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Laboratory Investigation

LABORATORY INVESTIGATION

Lidocaine inhibits cytoskeletal remodelling and human breast cancer cell migration

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Abstract

Background: The metastatic potential of breast cancer cells has been strongly associated with overexpression of the chemokine CXCL12 and the activity of its receptor CXCR4. Lidocaine, a local anaesthetic that can be used during breast cancer excision, inhibits the growth, invasion, and migration of cancer cells. We therefore investigated, in a breast cancer cell line, whether lidocaine can modulate CXCL12-induced responses.

Methods: Intracellular calcium, cytoskeleton remodelling, and cell migration were assessed *in vitro* in MDA-MB-231 cells, a human breast cancer epithelial cell line, after exposure to lidocaine (10 μM or 100 μM).

Results: Lidocaine (10 or 100 μM) significantly inhibited CXCR4 signalling , resulting in reduced calcium release (Fluo 340 nm/380 nm, 0.76 mean difference, $p<0.0001$), impaired cytoskeleton remodelling (F-Actin fluorescence mean intensity, 21 mean difference, $P=0.002$), and decreased motility of cancer cells, both in the scratch wound assay (wound area at 21 h, −19%, $P<0.0001$), and in chemotaxis experiments (fluorescence mean intensity, 0.16, $P=0.0047$). The effect of lidocaine was not associated with modulation of the CD44 adhesion molecule.

Conclusions: At clinical concentrations, lidocaine significantly inhibits CXCR4 signalling. The results presented shed new insights on the molecular mechanisms governing the inhibitory effect of lidocaine on cell migration.

Keywords: breast cancer; cell migration; CXCR4; CXCL12; lidocaine

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Editor's key points

- Breast cancer metastasis involves chemokine signalling that modulates cytoskeletal structure and motility, which might be sensitive to local anaesthetics.
- Lidocaine inhibited CXCR4 mediated migration of a human breast cancer cell line *in vitro* involving changes in intracellular calcium release and the actin cytoskeleton.
- These findings provide a molecular mechanism for possible beneficial effects of lidocaine in breast cancer surgery through reduced tumour cell migration and metastasis.
- Clinical studies are necessary to establish a clinical role for lidocaine in reducing metastasis and improving outcomes in cancer surgery.

Breast cancer is the most common diagnosed tumour and represents the second leading cause of death in women. Although chemotherapy, endocrine therapy, and radiotherapy are effective, surgical removal of the tumour is still the best option for a positive outcome.[1] Retrospective and clinical studies on patient cohorts undergoing cancer surgery suggest that perioperative use of regional anaesthesia and local anaesthetic agents might improve outcome.[2] Recent investigations have shown that in lung carcinoma, local anaesthetics have anti-metastatic potential through inhibition of cancer cell migration and of Src signalling.[3]

The metastatic potential of breast cancer has been strongly associated with overexpression of the chemokine CXCL12 and the activity of its receptor CXCR4.[4–7] Under homeostatic conditions, the CXCL12/CXCR4 axis plays key roles in development and immunity, while in cancer, it promotes tumour survival, invasion, and metastasis.[8,9] Chemokines have emerged as key controllers of integrin function and cell locomotion.[9] Chemokine receptors are differentially expressed by all leukocytes and many non-haematopoietic cells, including cancer cells, and constitute the largest branch of the γ subfamily of rhodopsin-like G protein-coupled receptors (GPCR), a receptor superfamily that represents the most successful target of small molecule inhibitors in modern pharmacology.[10,11] As local anaesthetics are known to modulate the activity of specific G-proteins,[12,13] we investigated whether lidocaine at clinical concentrations can modulate CXCR4 responses induced by CXCL12.

Methods

Cell line

MDA-MB-231 human breast cancer epithelial cells (CRM-HTB-26™ from American Type Culture Collection, Rockville, MD, USA), derived from pleural effusion, were cultured with Dulbecco's Modified Eagle Medium (DMEM) containing D-glucose 4.5 g L^{-1}, and glutaMAX (619650-026, GIBCO, ThermoFisher Scientific, Switzerland) supplemented with fetal bovine serum 10% (16000-044, GIBCO, ThermoFisher), and penicillin-streptomycin 1% (15070063, GIBCO, ThermoFisher Scientific). Cells were incubated under standard culture conditions (CO_2 5%, O_2 95%, 37°C), and experiments were performed with cells at 70%–100% confluence.

Reagents

CXCL12 was chemically synthesised as described,[14] and lidocaine was from Sintetica® (Rapidocain 10 mg ml^{-1}, Mendrisio, Switzerland).

Cell viability

MDA-MB-231 cells cultured in six-well plates were incubated for 24 h with lidocaine (1 nM, 1, 10, 100 μM) or hydrogen peroxide 2 mM (1-07209-0250, Merck, Kenilworth, NJ, USA), used as positive control, for 3 h. Cells were stained with Annexin V-fluorescein isothiocyanate (FITC) (556419, BD Pharmingen™, San Jose, CA, USA) and propidium iodide 50 μg ml^{-1} (556463, BD Pharmingen™, San Jose, CA, USA) for 15 min at room temperature and directly analysed by flow cytometry (BD Canto, BD Biosciences, San Jose, CA, USA). Percentage of viable, early/late apoptotic and necrotic cells was quantified by FlowJo software (Tree Star, Inc., Ashland, OR, USA).

Flow cytometric analysis

For detection of surface expression of CXCR4, CD44, and CD54 on MDA-MB-231 cells, the following antibodies were used: CXCR4-APC (555976, BD Pharmingen™), CD44-FITC (555478, BD Pharmingen™), CD54-PE (322707, BioLegend, San Diego, CA, USA). Cells were incubated for 30 min at 4°C following the manufacturer's instructions for each antibody. Samples were analysed by flow cytometry with BD Canto (BD Biosciences) with the FlowJo software (Tree Star, Inc.). Relative mean fluorescence intensity was calculated as the ratio between stained and unstained samples.

Scratch wound assay

Migration of MDA-MB-231 cells stimulated by CXCL12 100 nM was assessed in the presence or absence of lidocaine 10 μM or 100 μM. Cells were grown in six-well plates until confluence for 24 h. A scratch was created in each well using a small pipette tip.[15] For the experiments with the CD44 blocking antibody (MA4400, Invitrogen, Waltham, MA, USA) used at 10 μg ml^{-1}, cells were grown in 24-well plates until confluence. Stimulation and scratch were performed as described above. Images were recorded with a BD pathway 855 imager for 24 h maintaining cells as described above, at 10× magnification. The scratch wound area, expressed as percentage of the area at time 0, was quantified using the open-source image analysis software Fiji[16] and normalised to time 0 for each condition.

Chemotaxis assays

Real-time cell migration of MDA-MB-231 cells was measured using the μ-Slide chemotaxis system from Ibidi (80326, Martinsried, Germany), according to the manufacturer's instructions. Briefly, MDA-MB-231 cells were seeded at 4×10^6 cells ml^{-1} in chemotaxis medium [DMEM, fetal bovine serum 1%, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) 20 mM, pH 7.4] in the central channel of the chemotaxis slide, and were cultured in standard conditions for 8 h to allow adherence. Chemoattractant gradients were generated by applying the following stimuli in the reservoirs of the chemotaxis slide: chemotaxis medium, CXCL12 100 nM, or CXCL12 100 nM with lidocaine 100 μM. Phase contrast images were recorded for 18 h with a time lapse of 15 min using the ImageXpress Micro 4 Imager (Molecular Devices, San Jose, CA, USA) equipped with an incubation system set to CO_2 5%, O_2 95%, 37°C, and with a 4× objective. Single cell tracking was performed selecting the centre of mass in each frame using the manual tracking plug-in tool for the software ImageJ. Spider plots representing the trajectories of tracked cells, forward migration indexes, accumulated distance, and cell velocity were obtained using the chemotaxis and migration plug-in tool from Ibidi.