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Differential effects of serum from patients administered distinct anaesthetic techniques on apoptosis in breast cancer cells *in vitro*: a pilot study

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Editor's key points

- Some data suggest that anaesthetic technique might affect cancer outcome.
- Serum was obtained from a subset of patients enrolled in a larger clinical trial who were randomized to receive propofol-paravertebral or sevoflurane-opioid based anaesthesia.
- Postoperative serum from the sevoflurane anaesthesia group reduced cancer cell apoptosis *in vitro*. These preliminary findings suggest that anaesthetic technique might affect cancer cell metastatic potential during cancer surgery.

Background. *In vitro* and retrospective clinical studies suggest an association between anaesthetic technique during primary breast cancer surgery and cancer outcome. Apoptosis is an important step in the mechanism of breast cancer metastasis, but whether it is influenced by anaesthetic technique is unknown. Using serum from breast cancer surgery patients randomized to receive distinct anaesthetic techniques, we investigated its effect on apoptosis in oestrogen receptor (ER)-negative breast cancer cells *in vitro*.

Methods. Women with biopsy-proven breast cancer were randomized to receive either propofol general anaesthesia with paravertebral analgesia (PPA) or standard sevoflurane general anaesthesia with opioid analgesia (SGA) in an ongoing, prospective clinical trial (NCT 00418457). Serum from a randomly selected subset of these patients (10 PPA and 10 SGA) who had donated 20 ml venous blood immediately before anaesthetic induction and at 1 h after operation was exposed to ER-negative MDA-MB-231 cells. Apoptosis was measured using ApoLive-Glo Multiplex Assay™.

Results. Exposure of MDA-MB-231 cells to postoperative serum of PPA patients resulted in higher luminescence ratio (apoptosis) than SGA patients, median (25–75%), 0.40 (0.35–0.43) compared with 0.22 (0.21–0.30), respectively (P=0.001). The luminescence ratio of postoperative serum from SGA was reduced compared with preoperative SGA 0.22 (0.21–0.30) compared with 0.3 (0.25–0.35) (P=0.045).

Conclusions. Serum from patients given sevoflurane anaesthesia and opioids for primary breast cancer surgery reduces apoptosis in ER-negative breast cancer cells to a greater extent than serum from patients given propofol–paravertebral anaesthesia. Anaesthetic technique might affect the serum milieu in a manner that impacts cancer cell apoptosis, and thereby tumour metastasis.

Keywords: anaesthesia, general; anaesthesia, paravertebral; anaesthesia, regional; apoptosis; breast cancer

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Breast cancer is one of the main causes of cancer-related death in women.[1] Associated morbidity and mortality relates mainly to metastatic disease and not the primary tumour. The process of metastasis is complex, with tumour cells requiring the ability to resist apoptosis, survive conditions of stress, seed, proliferate, and induce angiogenesis.[2]

A number of perioperative factors during primary breast cancer surgery, including anaesthetic technique, might influence whether minimal residual micrometastases are eliminated by the immune system or become full-blown metastatic disease.[3–7] More than 50% of primary neoplasms have defects in cellular apoptotic mechanisms and the process of apoptosis is a key regulator of cancer cell growth, including metastatic potential. Apoptosis is influenced by multiple factors, including immune cytokine signalling.[8 9] In the perioperative period, apoptosis in minimal residual cancer (including micrometastatic deposits, shed tumour cells at the time of surgery, and circulating cancer cells) could plausibly be influenced by many factors, including perioperative immune suppression, the stress response to surgery, acute pain, and opioids. All of these factors can potentially be modified by anaesthetic technique.[7] We have previously studied

serum from breast cancer patients as a marker of the overall effect of anaesthesia and surgery on patients' physiological status, and showed that serum from breast cancer patients differentially affected breast cancer cell functional biology.[10]

The expression of oestrogen receptors (ERs) in breast cancer cells is an important predictor of response to therapy and prognosis.[11] ER-negative breast cancer tends to be associated with more resistance to apoptosis and with poorer outcomes in the clinical context. Therefore, we investigated the potential effect of serum from breast cancer patients who had received different anaesthetic techniques on ER-negative breast cancer cell apoptosis *in vitro*. The primary endpoint was the effect of postoperative serum from breast cancer surgery patients who had received different anaesthetic techniques on apoptosis compared with the effect of serum taken before operation from the same patients.

Methods

Patient selection

After approval from the Ethics Committee of the Mater Misericordiae University Hospital and written informed consent, women undergoing surgery for biopsy-proven primary breast cancer were randomized into an international, multicentre, prospective clinical trial (NCT 00418457). In our centre only, patients were also consented to contribute a sample of venous blood before operation, and at 1 h after operation for this study of the effect of anaesthetic technique on cancer cell biology. After centrifugation at 400*g*, serum was divided into multiple aliquots and stored at −20°C. Inclusion criteria were women aged 18–85 yr undergoing mastectomy or wide local tumour excision with or without axillary node sampling or excision (i.e. believed to be tumour stages 1–3, nodes 0–2). Exclusion criteria were prior breast cancer surgery (except diagnostic biopsy); presence of inflammatory breast cancer; ASA physical status IV; any contraindication to paravertebral anaesthesia; or any general anaesthetic agent.

Randomization

Patients had been randomly assigned for the long-term follow-up clinical trial (NCT 00418457) from a web-based system that automatically recorded their study number and study group allocation. Patients received either combined propofol general anaesthesia with paravertebral analgesia (PPA) or standard sevoflurane general anaesthesia with opioid analgesia (SGA). From the patients enrolled to each arm of this study at our centre who had contributed serum samples, we randomly selected 10 patients from each arm for inclusion in the present study.

Anaesthetic technique

For patients who received PPA, a catheter was positioned in the ipsilateral paravertebral space at the level of the second thoracic vertebra using a standard technique. A 20 ml bolus of levobupivacaine 0.25% was administered before surgery. Total i.v. general anaesthesia was then commenced using a target-controlled infusion of propofol (Diprifusor™). Fentanyl 1–3 μg kg^{-1} was administered at induction. Maintenance of the airway was through a laryngeal mask airway (LMA) with patients breathing spontaneously. Postoperative analgesia was a continuous infusion of levobupivacaine 0.25% at 5–10 ml h^{-1} via paravertebral catheter. Paravertebral catheters were removed at 24 h. Rescue analgesia if needed was triggered by a visual analogue scale (VAS) pain score ≥3, consisting of morphine 0.1 mg kg^{-1} i.m. every 3–4 h as required.

In the SGA group, anaesthesia was induced with fentanyl 1–2 μg kg^{-1} and propofol 1.5–2 mg kg^{-1}. Anaesthesia was maintained with sevoflurane (end-tidal concentrations 1–3%) in oxygen/air mixture. Intraoperatively, morphine 0.1–0.15 mg kg^{-1} was given at the discretion of the anaesthetist. Patients received postoperative patient-controlled analgesia morphine, bolus 1 mg, lockout 6 min, and 4 h dose limit 30 mg. Paracetamol 1 g i.v. was given to all patients during surgery. Venous blood was sampled before and 1 h after surgery. Samples collected before surgery and 1 h after surgery were used for further analysis in this study.

Cell culture

The ER-negative MDA-MB-231 cell line from European Collection of Cell Cultures (ECACC) was used for analysis of cell viability and apoptosis. Cells were cultured in Leibovitz's L-15 medium to which 10% fetal bovine serum, L-glutamine, and 1% penicillin–streptomycin solution was added. Cells were incubated at 37°C in air with 5% CO_2.

Cell viability and apoptosis assay

ApoLive-Glo™ Multiplex Assay from Promega (Southampton, UK) was used to evaluate cell viability and apoptosis. Cells were cultured in L-15 Medium (Leibovitz) supplemented with 10% FBS, 1% penicillin–streptomycin solution, and L-glutamine at 37°C, with 5% CO_2 for 48 h. They were then harvested by trypsinization, resuspended in medium, and added to opaque, clear bottom 96-well plates at a density of 5000 cells per well. Culture plates were subsequently incubated in full medium for 24 h at 37°C to allow cell attachment. Thawed serum were diluted in medium to produce 10% serum concentrations as previously described.[10] Serum was added in triplicate and culture plates incubated for a further 24 h. Viability Reagent (20 μl) was then added to the wells, and mixed by orbital shaking. Plates were incubated for 30 min at 37°C and fluorescence measured at $400_{Ex}/505_{Em}$ nm (viability). Caspase-Glo 3/7 reagent (100 μl) was then added and mixed briefly by orbital shaking (300–500 rpm for ~30 s). Plates were then left for 30 min at room temperature. Luminescence was analysed using GloMax®-Multi Microplate Multimode Reader (Glomax, Promega, Southampton UK).

Statistical analysis

GraphPad Prism v 6 (GraphPad, San Diego, CA, USA) was used for analysis. For parametric continuous data, the unpaired *t*-test was used for comparisons between the groups regarding patient characteristic data. Differences in categorical data were tested using the Fisher exact test. Luminescence data were normalized to control luminescence without serum, and