BJA

British Journal of Anaesthesia, 121 (4): 962–968 (2018)

doi: 10.1016/j.bja.2018.07.015
Advance Access Publication Date: 16 August 2018
Translational Studies

Lidocaine inhibits cytoskeletal remodelling and human breast cancer cell migration

G. D'Agostino[1,*,#], A. Saporito[2,*,#], V. Cecchinato[1], Y. Silvestri[1], A. Borgeat[3], L. Anselmi[2] and M. Uguccioni[1,4]

[1]Institute for Research in Biomedicine, Faculty of Biomedical Sciences, Università della Svizzera italiana, Bellinzona, Switzerland, [2]Service of Anaesthesia, Bellinzona Regional Hospital, Bellinzona, Switzerland, [3]Universitaetsklinik Balgrist, University of Zurich, Zurich, Switzerland and [4]Humanitas University, Department of Biomedical Sciences, Pieve Emanuele, Milan, Italy

*Corresponding authors. E-mails: gianluca.dagostino@irb.usi.ch, andrea.saporito@eoc.ch

#Both authors contributed equally to this work.

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; cytoskeleton; lidocaine; metastasis; regional anesthesia

Editorial decision: 20 July 2018; **Accepted:** 20 July 2018

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 Bioscences) 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.

Actin polymerisation

Cells grown on poly-D-lysine-coated dishes (P35GC-0-14C, MatTek Corporation, Ashland, MA, USA) were pre-treated with lidocaine 10 μM or 100 μM for 24 h under culture conditions. After washing with phosphate buffered saline, cells were stimulated with complete medium in the presence or absence of CXCL12 100 nM for 15 s, fixed in paraformaldehyde 4% in phosphate buffered saline for 12 min on ice, and permeabilised with Triton-X-100 0.01% for 2 min on ice. Filamentous actin was stained using phalloidin-FITC (4 μg ml^{-1}, P-5282, Sigma Aldrich, Saint Louis, MO, USA) for 30 min at room temperature. Samples were analysed by confocal microscopy (Leica SP5, Heerbrugg, Switzerland) at 63× magnification. Mean fluorescence intensity was quantified using the open-source image analysis software Fiji.[16]

Intracellular Ca^{2+} measurement

MDA-MB-231 cells (0.2×10^6) were seeded on poly-D-lysine-coated dishes (P35GC-0-14C, MatTek Corporation) and were pre-treated with lidocaine 10 μM or 100 μM for 24 h under culture conditions. To block the Gαi subunit of CXCR4, pertussis toxin from *Bordetella pertussis* (PTX) at 1 μg ml^{-1} (P7208, Sigma Aldrich) was applied to the cells 2 h before Ca^{2+} measurements. Cells were supplemented with FURA-2-acetoxymethyl ester (Sigma Aldrich) 1 μM, incubated for 20 min at 37°C, and washed with a buffer containing NaCl 136 mM, KCl 4.8 mM, HEPES 20 mM, and $CaCl_2$ 1 mM, pH 7.4. Images were recorded on an inverted microscope at 40× magnification (Axiovert 200; Carl Zeiss, Oberkochen, Germany) with excitation at 340 and 380 nm using the Polychrom V illumination system from TILL photonics GmbH (Gräfelfing, Germany). Chemokine was injected after 60 s, and recording continued up to 150 s. The uncalibrated 340/380 ratio provides a relative measure of cytoplasmic-free Ca^{2+} concentration.

Statistical analysis

Data are presented as mean (standard deviation). Normal distribution was assessed using Shapiro-Wilk testing. Normally distributed data were analysed with Student's *t*-test (statistical significance between two groups), or two-way analysis of variance followed by Dunnett's multiple comparisons adjustment (statistical significance between more than two groups).

Results

We first evaluated lidocaine toxicity on MDA-MB-231 cells. Cells were incubated with different concentrations of lidocaine (1 nM, 1, 10, 100 μM) and induction of apoptosis or necrosis was assessed after 24 h. Lidocaine did not induce apoptosis or necrosis at any of the concentrations tested (Fig. 1).

Lidocaine effects on tumour cell migration

We then investigated whether lidocaine alters MDA-MB-231 chemokinesis or chemotaxis induced by CXCL12, the chemokine agonist of the CXCR4 receptor. For this we used an *in vitro* scratch wound assay, a technique widely applied for testing *in vitro* cell migration of epithelial cancer cells,[15] and an *in vitro* chemotaxis assay that allows tracking directional cell migration in response to a chemotactic gradient. CXCL12 induced significant MDA-MB-231 migration both in the scratch wound assay (9 h, $P=0.0372$; 12 h, $P=0.0003$; 15, 18, 21 h, $P<0.0001$) and chemotaxis assay ($P=0.0047$), supporting the relevance of the CXCL12/CXCR4 axis in tumour cell migration (Fig. 2). As shown in Fig. 2A, lidocaine prevented closure of the scratch area induced by CXCL12 at 10 μM or 100 μM ($P<0.0001$). Moreover, lidocaine abrogated CXCL12 mediated chemotaxis as demonstrated by a significant reduction in the forward migration index ($P=0.0446$), without altering cell velocity and cell accumulated distance (Fig. 2B and C). These effects were not as a result of changes in CXCR4 surface expression or alterations in receptor trafficking as assessed by flow cytometry (data not shown).

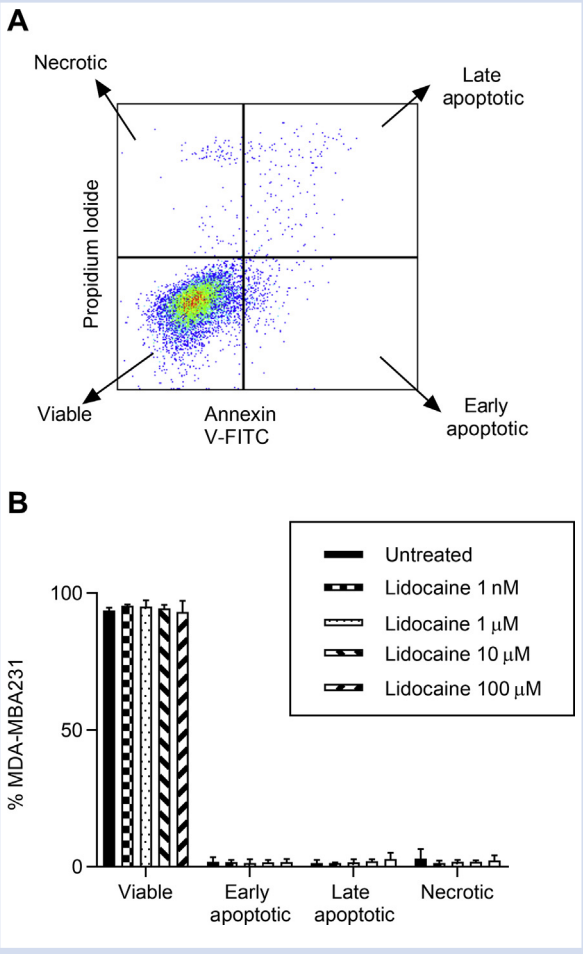

Fig 1. MDA-MB-231 cell viability after lidocaine treatment. (A) Gating strategy to assess cell viability. One representative plot obtained from cells treated with lidocaine 100 μM is shown. (B) Percentage of viable, apoptotic and necrotic MDA-MB 231 cells treated for 24 h with different lidocaine concentrations. Mean (standard deviation) of three independent experiments. FITC, fluorescein isothiocyanate.

Different adhesion molecules expressed on epithelial cells can play a role in cancer cell invasion and metastasis. Therefore, the effect of lidocaine treatment was assessed on the expression of CD44 and CD54 [intercellular adhesion molecule 1 (ICAM-1)] after stimulation with CXCL12. In comparison to untreated cells, lidocaine pre-treatment induced an upregulation of CD44 ($P=0.0226$), which was observed also after

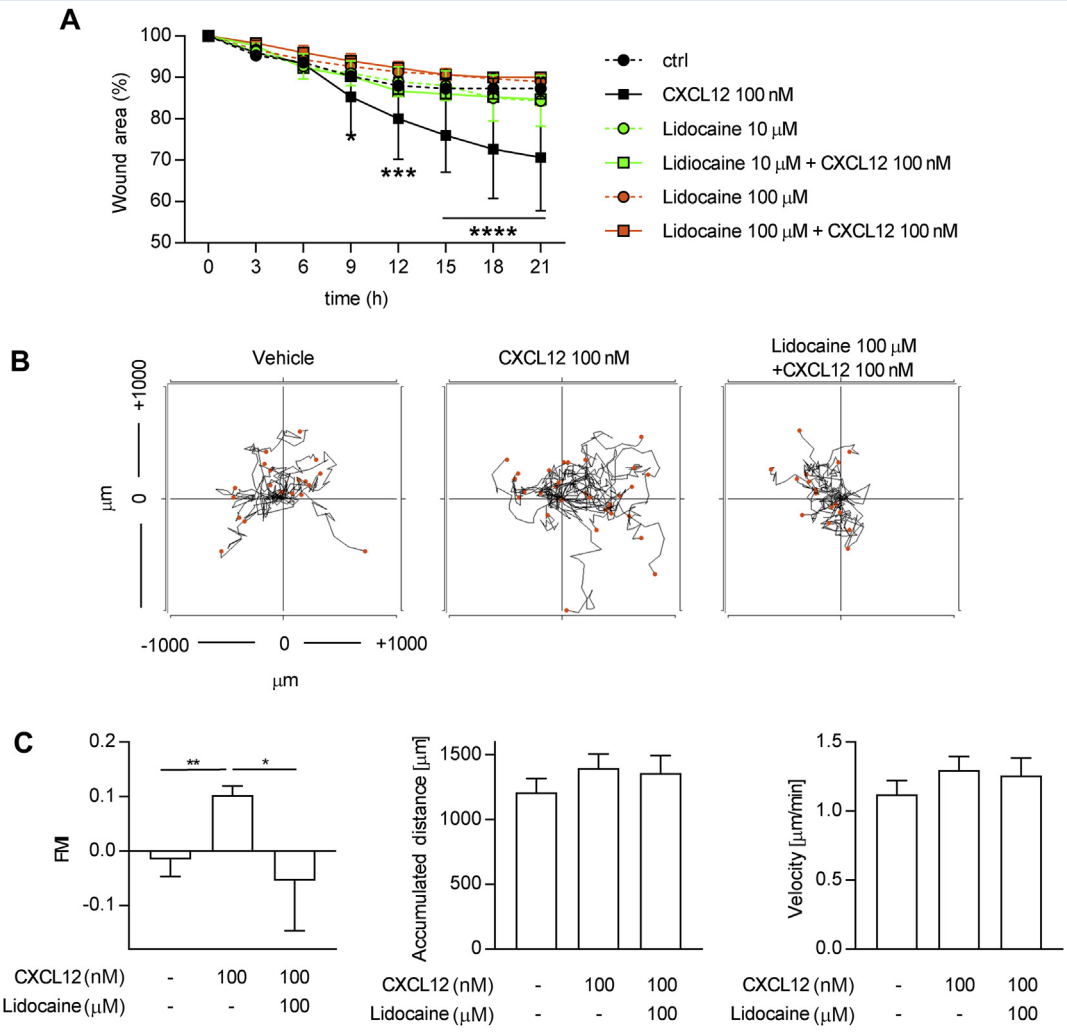

Fig 2. Lidocaine treatment inhibits CXCL12-induced *in vitro* migration of MDA-MB-231 cells. (A) Quantification of MDA-MB-231 cell motility, expressed as percentage of the uncovered scratch wound area respective to time 0. Mean (standard deviation) of three independent experiments of cells treated with lidocaine 10 μM (green) or 100 μM (red), in the presence or absence of CXCL12 100 nM. Statistical analysis was by two-way analysis of variance (ANOVA). (B) Aggregated trajectories of individual cells in the presence of vehicle, CXCL12 100 nM, or CXCL12 100 nM with lidocaine 100 μM. Red dots represent the position reached by each cell at the last time point recorded. A representative result, out of three independent experiments, is shown. (C) Quantification of MDA-MB-231 chemotaxis expressed as forward migration index (FMI), accumulated distance, and cell velocity. Statistical analysis was by Student's *t*-test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

stimulation with CXCL12 (Fig. 3A, $P=0.0010$), whereas no significant differences were detected in CD54 expression (data not shown). To understand whether lidocaine-mediated CD44 overexpression is essential for inhibition of MDA-MB-231 migration, the effect of CD44 blockade was tested in the scratch wound assay. The CD44-neutralising antibody did not restore cell migration in CXCL12 stimulated cells in the presence of lidocaine (Fig. 3B), indicating that modulation of this adhesion molecule is not essential for the observed effect, and suggesting that lidocaine directly alters the response to CXCL12.

Lidocaine effects on cytoskeleton remodelling

Remodelling of the cytoskeleton, supported by actin polymerisation, is a crucial process for correct polarisation of cells and motility during cell migration.[17] CXCR4 triggering by CXCL12 induces Ca^{2+} mobilisation from intracellular stores as a result of the activation of the Gαi pathway.[18] This is an essential pathway for modulation of proteins with Ca^{2+}-dependent activity that are involved in remodelling of the cytoskeleton. CXCL12 triggering of CXCR4 resulted in an intracellular Ca^{2+}increase, which could be blocked by the Gαi inhibitor pertussis toxin. Lidocaine pre-treatment partially inhibited the Ca^{2+} mobilisation induced by CXCL12 (Fig. 4A).

We hypothesised that lidocaine inhibition of intracellular Ca^{2+} increase impairs actin polymerisation, explaining the observed reduction in cell migration. Therefore, a specific staining for filamentous actin was performed in MDA-MB-231 cells pre-treated with lidocaine, and stimulated with CXCL12. Exposure of untreated MDA-MB-231 cells to the chemokine

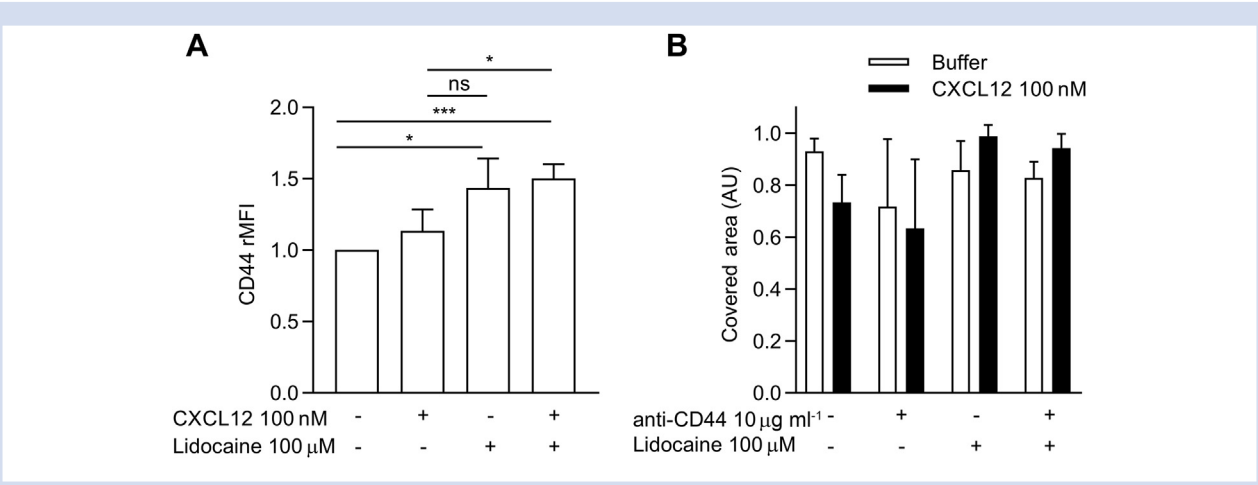

Fig 3. Effect of lidocaine on CD44 expression, and relevance of CD44-induced adhesion on cell migration in lidocaine treated cells. (A) Lidocaine induced CD44 up-regulation in the absence or presence of CXCL12. Relative mean fluorescence intensity (rMFI) is shown from three independent experiments. Statistical analysis was with one-way analysis of variance (ANOVA). (B) CD44-mediated adhesion is not required to inhibit CXCL12-induced migration in cells treated with lidocaine. Quantification of migration of MDA-MB-231 cells is expressed in arbitrary units (AU). Covered area was normalised to respective time 0. Mean (standard deviation) of three independent experiments pre-treating cells with lidocaine 100 μM in the presence or absence of a neutralising anti-CD44 antibody. *P<0.05, ***P<0.001.

resulted in rapid rearrangement of the cortical actin fibres, with pronounced actin polymerisation (Fig. 4B). Lidocaine pre-treatment at 10 and 100 μM inhibited cytoskeleton rearrangement after CXCL12 stimulation (Fig. 4B), supporting the results obtained in the migration assay. Quantification of mean fluorescence intensity of phalloidin, which indicates levels of actin polymerisation, confirmed the above data, showing a significant decrease when cells pre-treated with lidocaine at 10 or 100 μM were stimulated with CXCL12 (Fig. 4C, $P=0.0011$, $P=0.0020$, respectively). Of note, high lidocaine concentrations (100 μM) also decreased basal actin polymerisation compared with untreated cells (Fig. 4C, $P=0.0202$).

Discussion

We show that lidocaine, at clinical concentrations, inhibits CXCL12-induced CXCR4 signalling, which impairs the essential cascade of cytoskeleton remodelling, leading to a reduced migration of breast cancer cells.

Epidemiological studies have suggested an association between regional anaesthesia and a reduction in the incidence of cancer recurrence.[2] The underlying mechanism is still unknown. One report suggested a possible systemic effect of reabsorbed local anaesthetic in preventing invasiveness of tumour cells released from the primary tumour during surgery.[19] This hypothesis has been supported by *in vitro* studies, demonstrating that lidocaine affects motility of a lung cancer cell line by reducing ICAM-1 and Src phosphorylation after tumour necrosis factor stimulation. The reduced activity of ICAM-1 on tumour cells could inhibit their adhesion to vascular endothelium, thus preventing migration into tissues.[3] And in a murine model of breast cancer metastasis, lidocaine reduced pulmonary metastasis, perhaps by anti-inflammatory or anti-angiogenic mechanisms.[20] Recent studies have shown that, in septic patients, lidocaine can block neutrophil migration by inhibiting cell arrest and transmigration through endothelial cells, affecting G-protein signalling, without modulating expression of adhesion molecules.[21] The present study, focusing on breast tumour cells, shows that lidocaine, at clinical concentrations, can inhibit chemokine-induced cancer cell migration by directly inhibiting the activity of CXCR4. These results agree with studies performed on different cell types,[3,21–23] showing that lidocaine affects the activity of the chemokine class of GPCR, and providing new insights into the molecular mechanisms governing its inhibitory effect on cell migration.

Increasing evidence indicates that different classes of anaesthetics can exert either pro- or anti-metastatic effects, which depend on cancer cell type, dosage, and administration protocol.[24] As an example, a potential pro-metastatic role of volatile anaesthetics involving CXCR2 expression has been described in ovarian cancer cells.[25] Our data support further studies to validate the role of lidocaine as an anti-metastatic agent, and for reconsidering the perioperative treatment in breast cancer. Indeed, perioperative continuous i.v. infusion of lidocaine has been safely used to reduce systemic inflammation and bowel dysfunction after abdominal surgery.[26]

The chemokine system represents a fundamental communication bridge between cancer and stromal cells, essential in maintaining and supporting tumour growth and metastasis. The chemokine receptor CXCR4 and its ligand CXCL12, are widely expressed in several types of tumours, and are both targeted in cancer therapy.[27] Numerous reports highlight the crucial involvement of the CXCL12/CXCR4 chemokine axis specifically in breast cancer cell metastasis to bone, lung, and brain.[4,28] In our work, lidocaine at clinical concentrations was able to block CXCR4-mediated cell migration, acting on the downstream signalling cascade. Lidocaine, by inhibiting CXCL12-dependent Ca^{2+} release and actin polymerisation, impaired the cytoskeleton remodelling required for directional cell migration. Of note, high doses of lidocaine induced the loss of cortical actin stress fibres, suggesting alteration of the basal cellular cytoskeleton architecture.

Adhesion molecules, expressed on tumours, mediate the interaction between cancer cells and the microenvironment,

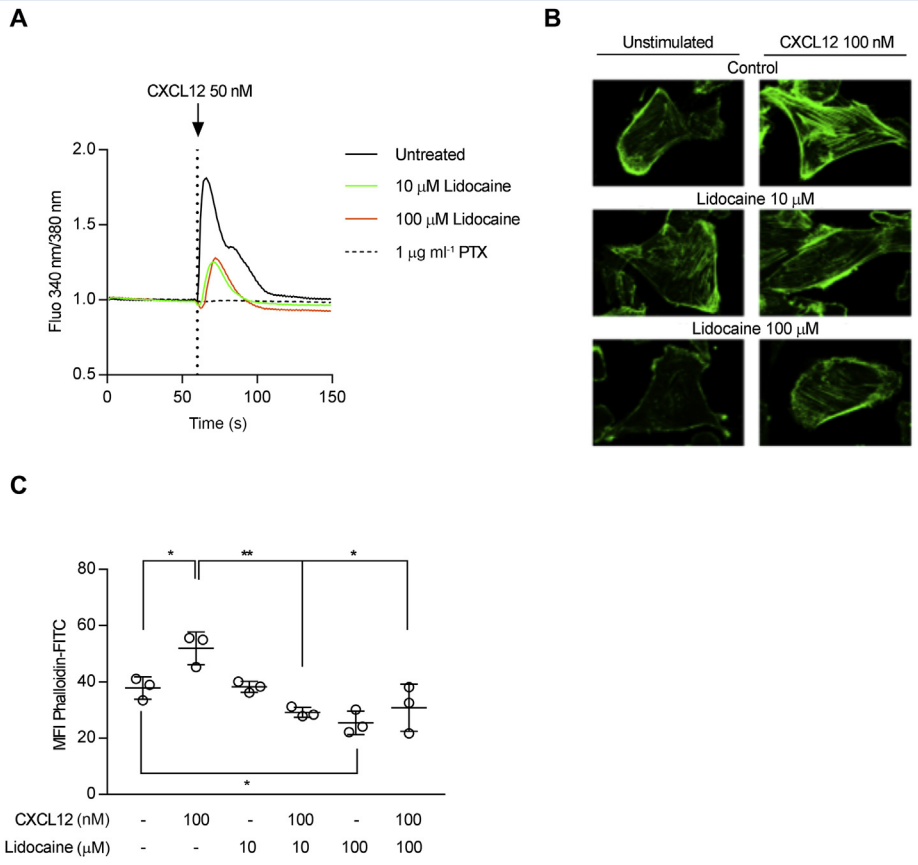

Fig 4. Effect of lidocaine treatment on intracellular Ca^{2+} increase and actin polymerisation after CXCL12 stimulation. (A) Changes of intracellular Ca^{2+} concentration in response to CXCL12 stimulation in cells pre-incubated with lidocaine 10 μM (green) or 100 μM (red) for 24 h. As a control, cells were pre-treated with pertussis toxin 1 μg ml^{-1} for 2 h. Data are presented as ratio of fluorescence (Fluo 340 nm:380 nm) values over time. A representative result, out of three independent experiments, is shown. (B) CXCL12-mediated-actin polymerisation measured by confocal microscopy using phalloidin coupled to fluorescein isothiocyanate (FITC). Cells, pre-incubated with lidocaine 10 μM or 100 μM for 24 h, were stimulated with CXCL12 100 nM. Images of cell associated filamentous actin (green) were acquired for unstimulated (left panels) or CXCL12-stimulated (right panels) cells. One representative experiment out of three is shown. (C) Quantification of actin polymerisation was performed measuring mean fluorescence intensity (MFI) values of phalloidin for each experiment acquiring at least 100 cells. Data are shown as mean (standard deviation) of three independent experiments. Statistical analysis was by one-way analysis of variance (ANOVA). *$P<0.05$.

sustaining tumour progression and metastasis.[29] We found that in MDA-MB-231 cells, lidocaine treatment promotes upregulation of CD44 expression, a transmembrane glycoprotein important for cancer interaction with hyaluronic acid, an essential component of the extracellular matrix. Fuchs and colleagues[30] pointed out a regulatory interplay between CD44 and the CXCL12/CXCR4 axis, describing a direct role of CD44 in the signalling of this chemokine pathway. Our results show that the blockade of CD44 does not restore CXCL12-mediated cell migration in MDA-MB-231 breast cancer cells, demonstrating that the lidocaine effect is CD44-independent.

In conclusion, lidocaine can block a pathway involved in cancer progression and metastasis, which opens the way for further investigations of the activity of lidocaine on cells of the immune system that might contribute to improved cancer outcomes. Studies performed *ex vivo* on tumour and infiltrating cells isolated after surgical removal from patients treated with lidocaine and clinical trials are needed to confirm these *in vitro* findings to establish a role for local anaesthetics in cancer therapy.

Authors' contributions

Study supervision: M.U.
Study design: G.D'A., A.S., V.C., M.U.
Study conduct: G.D'A., V.C., Y.S.
Data collection: G.D'A., V.C., Y.S.
Data analysis: G.D'A., V.C., A.S., M.U.
Critical revision of the data and writing of the manuscript: G.D'A., A.S., V.C, A.B., L.A., M.U.
Final approval of the manuscript: G.D'A., A.S., V.C, Y.S., A.B., L.A., M.U.

Acknowledgements

We thank J. Aguirre for critical discussion, G. Danelon for excellent technical assistance, and D. Jarrossay and R. D'Antuono for assisting in image analysis.

Declaration of interest

The authors declare that they have no conflicts of interest.

Funding

Swiss National Science Foundation (3100A0-143718/1 to M.U.) and San Salvatore Foundation (to M.U).

References

1. Velloso FJ, Bianco AF, Farias JO, et al. The crossroads of breast cancer progression: insights into the modulation of major signaling pathways. *Onco Targets Ther* 2017; **10**: 5491–524
2. Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? *Anesthesiology* 2006; **105**: 660–4
3. Piegeler T, Votta-Velis EG, Liu G, et al. Antimetastatic potential of amide-linked local anesthetics: inhibition of lung adenocarcinoma cell migration and inflammatory Src signaling independent of sodium channel blockade. *Anesthesiology* 2012; **117**: 548–59
4. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; **410**: 50–6
5. Murphy PM. Chemokines and the molecular basis of cancer metastasis. *N Engl J Med* 2001; **345**: 833–5
6. Zlotnik A, Burkhardt AM, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. *Nat Rev Immunol* 2011; **11**: 597–606
7. Mukherjee D, Zhao J. The role of chemokine receptor CXCR4 in breast cancer metastasis. *Am J Cancer Res* 2013; **3**: 46–57
8. Smith MC, Luker KE, Garbow JR, et al. CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Res* 2004; **64**: 8604–12
9. Bachelerie F, Ben-Baruch A, Burkhardt AM, et al. International Union of Pharmacology. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. *Pharmacol Rev* 2014; **66**: 1–79
10. Wells TN, Power CA, Shaw JP, Proudfoot AE. Chemokine blockers–therapeutics in the making? *Trends Pharmacol Sci* 2006; **27**: 41–7
11. Schall TJ, Proudfoot AE. Overcoming hurdles in developing successful drugs targeting chemokine receptors. *Nat Rev Immunol* 2011; **11**: 355–63
12. Benkwitz C, Garrison JC, Linden J, Durieux ME, Hollmann MW. Lidocaine enhances Galphai protein function. *Anesthesiology* 2003; **99**: 1093–101
13. Hollmann MW, Herroeder S, Kurz KS, et al. Time-dependent inhibition of G protein-coupled receptor signaling by local anesthetics. *Anesthesiology* 2004; **100**: 852–60
14. Clark-Lewis I, Moser B, Walz A, Baggiolini M, Scott GJ, Aebersold R. Chemical synthesis, purification, and characterization of two inflammatory proteins, neutrophil activating peptide 1 (interleukin-8) and neutrophil activating peptide 2. *Biochemistry* 1991; **30**: 3128–35
15. Liang CC, Park AY, Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc* 2007; **2**: 329–33
16. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 2012; **9**: 676–82
17. Thelen M, Stein JV. How chemokines invite leukocytes to dance. *Nat Immunol* 2008; **9**: 953–9
18. Roland J, Murphy BJ, Ahr B, et al. Role of the intracellular domains of CXCR4 in SDF-1-mediated signaling. *Blood* 2003; **101**: 399–406
19. Biki B, Mascha E, Moriarty DC, Fitzpatrick JM, Sessler DI, Buggy DJ. Anesthetic technique for radical prostatectomy surgery affects cancer recurrence: a retrospective analysis. *Anesthesiology* 2008; **109**: 180–7
20. Johnson MZ, Crowley PD, Foley AG, Xue C, Connolly C, Gallagher HC, Buggy DJ. Effect of perioperative lidocaine on metastasis after sevoflurane or ketamine-xylazine anaesthesia for breast tumour resection in a murine model. *Br J Anaesth* 2018; **121**: 76–85
21. Berger C, Rossaint J, Van AH, Westphal M, Hahnenkamp K, Zarbock A. Lidocaine reduces neutrophil recruitment by abolishing chemokine-induced arrest and transendothelial migration in septic patients. *J Immunol* 2014; **192**: 367–76
22. Li CY, Tsai CS, Hsu PC, Chueh SH, Wong CS, Ho ST. Lidocaine attenuates monocyte chemoattractant protein-1 production and chemotaxis in human monocytes: possible mechanisms for its effect on inflammation. *Anesth Analg* 2003; **97**: 1312–6
23. Jiang Y, Gou H, Zhu J, Tian S, Yu L. Lidocaine inhibits the invasion and migration of TRPV6-expressing cancer cells by TRPV6 downregulation. *Oncol Lett* 2016; **12**: 1164–70
24. Yang W, Cai J, Zabkiewicz C, Zhang H, Ruge F, Jiang WG. The effects of anesthetics on recurrence and metastasis of cancer, and clinical implications. *World J Oncol* 2017; **8**: 63–70
25. Iwasaki M, Zhao H, Jaffer T, et al. Volatile anaesthetics enhance the metastasis related cellular signalling including CXCR2 of ovarian cancer cells. *Oncotarget* 2016; **7**: 26042–56
26. Kranke P, Jokinen J, Pace NL, et al. Continuous intravenous perioperative lidocaine infusion for postoperative pain and recovery. *Cochrane Database Syst Rev* 2015: CD009642
27. Guo F, Wang Y, Liu J, Mok SC, Xue F, Zhang W. CXCL12/CXCR4: a symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. *Oncogene* 2016; **35**: 816–26
28. Chiang AC, Massague J. Molecular basis of metastasis. *N Engl J Med* 2008; **359**: 2814–23
29. Farahani E, Patra HK, Jangamreddy JR, et al. Cell adhesion molecules and their relation to (cancer) cell stemness. *Carcinogenesis* 2014; **35**: 747–59
30. Fuchs K, Hippe A, Schmaus A, Homey B, Sleeman JP, Orian-Rousseau V. Opposing effects of high- and low-molecular weight hyaluronan on CXCL12-induced CXCR4 signaling depend on CD44. *Cell Death Dis* 2013; **4**: e819

Handling editor: H.C. Hemmings Jr