Anti-inflammatory Agent Indomethacin Reduces Invasion and Causes Metabolic Changes in a Human Breast Cancer Cell Line.

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Introduction: The typically low ratio of phosphocholine (PC) to glycerophosphocholine (GPC) in human mammary epithelial cells (HMECs) is reversed with oncogenic transformation [1]. The breakdown of phosphatidylcholine by phospholipase A2 releases arachidonic acid, a fatty acid essential for the production of eicosanoids by the action of lipo- and cyclooxygenases. Eicosanoid expression is increased in inflammation. Thus, the phospholipid metabolism of cells is coupled to the inflammatory response. We observed that treatment of malignant HMECs with the anti-inflammatory agent and non-specific COX inhibitor indomethacin significantly reduced the PC/GPC ratio toward a value more characteristic of non-malignant HMECs [2, 3]. Using the Metabolic Boyden Chamber [4], we determined the effects of indomethacin on invasion and metabolism of the human breast cancer cell line, MDA-MB-435.

Materials and Methods: Immobilized MDA-MB-435 cells were layered on either side of an ECM gel chamber in a customized NMR tube and perfused continuously with DMEM or RPMI 1640 supplemented with 9 % fetal bovine serum, 90 U/ml Penicillin, 90 µg/ml Streptomycin, and 10 mM HEPES. In treatment experiments 2 or 3 21-days-release biodegradable indomethacin pellets (0.5 mg/pellet, IRA, FL, USA), were positioned in the upper cell layer approximately 4 mm above the ECM gel. The resulting global concentration of indomethacin in approximately 420 ml perfusion medium per day was 0.48 µM and 0.32 µM for 3 and 2 pellets respectively. Cell invasion was detected by T_1-weighted ^{1}H MR images and quantified from changes in the intracellular water profiles. Cell metabolism was studied by 1D ^{1}H SI of 310 µm thick slices along the sample and over the entire sample by 1D ^{1}H and ^{31}P MR spectroscopy. The temperature was kept at 37ºC and the oxygen tension ≥ 20%.

Results:

DMEM

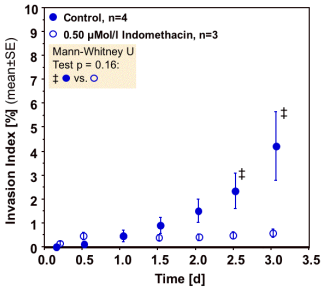

Figure 1: Invasion Index I(t) for indomethacin-treated and untreated MDA-MB-435 cells, cultured and perfused with DMEM as basal culture and perfusion medium

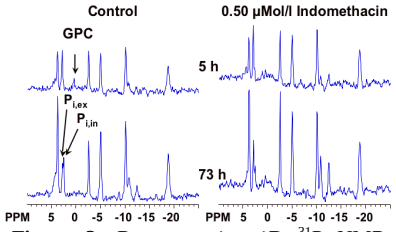

Figure 3: Representative 1D ^{31}P NMR spectra from MDA-MB-435 cells using DMEM as basal medium, under control conditions and with indomethacin treatment.

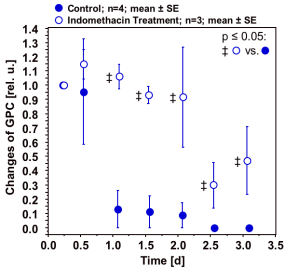

Figure 5: Relative changes of GPC during the time course of the experiments; differences between treated and untreated MDA-MB-435 cells in DMEM are considered significant for p≤0.05 (Mann-Whitney-U-test).

RPMI1640

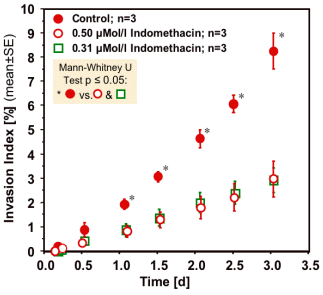

Figure 2: I(t) for MDA-MB435 cells treated with 2 different doses of indomethacin or untreated, cultured and perfused with RPMI 1640 as basal medium.

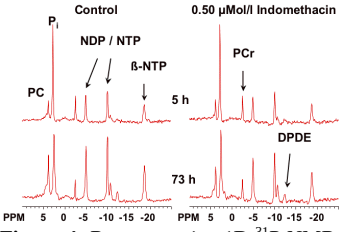

Figure 4: Representative 1D ^{31}P NMR spectra from MDA-MB-435 cells using RPMI1640 as basal medium, under control conditions and with indomethacin treatment.

MDA-MB-435 cell invasion was almost entirely inhibited following treatment with indomethacin using DMEM as basal medium **(Fig. 1).** Compared to the use of DMEM as culture and perfusion medium, the use of RPMI 1640 increased the invasion of untreated MDA-MB-435 cells **(Fig.1** and **Fig. 2)**. Indomethacin treatment of MDA-MB-435 cells in RPMI 1640 reduced their invasive capacity by approximately 70% but did not inhibit invasion completely **(Fig. 2)**. For the 2 doses of indomethacin tested, no differences in the reduction of invasion of MDA-MB-435 cells could be observed **(Fig. 2)**. **Figure 3** and **Fig. 4** show representative ^{31}P NMR spectra of untreated and treated MDA-MB-435 cells, using either DMEM **(Fig. 3)** or RPMI 1640 **(Fig.4)** as basal medium. Signals assigned are: PC, phosphocholine; Pi, inorganic phosphate; Pi,ex, extracellular Pi; Pi,in, intracellular Pi; GPC, glycerophosphocholine; PCr, phosphocreatine; NTP, nucleoside triphosphate; NDP, nucleoside diphosphate; DPDE, diphosphodiester. The ^{31}P MR spectra demonstrate the stability of energy levels and pH over the time course of the experiments. For control MDA-MB-435 cells in DMEM, the phospholipid metabolite GPC could be detected at the start of the experiments and then decreased close to the detection limit after 0.5 days; during treatment with indomethacin, GPC remained constant for 2 days and dropped on day 3 by approximately 55 % **(Fig. 5)**. MDA-MB-435 cells cultured and perfused with RPMI 1640 exhibited almost no detectable GPC in 1D ^{31}P NMR spectra **(Fig. 4)**. Representative LacTG levels of 1D ^{1}H Spectroscopic Imaging (SI) MR spectra along the sample for MDA-MB-435 cells cultured and perfused with RPMI 1640 are shown in **Figure 6**. Intracellular LacTG levels appeared to increase after indomethacin treatment **(Fig.6)**.

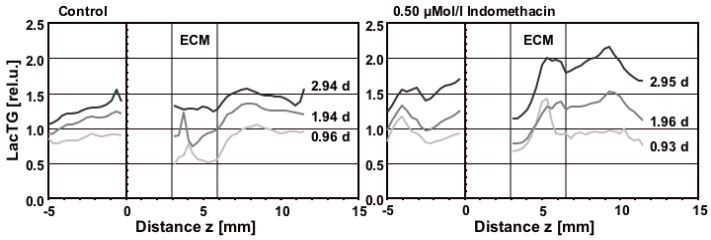

Figure 6: Representative LacTG levels along the sample obtained from 1D ^{1}H SI MR spectra of 310 µm thick slices of untreated and treated MDA-MB-435 cells using RPMI 1640 as basal medium. LacTG levels appear to increase with time. The effect appears to be enhanced with indomethacin treatment.

Discussion: Compared to control cells, indomethacin treatment reduced invasion of the human breast cancer cell line MDA-MB-435 independent of the choice of DMEM or RPMI 1640 as culture and perfusion medium. These results are consistent with previous observations: MDA-MB-435 tumors displayed reduced growth and metastasis to the lung following treatment with indomethacin [5]. Eighteen hours of treatment with 50 µM indomethacin reduced the invasion, as measured by a Boyden chamber assay, of mouse melanoma cells and a human fibrosrcoma cell line [6]. We did not observe a dose dependency of indomethacin treatment of MDA-MB-435 cells for the two doses selected. Consistent with results obtained from cell extracts [2, 3], indomethacin treatment of MDA-MB-435 cells increased GPC compared to control cells. The higher GPC levels for lower I(t) agree with the previous finding that GPC was inversely related to invasion [7].

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References: **[1]** E.O. Aboagye and Z.M. Bhujwalla; *Cancer Res*, 59(1):80-84, 1999 **[2]** K. Natarajan, N. Mori, D. Artemov, E.O. Aboagye, V.P. Chacko, and Z.M. Bhujwalla; *Adv Enzyme Reg*, 40:271-284, 2000 **[3]** K. Natarajan, N. Mori, D. Artemov, and Z.M. Bhujwalla; *Neoplasia*, 4(5):409-416, 2002 **[4]** U. Pilatus, E. Ackerstaff, D. Artemov, N. Mori, R.J. Gillies, and Z.M. Bhujwalla; *Neoplasia*, 2(3):273-79, 2000 **[5]** J.M. Connolly, X.H. Liu, D.P. Rose; *Nutr Cancer*, 25:231-240, 1996 **[6]** R. Reich and G.R. Martin; *Prostaglandins*, 51:1-17, 1996 **[7]** E. Ackerstaff, D. Artemov, R.J. Gillies, and Z.M. Bhujwalla; ISMRM 2003, Poster #1316