S-16-2  Function of Vitamin A in Normal and Malignant Leukocytes

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I. INTRODUCTION

The anti-cancer action of retinoids (vitamin A and its metabolites) has successfully been shown in cell culture systems, with retinoic acid as one of the most active of the retinoids. In such studies, however, the fat soluble retinoic acid is dissolved in an organic solvent before it is added to the cells. Therefore, these in vitro studies do not take into account the transport to and uptake by the target cells that must occur in vivo. The body has a specific and high capacity transport system for retinol (bound to retinol-binding protein, RBP) and retinyl esters (bound to chylomicrons and their remnants), but not for retinoic acid and its synthetic analogs [1-2]. Recent studies shed some light on the in vivo transport of retinoids to cancer cells [3].

Newly absorbed retinol is transported from intestine mainly to the liver as retinyl esters in chylomicrons and their remnants [1]. In addition, retinol is mobilized from the hepatic store in perisinusoidal stellate cells and transported to extrahepatic target cells as retinol bound to retinol binding protein (RBP) [1]. Following a vitamin A rich meal containing about 1 mg retinol, peak plasma concentration of retinyl esters in chylomicrons and their remnants will be about 1-3 \( \mu \text{M} \). Intake of 15 mg retinol (in concentrated form) by adults, will result in a plasma retinol concentration of between 10-30 \( \mu \text{M} \) [3]. The plasma concentration of retinol bound to RBP is not regulated by the newly absorbed vitamin A, and is normally between 0.7 and 2.5 \( \mu \text{M} \) [1].

Small amounts (about 5 nM) of retinoic acid is normally found in plasma, but during retinoic acid therapy, the concentration may increase considerably. Following a single intake of 400 mg/m\(^2\) retinoic acid by an adult, peak concentration of about 5 \( \mu \text{M} \) was observed in plasma [3]. However, when daily treatment is continued for several years, as in cancer treatment, doses above 10 - 50 mg retinoic acid per day should not be used due to the toxic effects. By extrapolating these data, intake of 50 mg retinoic acid per square meter should result in a peak plasma concentration of about 0.6 \( \mu \text{M} \).

Vitamin A is involved in regulation of proliferation and differentiation of different types of cells such as leuko-cytes. We have studied the effects of physiological and pharmacological concentrations of retinol-RBP and chylomicon remnant retinyl esters on human peripheral B-lymphocytes, and myeloid and lymphoid leukemic cells.
II. CHYLOMICRON REMNANT RETINYL ESTERS AND RETINOL-RBP INHIBIT B-CELL ACTIVATION

As a basis for our studies on the effects of retinoids on human B-lymphocytes, we isolated pure B cells from normal blood donors. Using positive selection with immunomagnetic beads, we reproducibly obtain cells with less than 0.5% contaminating T cells and monocytes. B-cells were either cultivated in medium alone, or activated with anti-μ or SAC. Both chylomicron remnant retinyl ester (0.1-1.0 μM) and retinol bound to RBP (1 μM) markedly inhibited DNA synthesis of the cells [4]. Retinol-RBP inhibited the cells even greater than the same concentration of free retinol dissolved in ethanol. In this study we have shown that vitamin A in physiological doses and also bound to its physiological carriers, markedly impair normal B-lymphocyte activation, cytokine production, and differentiation [4].

III. EFFECT OF CHYLOMICRON REMNANT RETINYL ESTERS, RETINOL-RBP AND ALBUMIN BOUND RETINOIC ACID ON GROWTH AND DIFFERENTIATION OF HL60 CELLS

Retinoic acid was pre-equilibrated with albumin (which is the proposed transport molecule for retinoic acid) before addition to the cells. Whereas no specific uptake mechanism for the retinoic acid transport protein is described, retinol bound to RBP as well as retinyl ester in lipoproteins may be taken up by surface receptors [1].

The results show that pharmacological concentrations (10 μM) of chylomicron remnants retinyl ester completely block the proliferation of the cells, and induce differentiation in 60% of the cells after 5 days [5]. Normal physiological concentrations of chylomicron remnant retinyl ester (1 μM) induced differentiation in about 25% of the cells after 5 days. Retinol bound to RBP reduced the proliferation of HL-60 cells, but did not induce differentiation of the cells. At concentrations which may be obtained in plasma during retinoic acid treatment (0.6 μM), retinoic acid bound to albumin was about as effective as retinyl ester (10 μM) in chylomicron remnants [5].

IV. EFFECT OF CHYLOMICRON REMNANT RETINYL ESTERS ON SOME MYELOID AND LYMPHOID CELL-LINES

We have studied the effects of retinyl esters in chylomicron remnants on cell growth and differentiation of myeloid and lymphoid leukemic cell-lines. Ten μM retinyl ester in chylomicron remnants effectively reduced proliferation of the myeloid leukemic cell lines U937 and KG-1, and induced differentiation of 53% of the U937 cells in 5 days [6]. While no effect on cell growth of the lymphoid cell lines Daudi, Raji and SOS was observed, 10 μM retinyl esters in chylomicron remnants reduced the growth of the B lymphoid cell line Reh with more than 50% [6].

V. EFFECT OF CHYLOMICRON REMNANT RETINYL ESTERS ON PRIMARY CULTURES OF LEUKEMIC CELLS

Primary cell cultures from six patients with acute leukemia (4 myeloid and 2 lymphocytic) were incubated with chylomicron remnant retinyl esters and proliferation was measured by means
of thymidine incorporation. Among the myeloid leukemic cells, the monomyelocytic, the two promyelocytic and the monoblastic leukemic cells were growth inhibited. Chylomicron remnants had no effect on the growth of the c-ALL primary culture, but reduced proliferation of the T-ALL primary culture with about 20% after 48 hours [6].

VI. CONCLUDING REMARKS

One reason that most reports have chosen retinoic acid and not retinol in clinical trials is the so-called "therapeutic index" [3]. The therapeutic index for retinoids was defined as the ratio between the lowest daily intraperitoneal dose causing hypervitaminosis A in rats, and the therapeutic dose given intraperitoneally once a week for two weeks causing a 50% regression of established rat papillomas. This index is based on intraperitoneal injection of the retinoid, an administration route that is not used for these agents in humans. Furthermore, such administration does not allow the retinoids to utilize the normal plasma transport system. Hence, the therapeutic index is not very useful when considering which retinoid should be taken orally by patients.

The toxicity of retinoids, which include the teratogenic effect, is a main handicap to their practical use. In animal models, retinol is 10-100 times less toxic than retinoic acid and its synthetic retinoids [3]. It is suggested that retinol's ability to utilize its specific transport system in the body explains its lower toxicity.

Taken together, although retinoic acid is more active than retinol in in vitro assays, and retinoic acid seems to be the active metabolite in the mechanism of action of retinoids, it is still reasonable to use retinol in long term treatment of some cancers: First, more retinol than retinoic acid is found in plasma under physiological as well as pharmacological conditions. Second, myeloid leukemic cells express specific uptake mechanisms for retinol and lipoprotein bound retinyl ester, whereas no such mechanism has been described for retinoic acid. Third, physiological and pharmacological concentrations of retinyl esters in chylomicron remnants are at least as active as retinoic acid in reduction of proliferation and induction of differentiation. And finally, retinol is much less toxic than retinoic acid.

Promising results have been obtained in an initial clinical trial where children with acute myeloid leukemia in remission (induced by cytostatics) have been treated with high doses of retinol in periods up to three years [7]. This treatment increases plasma concentration of chylomicron remnant retinyl esters considerably, and probably prevents the few remaining leukemic clones to cause relaps. Therefore, the antitumor effect of vitamin A in this study may be mediated via retinyl ester bound to chylomicron remnants rather than retinol bound to retinol-binding protein or free retinoic acid.

Recently, retinoic acid has been used with success in the induction of remission in patients with acute promyelocytic leukemia [8-10]. In future clinical trials it would be interesting to test whether long term treatment of such patient with retinol (after induction of remission with retinoic acid)
VII. REFERENCES


