Development of a FLAP competition binding assay using human HL-60 cells.

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Leukotriene synthesis involves an interaction of 5-lipoxygenase (5-LO) with membrane-bound 5-LO-activating protein (FLAP) converting arachidonic acid to leukotriene A4 (LTA4). Here we describe the development of FLAP filtration binding assay using HL-60 cell membranes suitable for the detection of unlabelled compounds which bind to FLAP. FLAP mRNA and protein expression were compared in HL-60 and THP-1 cells following stimulation with Phorbol 12-myristate 13-acetate (PMA). In the case of HL-60 cells PMA produced a significant improvement in the assay window comparable to the traditionally used human polymorphonuclear (PMN) cell membranes. PMN cells extracted from donor blood are costly and inconvenient to obtain, therefore replacement with PMA treated HL-60 cells is advantageous.

Crude membrane preparations were made from primary human PMN cells, HL-60 and THP-1 cells. PMA treated cells were cultured with and without PMA (32ng/ml) for three days before harvesting. Relative FLAP mRNA levels were determined using RT PCR. FLAP protein levels (Bmax) were determined using a membrane protein concentration of 10µg/well in a standard radioligand saturation binding assay format using [^{3}H]-MK886, a selective FLAP ligand. Subsequently a fixed concentration of [^{3}H]-MK886 (6nM), and membrane (10µg/well) was used in a 96-well competition binding assay format to determine the affinity of known FLAP ligands (see Charleson et al., 1992).

Membrane source	**Tissue Bmax (pmol/mg)**	**Relative FLAP levels compared to untreated controls**	**FLAP assay pK_d**	**Relative FLAP mRNA levels compared to untreated controls**
PMN	38 ± 3.5 (27; 50)	-	7.72 ± 0.01 (7.69; 7.74)	-
HL-60 untreated	4.4 ± 0.7 (2.1; 6.7)	1	7.79 ± 0.07 (7.54; 8.03)	1
HL-60 PMA treated	51 ± 8.2 (30; 72)	11.6	7.67 ± 0.07 (7.49; 7.86)	24

THP-1 untreated	5.9 ± 0.8 (4.1; 7.7)	1	7.73 ± 0.03 (7.67; 7.79)	1
THP-1 PMA treated	13 ± 0.7 (11; 15)	2.2	7.71 ± 0.04 (7.59; 7.82)	14

Table 1. Comparison of FLAP B_{max} and mRNA levels in THP-1 and HL-60 crude cell membranes following treatment with PMA. Data are expressed as mean ± SEM with 95% confidence intervals in parentheses.

RT PCR analysis revealed a 14-fold and 24-fold increase in FLAP mRNA levels in response to PMA exposure in THP-1 and HL-60 cells respectively. Saturation binding showed a 2.2-fold and 11.6-fold increase in FLAP protein levels in response to PMA treatment of THP-1 and HL-60 cells respectively. FLAP protein expression in PMN cell membranes was significantly greater (One-way ANOVA, $p<0.05$) than that measured in PMA stimulated THP-1 membranes but not significantly different from that measured in PMA stimulated HL-60 membranes. In all cases the measured K_d of [^{3}H]-MK886 was found to be equivalent in each tissue tested and comparable to literature values. The measured K_i values of the standard FLAP ligands also closely matched literature values. The greater expression of FLAP protein in PMN cells and PMA stimulated HL-60 cells translated to a more robust competition binding assays and with lower Totals/NSB ratios.

In conclusion we demonstrate that PMA stimulated HL-60 cells (THP-1 cells to a lesser extent) are a convenient alternative to PMN cells for development of FLAP competition binding assays.

Reference:

Charleson *et al*., (1992) *Mol. Pharmacol.* **41**, 873-879