the Western world.

Tumour monotherapy with retinoids

A number of laboratory and clinical studies have been undertaken to test the efficacy of retinoids as a single-agent therapy for established tumours. Even at dose levels associated with troublesome toxicity results have proved disappointing in all but a few situations. The topical application of retinoic acid to basal cell carcinoma and hyperkeratotic skin lesions has proved useful. The use of retinoids in the treatment of metaplasia is considered worthy of investigation.

Adjuvant therapy with retinoids

With the experimental demonstration of the radiation-sensitizing effect of vitamin A, a number of adjuvant clinical studies have been undertaken of retinoids used in conjunction with radiation or chemotherapy. No significant benefit over controls has been demonstrated, and some studies were terminated because of unacceptable side-effects (Hoffmann-La Roche & Co. — personal communication).

Conclusion

Despite the encouraging experimental evidence of the antitumour properties of vitamin A, its use in the treatment of cancer is disappointingly limited. This may be due to inadequate dosage in the face of intolerable side-effects. The development of a suitable analogue of potent vitamin A action and reduced toxicity is a hopeful prospect. Studies using ß-carotene and other available compounds in the reversal of epithelial metaplasia and other precancerous conditions are currently under way and are of interest.

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The mechanical transmission of hepatitis B virus by the common bedbug (*Cimex lectularius* L.) in South Africa

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Summary

Tests for both hepatitis B surface antigen (HBsAg) and hepatitis e antigen (HBeAg) were carried out on wild-caught and laboratory-colonized bedbugs (*Cimex lectularius* L.), the latter after hepatitis B virus (HBV)-positive blood-meals. Positivity for both antigens was interpreted as an indication of HBV infectivity. Of 22 pools in which were tested 211 bugs collected in the northern Transvaal, 18 were HBsAg-positive and 17 HBeAg-positive, with estimated infection rates of 156,7 and 137,7 per 1000 bugs respectively. Passage of HBV in bugs, allowing an extrinsic incubation period of 57-69 days, resulted in 19 out of 25 bugs being positive for HBsAg after the first passage; only a small number of these were positive for HBeAg. After the second passage all bugs tested were HBsAg-negative, showing that the virus had disappeared. Tests on the salivary glands and carcass of each bug at intervals up to 31 days after an infective meal showed a positivity rate of 98% (HBsAg) and 17% (HBeAg) for carcasses and 20% (HBsAg) and 0% (HBeAg) for salivary glands. Attempts to detect HBV particles in the salivary glands by electron microscopy failed. Bugs were shown to continue to excrete HBsAg in their faeces up to the 42nd day, and both HBsAg and HBeAg together up to the 30th day. HBsAg particles were only detected by electron microscopy in faeces harvested on the 10th day. The results as a whole indicate that no biological multiplication of virus occurs in *C. lectularius* but that mechanical transmission from insects to man could occur by: (*i*) contamination of a person when crushing infective bugs; (*ii*) contamination from infected faeces; and (*iii*) infection by bite due to regurgitation or interrupted feeding.

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Studies already reported from our laboratory have provided a considerable amount of evidence to incriminate the common bedbug, *Cimex lectularius* L., as a vector of human hepatitis B virus (HBV) in South Africa. High positivity rates for hepatitis B surface antigen (HBsAg) were shown in bugs collected from huts located in the northern Transvaal.[1] In addition, the results of laboratory experiments indicated that the bug probably transmits infection mechanically between humans and that the virus does not multiply biologically in the insect.[2,3] Since that work was carried out a radio-immunoassay test for hepatitis e antigen (HBeAg) has also become available. Since data obtained for both HBsAg and HBeAg on a specimen gives a firmer indication that it contains infectious HBV,[4,5] further studies were conducted incorporating both types of test. We thought this should provide stronger evidence as to whether biological or mechanical transmission occurs. The results of this work are reported in this paper.

Further batches of bugs which had been collected in the field at Louis Trichardt in the northern Transvaal and stored at −20°C were chosen for testing because our previous work[1] had shown that the highest infection rate occurred in bugs collected in villages at this particular locality. We also repeated our serial passage of HBV in bugs to see whether the virus disappeared during passage, which would indicate a lack of multiplication. This time we allowed a longer extrinsic incubation period of about 60 days, which is within the range for the duration of HBV incubation in the human host, so as to give the maximum opportunity for viral multiplication to occur. Furthermore, we tested both the salivary glands and the remainder of each insect at intervals after an infective meal to ascertain whether the virus could be replicating in these glands as in the case of an arbovirus. Lastly, we collected and tested faeces from bugs after an infective feed to investigate whether virus was excreted in them making them a source of infection.