Hypervitaminosis A: side-effects of retinoids

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High doses of retinoids are useful in the therapy of non-neoplastic dermatological diseases like acne, psoriasis and other keratinizing dermatoses (Peck, 1981, 1983; Bollag, 1983). They also possess anti-carcinogenic activities (Lotan, 1980; Sporn, 1983). The toxicity of retinoids, however, is the main handicap to their practical use (Bollag, 1983). Here the main symptoms of hypervitaminosis A, which includes teratogenicity, are presented. In addition, retinoids induce degradation of fetal cartilage in vitro, which may be related to their teratogenicity.

Hypervitaminosis A as side-effects of retinoids

The most characteristic effects of chronic hypervitaminosis A in laboratory animals are weight loss, erythema, desquamation of skin, hair loss and alteration of the skeletal system including bone fractures (Fig. 1; Kamm et al., 1984). In man the main symptoms are changes in the skin and mucous membranes, headache and pains in joints and bones (Bollag, 1983). In searching for new and better retinoids with a higher dissociation between any of the known therapeutic properties and the undesirable side-effects, a sulphur-containing retinoid devoid of bone toxicity in rats was recently found (Kistler et al., 1982). This finding indicates that it could be possible to find retinoids with a more selective action.

In addition to the side-effects mentioned, high doses of retinoids are teratogenic in animals (Geelen, 1979) and man (Happle et al., 1984; Lammert et al., 1985). As a result of the inadvertent use of the two retinoids which have been marketed up to now, isotretinoin (13-cis-retinoic acid) and etretinate, malformed infants have been born.

Cartilage degradation in vitro: mechanism of action

In fetal epiphyseal cartilage, retinoic acid induces the release of proteoglycan (Fig. 2) which represents cartilage matrix degradation (Kistler & Galli, 1979). This is followed by a marked shortening of the bones (Fig. 2), which correlates with cartilage tissue breakdown, including loss of DNA, RNA and protein (Kistler & Galli, 1979). To study how the release of proteoglycan and/or the bone-shortening induced by retinoic acid in cultured fetal rat bones are mediated, we used specific inhibitors (Table 1). Actinomycin D, cordycepin and cr-amanitin, three inhibitors of RNA synthesis which affect transcription at different levels, depress the retinoic acid-induced release of proteoglycan (Kistler, 1978). Cycloheximide, an inhibitor of protein synthesis, suppresses both the retinoid-induced release of proteoglycan (Kistler, 1978) and bone-shortening in a dose-dependent manner (Fig. 2). Tunicamycin, which selectively inhibits glycosylation of the asparagine residues in proteins, prevents bone shortening but does not affect the retinoic acid-induced proteoglycan release (Kistler, 1982). Furthermore, retinoic acid specifically changes the [3H]leucine incorporation pattern as revealed by gel electrophoresis but decreases the overall incorporation of radiolabelled leucine and mannose into acid-precipitable material (Kistler, 1982). Thus, retinoic acid-induced cartilage degradation requires RNA, protein and glycoprotein synthesis and specifically changes the protein synthesis pattern.

From our observation that EDTA completely blocks retinoic acid-induced cartilage degradation (Kistler & Galli, 1979) we suggest that metalloproteinasises may be involved in the degradation process. The inhibitory effect of EDTA was confirmed by Hembry et al. (1982) but they attributed this suppression to reduced cell viability assessed by the reduced capacity to incorporate [3H]leucine. However, we reported that Zn2+ but not Ca2+ reverses the inhibitory effect of EDTA (Kistler & Galli, 1979) and abolishes the EDTA-induced suppression of [3H]leucine and [14C]mannosine incorporation in the presence of retinoic acid (Kistler, 1982).
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Fig. 1. Hypervitaminosis A in rats

Note erythema and encrustations around eyes, nose and mouth, rough and lustreless hair and hairless patches, and alterations of the skeletal system consisting of marked bone remodelling leading to fractures (arrow) and subsequent healing processes (arrowhead).

Furthermore, the inhibition by EDTA of retinoic acid-induced proteoglycan release is reversible (A. Kistler, unpublished work). Thus, it is unlikely that the inhibition by EDTA was caused by a reduced cell viability. Nevertheless, the identity of the enzymes responsible for retinoic acid-induced cartilage matrix degradation remains obscure.

In recent years the role of protein kinases in the transduction of extracellular signals has been emphasized. It is suggested that the control of gene expression by protein kinases may integrate a receptor cascade system consisting of membrane phospholipid degradation producing inositol 1,4,5-triphosphate and diacylglycerol, and involving Ca\(^{2+}\), calmodulin, arachidonic acid, prostaglandins, cyclic AMP and cyclic GMP (Michell, 1983; Berridge, 1984, Nishizuka, 1984).

At first we tested if cyclic AMP, a second messenger which has been proposed as a mediator of retinoic acid effects (Lotan et al., 1978), may be involved in the retinoic acid response in cartilage. Neither forskolin (A. Kistler, unpublished work), a potent activator, nor LiCl (Kistler & Galli, 1979), an inhibitor of adenylate cyclase, affects retinoic acid-induced cartilage degradation. In addition, cyclic AMP also has no effect, indicating that in cartilage cyclic AMP is not involved in mediating tissue changes induced by retinoic acid (Kistler & Galli, 1979).

Next we investigated if Ca\(^{2+}\) and calmodulin were involved in the mediation of the retinoic acid response in fetal cartilage.
The divalent cation ionophore A23187, which increases intracellular Ca\(^{2+}\) entry, reversibly suppressed both the release of proteoglycan and the subsequent bone-shortening, indicating that Ca\(^{2+}\) is involved in the action of retinoic acid (Kistler, 1984). Trifluoperazine and the naphthalenesulphonamide W-7, two calmodulin antagonists, do not affect the retinoic acid-induced proteoglycan release but inhibit the bone-shortening, suggesting that calmodulin is required for the tissue breakdown processes (Kistler, 1985a). Thus, it appears that both Ca\(^{2+}\) and calmodulin may be involved in the mediation of retinoic acid action in cartilage.

Recently we studied the possible involvement of proteoglycans in retinoid action. Whereas indomethacin, an inhibitor of prostaglandin synthesis, does not affect the retinoic acid response in cartilage, the cyclohexanetrione Ro 31-0521, which stimulates prostaglandin synthesis (N. A. Roberts, personal communication), reversibly inhibits retinoic acid-induced cartilage degradation in vitro (Kistler, 1986). Furthermore, the cyclohexanetrione suppresses the congenital forelimb malformations induced by retinoic acid in rats. Thus, assuming that stimulation of prostaglandin synthesis is the main biological activity of the cyclohexanetrione, our findings suggest that proteoglycans may be involved in mediating retinoid action.

In summary, our results suggest that retinoic acid may influence cellular differentiation by modifying gene transcription and thereby affecting the normal biochemical expression of differentiation and morphogenesis. In addition, we suggest that retinoids may induce cartilage degradation via Ca\(^{2+}\) and calmodulin and involving proteoglycans. This possibility opposes the hypothesis that retinoids may influence cellular regulation via intracellular retinoid-binding proteins but supports the possibility that intracellular Ca\(^{2+}\) mobilization and protein kinases may be involved in the mediation of retinoid effects.

Relevance of retinoid-induced cartilage degradation

What is the relevance of retinoid-induced cartilage degradation? From the above discussion it is obvious that the release of proteoglycan from cartilage and the actual breakdown of cartilage itself is a well-controlled tissue degradation process. As shown above, the cyclohexanetrione Ro 31-0521 inhibits the retinoic acid-induced cartilage degradation in vitro and suppresses congenital malformations in vivo, indicating that these two retinoid effects may be related. This is further supported by our findings that (1) retinoids inhibit chondrogenesis in limb bud cell cultures (Kistler, 1985b), a test system which was proposed as useful for measuring the teratogenic potential of compounds in vitro (Wilkinson et al., 1980; Hassell & Horigan, 1982; Guntakattta et al., 1984), that (2) there is a good correlation for retinoids with a carboxyl end group between the activity in limb bud cells in vitro and induction of cartilage degradation in fetal bone cultures, and (3) that the activity of the above retinoids in limb bud cells in vitro correlates well with their teratogenicity in vivo (A. Kistler, unpublished work). Thus, retinoids alter normal growth and cell differentiation in all three test systems and the good structure–activity relationship between all three systems suggests that they may be related.

Therefore, elucidation of the mechanism of action of retinoids on fetal cartilage and chondrogenesis in vitro may help to understand how retinoids alter normal differentiation processes in the embryo which finally result in congenital malformations. Furthermore, it could help to elucidate the mechanism of action of the therapeutic effects of retinoids, which is currently unknown.

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Retinoids and cancer

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An adequate intake of those retinoids which together comprise natural vitamin A is essential for normal differentiation of epithelial tissues. Either dietary excess or deficiency will cause abnormal differentiation and associated failure of normal epithelial function with an increased risk for a variety of pathological conditions. Abnormal patterns of epithelial differentiation are also seen during carcinogenesis. Lasnitzki (1955) showed that abnormally differentiated prostatic cancer cells in culture could be restored to histological normality by addition of retinoic acid to the culture medium, thus suggesting a possible therapeutic role for vitamin A, and subsequently natural retinoids were shown to be potentially anti-carcinogenic in vivo (Bollag, 1972). More recently, consideration of epidemiological data suggested that people with below average serum retinol levels were at increased risk for cancer (Bjelke, 1975; Kark, 1980; Wald et al., 1980) but further investigation indicated that the relationship was not necessarily causal (Willett et al., 1984). The systemic toxicity of high dietary levels of vitamin A precludes its clinical use as an anti-cancer agent. By contrast, its major natural precursor, b-carotene, is not toxic; its conversion to retinol is physiologically controlled and even very high dietary levels of b-carotene can only maintain, or restore to normal, plasma retinol levels (Willett et al., 1983).

Synthetic retinoids

Because the release of natural retinoids from the liver into the circulation is physiologically regulated, moderate increases in dietary intake of vitamin A are not reflected by increases in retinol levels in the blood or tissues. After excessive doses, unbound natural retinoids in the circulation cause systemic toxicity but still without any selective increase in concentration in the epithelial tissues which are the target organs for carcinogens. Interest has been directed, therefore, to the synthetic analogues which, by chemical substitution of the polar terminal group and alteration of the aliphatic carbon skeleton, can be rendered less toxic than the parent compounds (Sporn et al., 1979). Many of these synthetic retinoids are neither stored in the liver nor under the same physiological regulation as their natural precursors; consequently tissue levels can be influenced directly by their dietary intake.

Anti-cancer activity of synthetic retinoids. In the last 10 years several synthetic retinoids have been shown to modulate carcinogenesis in experimental animal cancer models (Sporn et al., 1976; Sporn & Newton, 1979). Not all retinoids have anti-carcinogenic activity but some reduce cancer incidence in the mouse skin and delay, but do not absolutely prevent, the development of chemically induced cancers in the rat and mouse bladder, mammary gland and other organs (Sporn et al., 1977; Moon et al., 1979; Sporn & Newton, 1979; Slaga et al., 1980; Hicks et al., 1982; Moon & McCormick, 1982; Hicks, 1983a; Slaga, 1983; Moon & Itri, 1984). It is important to note that in most of these systems retinoids do not reduce the rate of growth of existing cancers and cannot be used like cytotoxic drugs to treat established malignancies. They antagonize the early promotion stages of carcinogenesis thereby delaying the recurrence or growth of new tumours from previously initiated epithelial cells. In the urinary bladder, for example, where papillary transitional cell carcinoma develops by a multi-stage system analogous to that in the mouse skin (Hicks et al., 1978; Hicks, 1980; 1981; 1982) several synthetic retinoids can reduce the incidence of experimentally induced urothelial cancer (Sporn et al., 1977; Becci et al., 1978, 1981; Thompson et al., 1981; Moon & McCormick, 1982; Hicks, 1983a; Hicks et al., 1982, 1985). This is most readily demonstrated in short-term trials, using high doses of a selective bladder carcinogen such as N-butyl-N(4-hydroxybutyl)nitrosamine (BBN) or N-methyl-N-nitrosourea as the inducing agent. If the trials are continued long enough, however, the neoplastic cells escape retinoid control and tumour growth will progress even in the continued presence of the retinoid (Hicks et al., 1982). Nevertheless, retinoid treatment confers a real advantage on carcinogen-treated animals by increasing their average survival time by the same amount as it increases the latent period between carcinogen exposure and tumour growth (Hicks et al., 1982, 1985).

Although papillary transitional cell carcinoma develops by a multi-stage mechanism, it is probable that development of the flat invasive form of the disease follows an alternative pathway not involving clonal expansion of previously initiated and promoted cells (Hicks et al., 1985). We have already shown that some retinoids, even though they reduce the incidence of papillary transitional cell carcinoma in both rat and mouse, do not inhibit the flat invasive form of bladder cancer (Hicks et al., 1985). Nevertheless, retinoid therapy is a potentially valuable new approach to the clinical management of bladder cancer since most patients, approx. 90%, present in the first instance with the recurrent papillary disease which, by analogy with rodent models, should be susceptible to control by retinoids.

Factors affecting the clinical use of retinoids as cancer chemopreventive agents

Differing biological activities. If specific retinoids are to be used intelligently in the clinical management of bladder cancer, then the scientific basis of their activities must be understood and their limitations, as well as their therapeutic advantages, appreciated (Hicks, 1983a). Quite apart from any considerations of toxicity it is important to remember that not all retinoids are equally effective as anti-carcinogenic or chemopreventive agents. Many retinoids are without demonstrable anti-carcinogenic activity in rodent

Abbreviations used: BBN, N-butyl-N-(4-hydroxybutyl)nitrosamine; HPR, N-(4-hydroxyphenyl)retinamide; 13-NER, 13-cis-N-ethylretinamide; NSAIDs, non-steroidal anti-inflammatory drugs.

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