BJA Advance Access published June 19, 2014

British Journal of Anaesthesia Page 1 of 7
doi:10.1093/bja/aeu201

BJA

Lidocaine and ropivacaine, but not bupivacaine, demethylate deoxyribonucleic acid in breast cancer cells *in vitro*

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Editor's key points

- Lidocaine demethylates deoxyribonucleic acid (DNA) in breast cancer cells, which may have therapeutic potential.
- This cell culture study evaluated the use of bupivacaine and ropivacaine for demethylation in breast cancer cells and also whether lidocaine and the chemotherapy agent 5-aza-2′-deoxycytidine (DAC) have additive demethylating effects.
- Ropivacaine but not bupivacaine demethylates DNA in breast cancer cells and lidocaine and DAC have additive demethylating effects.

Background. Lidocaine demethylates deoxyribonucleic acid (DNA) in breast cancer cells. This modification of epigenetic information may be of therapeutic relevance in the perioperative period, because a decrease in methylation can reactivate tumour suppressor genes and inhibit tumour growth. The objectives of this study were to determine the effect of two amide local anaesthetics, ropivacaine and bupivacaine, on methylation in two breast cancer cell lines and to detect whether the combination of lidocaine with the chemotherapy agent 5-aza-2′-deoxycytidine (DAC) would result in additive demethylating effects.

Methods. Breast cancer cell lines BT-20 [oestrogen receptor (ER)-negative] and MCF-7 (ER-positive) were incubated with lidocaine, bupivacaine, and ropivacaine to assess demethylating properties. Then, we tested varying concentrations of lidocaine and DAC to assess whether their demethylating effects were additive. Cell numbers and global methylation status were analysed.

Results. Lidocaine decreased methylation in BT-20 and MCF-7 cells, ropivacaine decreased methylation in BT-20 cells, and bupivacaine had no demethylating effect. When combined, lidocaine and DAC had additive demethylating effects.

Conclusions. At clinically relevant doses, lidocaine and ropivacaine exert demethylating effects on specific breast cancer cell lines, but bupivacaine does not. The demethylating effects of lidocaine and DAC are indeed additive.

Keywords: cancer; demethylation; epigenetics; local anaesthetic, bupivacaine; local anaesthetic, lidocaine; local anaesthetic, ropivacaine

Accepted for publication: 18 April 2014

More than 100 yr ago, it was recognized that tumour surgery makes metastasis more likely.[1] The perioperative period of tumour surgery may well be a crucial time when patient outcomes can be decisively influenced.[2] Numerous perioperative factors that may explain tumour progression have now been identified. For example, resecting a primary tumour may promote the progression of distant metastases, or even induce tumour self-seeding, where circulating tumour cells re-infiltrate the original site of a resected tumour.[3] This concept is supported by recent evidence that the number of circulating tumour cells increases dramatically during the perioperative period.[4] One very important determinant of metastatic potential is the epigenetic signature of tumour cells.[5]

In a healthy body, epigenetic mechanisms are responsible for the stability and expression of human deoxyribonucleic acid (DNA) as they regulate the methylation of specific DNA regions.[6] However, epigenetic mechanisms are also increasingly recognized as pathogenic factors in several forms of cancer.[6] Specifically, increases in methylation levels can deactivate tumour suppressor genes and lead to the progression of cancer.[6 7] In these cases, decreasing methylation levels may be of therapeutic benefit. So, a new class of chemotherapeutics designed to demethylate tumour DNA has been introduced into clinical practice.[8]

We have recently shown that the prototype local anaesthetic, lidocaine, can reduce methylation of breast cancer cells at clinically relevant concentrations *in vitro*.[9] This study sought to determine whether similar demethylating effects could also be observed with two prototype long-acting local anaesthetics such as bupivacaine and ropivacaine which are typically used for perioperative neuraxial anaesthesia. In addition, local anaesthetics have been described as enhancing the tumoricidal effects of conventional chemotherapeutics,[10] and we wanted to investigate whether local anaesthetics would also

increase the demethylating effect of a prototypical demethylating chemotherapeutic, 5-aza-2′-deoxycytidine (DAC).

The aim of this study was therefore to test two hypotheses: (i) the local anaesthetics lidocaine, bupivacaine, and ropivacaine decrease methylation levels in tumour cells and (ii) local anaesthetics enhance the demethylating effects of DAC.

Methods

Cell culture

Human breast cancer cell lines BT-20 [oestrogen receptor (ER)-negative] and MCF-7 (ER-positive) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured according to ATCC recommendations. Amplification of 15 short tandem repeat loci and the gender-specific locus amelogenin was carried out in the Institute of Legal Medicine of the Medical University Innsbruck, Austria, to authenticate the cell lines. This was done using 10 ng of template DNA, applying the Geneprint PowerPlex 16 System (Promega, Madison, WI, USA) according to the manufacturer's recommendations, as previously described.[11]

Drug treatments

The following drugs were purchased from Sigma-Aldrich (Vienna, Austria): lidocaine *N*-ethyl bromide (L5783), bupivacaine hydrochloride monohydrate (B5274), and ropivacaine hydrochloride monohydrate (R0283), all dissolved in distilled water. We treated BT-20 and MCF-7 breast cancer cell lines with these local anaesthetics first alone and then in combination with varying concentrations of DAC for 72 h. The following concentrations were used: 10 and 100 μM lidocaine, 2 and 20 μM bupivacaine, 3 and 30 μM ropivacaine; 0.001, 0.02, 0.1, 0.2, 0.5, and 1 μM DAC. Twenty-four hours after seeding, the medium was removed and replaced with medium containing the drug solutions at the desired final concentration. DAC was dissolved in dimethyl sulfoxide to a final concentration of 10 mM, aliquoted, and stored at −20°C. Lidocaine, bupivacaine, and ropivacaine were dissolved in water to a final concentration of 1 M and 100 mM, respectively, aliquoted, and stored at −20°C. Whenever needed, a fresh aliquot was diluted to the desired final concentration.

Effect of local anaesthetics on cell viability

We analysed the effects of lidocaine in combination with DAC and bupivacaine and ropivacaine on cell viability in the human breast cancer cell lines BT-20 and MCF-7 during 72 h incubation by a colorimetric assay (M5655; Sigma, Vienna, Austria). The tetrazolium dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was dissolved in RPMI-1640 without phenol red. The assay was performed according to the manufacturer's instructions. Absorbance of converted dye was measured at a wavelength of 570 nm with background subtraction at 630–690 nm.[12]

Global genomic DNA hypermethylation

Global genomic 5-methylcytosine content was determined by quantitative MethyLight assay, specific for Chromosome 1 SAT2 repeat sequences.[13] We analysed the effects of 10 and 100 μM lidocaine, 2 and 20 μM bupivacaine, and 3 and 30 μM ropivacaine alone or in combination with 0.001, 0.02, 0.1, 0.2, 0.5, or 1 μM DAC, respectively, on the global DNA methylation status in BT-20 and MCF-7 breast cancer cells after 72 h. Genomic DNA from treated cells was extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany). Sodium bisulphite conversion of genomic DNA and MethyLight was performed as described previously.[14]

Additivity

We sought to determine whether the interaction between DAC and lidocaine as the prototype local anaesthetic was supra-additive. Our calculations were based on the Loewe isobolographic additivity model, which has been described as particularly useful when investigating the interplay between two toxic substances *in vitro*.[15] The half maximal DNA demethylation concentration (EC50) was calculated in BT-20 cells for DAC and lidocaine from at least seven independent experiments at different concentrations of DAC and lidocaine, resulting in a preliminary EC50 of 0.08 μM for DAC, and 77.3 μM for lidocaine (line of additivity). We assumed supra-additive effects if 50% of demethylation were achieved with a combination of concentrations significantly lower than those representing the line of additivity.

Statistics

Results are expressed as mean (SD). The Mann–Whitney *U*-test was used for the comparison of the various effects after the different treatments. *P*-values of <0.05 were considered statistically significant. SPSS 17.0 (IBM, Vienna, Austria) was used for statistical analyses.

Results

Effect of lidocaine, bupivacaine, and ropivacaine on cell viability

Treatment with 10 or 100 μM lidocaine alone had no cytotoxic effect. Lidocaine at concentrations of 10 and 100 μM did not increase cytotoxicity of DAC in either BT-20 (Fig. 1A) or MCF-7 (Fig. 1B) breast cancer cell lines. Similarly, treatment with bupivacaine or ropivacaine at doses equipotent to lidocaine showed no cytotoxic effect in either breast cancer cell line (Fig. 1C and D).

Effect of bupivacaine and ropivacaine on global genomic DNA methylation

Treatment with bupivacaine at 2 and 20 μM revealed no significant demethylating effect on global genomic DNA methylation in either breast cancer cell line BT-20 (Fig. 2A) or MCF-7 (Fig. 2B).

Treatment with ropivacaine for 72 h at concentrations of 3 or 30 μM decreased methylation in BT-20 cells (Fig. 2A; $P=0.003$