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A commentary on the inhibition by retinoids of leukotriene B_4 production in leukocytes

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The order of potency of retinoids as inhibitors of A23187-induced production of leukotriene B_4 (LTB_4) in human polymorphonuclear leukocytes (PMN) was retinoic acid > retinal > retinol. However, the conversion of exogenous arachidonate (AA) to LTB_4 by PMN homogenates was inhibited in the rank order retinol > retinal ≫ retinoic acid. The agreement between active concentrations of retinol in these two systems is consistent with this compound acting directly to inhibit AA metabolism: this is not so for the other retinoids. The order of potency for inhibition of phorbol dibutyrate (PDBu)-stimulated superoxide (O_2^-) production in HL60 granulocytes was retinol > retinoic acid ≫ retinal (inactive); neither retinol nor retinal displaced [^{3}H]PDBu from HL60 cells. We conclude that inhibition of LTB_4 production by retinoic acid and retinal is neither through inhibition of AA metabolism nor through inhibition of protein kinase C.

Leukotriene B_4; Retinoid; Superoxide; (Human leukocyte, HL60 cell)

1. INTRODUCTION

When PMN are stimulated at least two lipid pathways are activated. Diacylglycerol is produced from phosphatidylinositol 4,5-bisphosphate [1,2] and endogenous arachidonic acid (AA) is mobilised [3]. Diacylglycerol activates a calcium-requiring phospholipid-dependent protein kinase C (PKC) [1], while AA mobilisation results in the rapid production of 5-L-hydroxy-*cis*-8,11,14-*trans*-6-eicosatetraenoic acid (5-HETE) and leukotriene B_4 (LTB_4) [4]. Several studies have suggested links between these two pathways. The PKC activator 12-*O*-tetradecanoyl phorbol-13-acetate (PMA) acts synergistically with the calcium ionophore A23187 in the production of AA metabolites [5,6]. PMA also acts synergistically with lipoxygenase (LO) products to evoke cellular responses [7,8], and the LO product lipoxin has recently been reported to activate PKC [9].

Retinoids have a role in cancer chemotherapy and have been shown to inhibit the actions of tumour promotors both in vivo and in vitro [10,11]. In this context they have also been described as inhibitors of PKC [12,13]. Retinoids are effective in the treatment of inflammatory skin diseases such as psoriasis, and the ability of retinoids to inhibit the generation of the chemotactic LO product LTB_4 [14,15] has been implicated as a possible mode of action. Retinoids may therefore provide useful tools to investigate possible links between PKC activation and AA metabolism.

We have investigated the activities of retinoic acid, retinol, and retinal in inhibiting LTB_4 generation in both intact and homogenised PMN, and compared these with their activities against PDBu-stimulated O_2^- production in HL60 cells, a convenient and direct functional measurement of PKC activation [16].

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2. MATERIALS AND METHODS

2.1. *LTB_4 production*

Human PMN were prepared from freshly drawn blood. Erythrocytes were removed by sedimentation at 37°C with 7.5 ml of 2% (w/v) methyl cellulose/100 ml blood. PMN were recovered by centrifugation (250 × *g*, 10 min) and residual erythrocytes were removed by lysis in 0.82% (w/v) NH_4Cl + 5 mM KCl, pH 7.4. For intact-cell experiments PMN were washed in Hanks' balanced salt solution containing 1 mM $CaCl_2$ buffered to, pH 7.4, with 30 mM Hepes and were then suspended at 10×10^6 cells/ml. After equilibration at 37°C for 15 min, retinoids (Sigma) were added in 10 μl DMSO. After incubation for 5 min, reactions were initiated by the addition of the calcium ionophore A23187 in DMSO (final concentration 1 μM). Reactions were terminated after 5 min by centrifugation (1000 × *g*, 3 min) and the supernatants were decanted for measurement of LTB_4.

For homogenate experiments, the PMN were washed in 50 mM sodium phosphate buffer, pH 7.0, containing 1 mM EDTA and sonicated (3 × 30 s, MSF Soniprep). Incubations were performed in 0.5 ml containing 5×10^6 cell equivalents and 0.94 mM EDTA. Retinoids were pre-incubated for 5 min at 37°C with homogenate and reactions were started by the simultaneous addition of AA (Sigma) in ethanol (final concentration 5 μM) and $CaCl_2$ (final concentration 1 mM). Free Ca^{2+} concentration was calculated to be 60 μM. Reactions were terminated after 5 min by boiling.

LTB_4 was measured without extraction by specific radioimmunoassay [17]. Mean LTB_4 production in the absence of inhibitor was 2.67 ± 0.89 (SE) ng/10^6 cells in intact cells (n = 7) and 7.32 ± 2.39 ng/10^6 cell equivalents in homogenates (n = 3).

2.2. *Superoxide production*

HL60 granulocytes, grown in suspension culture in RPMI-1640 medium (Gibco) [16], were washed and resuspended at 0.3×10^6/ml in Hepes-buffered Hanks' solution (pH 7.4). PDBu-stimulated O_2^- production was measured spectrophotometrically as in [16]. Retinoids and PDBu (100 nM) were each added in ethanol such that the final concentration of ethanol did not exceed 0.2%. Mean PDBu-stimulated O_2^- release in the absence of inhibitor was 4.92 ± 0.24 (SE) nmol/10^6 cells per min (n = 3).

2.3. *[^{3}H]PDBu binding*

Displacement of [^{3}H]PDBu binding to undifferentiated HL60 cells by retinoids was measured as in [16].

3. RESULTS AND DISCUSSION

Retinoids inhibited ionophore-induced LTB_4 production in intact PMN (fig.1). Retinoic acid (IC_{50} 5 μM) was more potent than retinal (IC_{50} 15 μM) and retinol (IC_{50} 75 μM) was the least active. These results support the previously reported activity of retinoids although in rat PMN the rank order of potency was reported to be retinoic acid > retinol > retinal [14]. Our results in PMN homogenates show that inhibition of LTB_4 production by retinol might be attributable to a direct effect on one of the enzymes that metabolises AA to LTB_4. However, this is unlikely to be so for retinoic acid and retinal because the concentrations of these compounds required to inhibit LTB_4 pro-

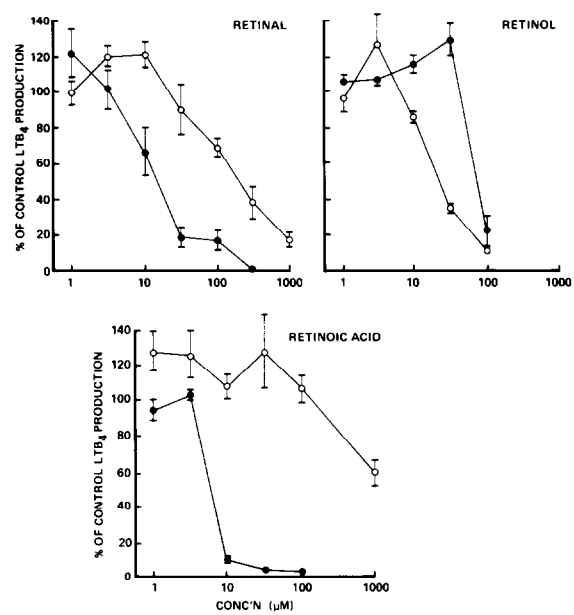

Fig.1. Inhibition by retinoids of LTB_4 production in intact (●) and homogenised (○) PMN. Each point represents mean ± SE from 3–5 separate experiments each in triplicate.

duction in PMN homogenates were 10–100-fold higher than were required in intact PMN. Therefore, the inhibition of ionophore-induced LTB_4 production in intact PMN by retinoic acid and retinal was probably due to interference with a cellular activation mechanism triggered by the ionophore.

Since it is known that PKC is activated by an elevation in intracellular calcium [18,19] and retinal has been described as an inhibitor of PKC [12,13], we compared the effects of retinoids on a leukocyte response stimulated by the PKC activator PDBu. PDBu-stimulated O_2^- production by HL60 granulocytes was inhibited by retinol (IC_{50} 20 μM) and retinoic acid (IC_{50} > 30 μM) (fig.2), but retinal was inactive at concentrations up to 30 μM. Thus, the rank order of potency of retinoids for inhibition in this system (retinol > retinoic acid ⩾ retinal) contrasted with their rank order of potency for inhibition of ionophore-induced LTB_4 production in PMN (retinoic acid > retinal > retinol). To characterise further the nature of the inhibition of PDBu-stimulated O_2^- release in HL60 cells, retinol and retinal were tested for displacement of [^{3}H]PDBu binding to intact HL60 cells (table 1). Neither compound displaced binding at concentrations up to 100 μM; if anything both compounds increased binding. Therefore, the inhibition by retinol of PDBu-stimulated O_2^- release cannot be explained by an interaction at the PDBu-binding site.

It could be argued that, since the data for LTB_4 production and O_2^- release were obtained using different cell types, direct comparison between the two systems can be made only with caution. However, HL60 cells are derived from human granulocytes and have been shown to be very similar to these both in terms of their functional responses [20,21] and their AA metabolism [22,23]. Since the rank orders of potency of retinoids for inhibition of LTB_4 production and inhibition of O_2^- release were not in agreement, we conclude that the two activities are unrelated, i.e. inhibition of ionophore-induced LTB_4 production does not involve inhibition of PKC. Retinol inhibits synthesis of LTB_4 from AA but retinal and retinoic acid probably interfere with a cellular activation process, resulting in a reduction of AA mobilisation [24]. Retinal inhibited PDBu-stimulated O_2^- release by HL60 cells, a PKC-dependent process, but this effect was not through interaction with the PDBu-binding site. The lack of effect of retinal in this system contrasts with the observation of Taffet et al. [13]. Thus, further investigation of the interaction between retinoids and PKC, especially the enzyme isolated from leukocytes, would be of interest.

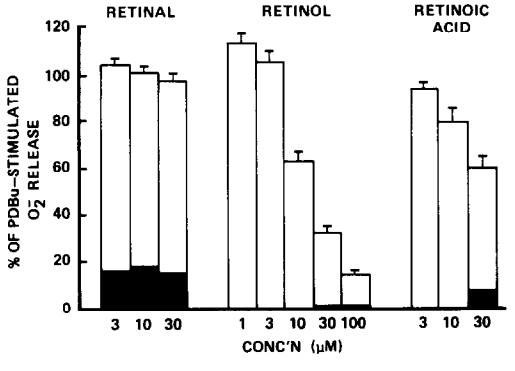

Fig.2. Inhibition by retinoids of PDBu-stimulated O_2^- release by HL60 cells. The bars represent means ± SE of 6–11 separate determinations. Shaded portions represent O_2^- release by retinoid alone.

Table 1

Displacement of [^{3}H]PDBu binding to intact HL60 cells by retinol and retinal

	[^{3}H]PDBu binding (fmol/10^6 cells)
Total binding	73.4 ± 10.7
Retinal	
1 μM	81.1 ± 15.6
10 μM	92.7 ± 20.8
100 μM	123.1 ± 30.8
Retinol	
1 μM	97.9 ± 24.5
10 μM	94.6 ± 19.5
100 μM	145.4 ± 29.0

Results are means ± SD from 3 experiments

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