Nociceptin/Orphanin FQ: protocol presentation of the first intrathecal use in humans

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Nociceptin/Orphanin FQ (N/OFQ) is the endogenous peptide agonist for the N/OFQ receptor NOP. Intrathecal (i.t.) injection of N/OFQ or NOP agonists produce anti-nociception in a range of experimental animal models, but supraspinal administration produces anti-opioid actions.1 There are several studies in non-human primates that show good analgesia with i.t. N/OFQ without the typical side-effects of MOP (μ) agonists such as morphine.2–4 We have a preparation of N/OFQ that has been used in humans via the intravesical route for detrusor instability.1 We aim to investigate the effects of this preparation via the i.t. route. We hypothesize that: (i) i.t. N/OFQ will cause significant analgesia, (ii) the analgesic effect of N/OFQ will be dose-dependent, (iii) there will be no itching, nausea/vomiting, or other side-effects associated with i.t. N/OFQ, (iv) i.t. N/OFQ will not affect cardiovascular or motor function, and (v) there will be no change in blood N/OFQ concentrations.

We will recruit patients with cancer-related pain of moderate-to-severe intensity [defined as visual analogue score (VAS) at rest of ≥4]. Patients may have already received morphine or i.t./epidural injections (analgesics, chemotherapeutic drugs, local anaesthetics, or steroids) as part of their routine therapy. The study will involve an overnight stay. A baseline thermal neurosensory testing study will be conducted before the i.t. injection using a Quantitative Sensory Analyser which will be repeated after i.t. injection and every 2 h until the return to baseline thermal sensitivity levels. Cardiovascular variables will be monitored. We will administer incremental doses of i.t. N/OFQ to sequential groups of six patients under sterile surgical conditions with all resuscitation equipment and drugs available. Blood samples (10 ml) will be collected through the indwelling i.v. cannula before, and at 1, 2, 6, and 12 h after i.t. injection and N/OFQ concentrations determined. The analgesic effect and duration will be monitored by recording VAS for pain intensity; lower limb muscle power will be assessed using the Bromage score. Patients will be discharged from hospital after 24 h observation and when the investigating team is satisfied, there are no residual effects. Every patient will receive a daily telephone follow-up for 5 days: a formal face-to-face interview will occur 1 week after the i.t. injection.

If we can demonstrate a good analgesic profile in humans, further work might include i.t. N/OFQ administration in combination with morphine as previous primate studies3 have demonstrated a synergistic analgesic action.

References
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3 Ko MC, Naughton NN. J Pain 2009; 10: 509–16

Accuracy and precision of intensive care medical staff using the Rockwood clinical frailty score

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The populations of European countries are becoming increasingly elderly. Admissions to critical care are following this trend with a gradual increase in those aged 80 yr and...
Interhemispheric EEG variability during physiological sleep measured using a bilateral bispectral index sensor

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Bispectral index (BIS) monitors are commonly used to measure anaesthetic depth. A new sensor has been developed that allows a BIS value to be calculated for both cerebral hemispheres. Previous work looking at BIS values in 10 sleep-deprived subjects (using two BIS sensors and machines in tandem)1 showed interhemispheric variability in only one individual. It is not known whether this difference was due to true variation or an error in processing EEG signals from two different sources. We aimed to assess the interhemispheric variability of BIS readings during sleep using an electrode designed to detect left to right EEG difference.

Approval was gained from the Ethical Committee of Sheffield University Medical School. Ten healthy, non-smoking, male volunteers with no pre-existing sleep disorders gave written, informed consent. Subjects had a bilateral BIS sensor applied and data were recorded for a whole night of sleep in their own home. Data were analysed by the Mann–Whitney U-test using SigmaStat software (Systat Software, Richmond, CA, USA) and the Bland–Altman plot.

Ten male subjects (aged 21–30) were studied; of which, three were left-handed. All subjects slept for at least 5 h (mean 419 min, range 332–439 min). In total, 4398 paired BIS readings were analysed, each representing 60 s of mean data. The median (IQR) BIS values were 73 (56.75–82) and 74 (58–82) for the left and right hemispheres, respectively (P=0.001) based on 4398 individual measurements, with wide variation demonstrated in the Bland–Altman plot (Fig. 1).

![Fig 1 Bland–Altman plot of BIS difference against the mean BIS value. Solid line represents the mean BIS difference, the dotted lines ±1.96 SD.](image-url)
Significant interhemispheric differences in BIS occur during normal sleep.

Reference
1 Dahoba AA, Xue JX, Xu GX. Minerva Anesthesiol 2011; 77: 388–93

Thoracic epidural or paravertebral block for post-thoracotomy analgesia
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Open thoracic surgery is painful and poor pain control contributes to postoperative morbidity and mortality. Both continuous paravertebral blocks (PVB) and thoracic epidural analgesia (TEA) are widely used as analgesia for this purpose. The relative effects of these two techniques on outcome however remain controversial.

The use of TEA has been declining and the use of PVB increasing in our hospital since 2007. Both techniques were equally used in July 2010. By December 2011, ≏70% of patients were receiving PVB. We retrospectively analysed the patient, disease, operative characteristics, and outcome data of the 1003 patients who had undergone open resective pulmonary surgery and received either a PVB or TEA between January 2009 and December 2011. We calculated the predictive postoperative (ppo) FEV1 for those patients undergoing either a lobectomy or a pneumonectomy. The patients with a ppo FEV1 of >50% and ppo FEV1 of <50% were separately analysed.

Patients receiving TEA (n=501) were older, median (inter-quartile range) age (yr) 68 (62–75) vs 67 (60–73) (P=0.01), had smoked for longer, median (inter-quartile range) pack years 30 (0–45) vs 20 (0–40) (P=0.01), and longer postoperative length of stay (days) median (inter-quartile range) 6 (5–10) vs 6 (4–8) (P=0.0001). For patients with a ppo FEV1 >50%, those receiving TEA (n=240) had smoked for longer median (inter-quartile range) pack years 30 (0–45) vs 18 (0–40) (P=0.01), were more likely to have undergone a pneumonectomy 5.4% vs 1.4% (P=0.01). The TEA patients had a higher mortality 3.3% vs 1.4% (P=0.14) and longer postoperative length of stay (days) median (inter-quartile range) 7 (5–10) vs 6 (4–8) (P=0.002) than those receiving PVB (n=283). Patient and outcome data for patients with a ppo FEV1 <50% are shown in Table 1.

This retrospective analysis did not show a clear superiority for either technique in patients with a ppo FEV1 <50%. This will help justify a randomized study of TEA vs PVB in patients with poor pulmonary function undergoing open resective pulmonary surgery.

Table 1. Patient and outcome data for patients with a predictive postoperative FEV1 <50%. Data are expressed as median (inter-quartile range) or as a percentage

<table>
<thead>
<tr>
<th>Variable</th>
<th>Epidural (n=102)</th>
<th>Paravertebral (n=65)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppo % FEV1</td>
<td>40 (36–45)</td>
<td>43 (36–46)</td>
<td>0.03</td>
</tr>
<tr>
<td>Alcohol excess (%)</td>
<td>2</td>
<td>9.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>67 (62–73)</td>
<td>68 (60–74)</td>
<td>0.37</td>
</tr>
<tr>
<td>Smoking (pack years)</td>
<td>30 (0–48)</td>
<td>20 (0–40)</td>
<td>0.16</td>
</tr>
<tr>
<td>Respiratory complication (%)</td>
<td>7.8</td>
<td>12.3</td>
<td>0.34</td>
</tr>
<tr>
<td>Any complication (%)</td>
<td>28.4</td>
<td>36.9</td>
<td>0.25</td>
</tr>
<tr>
<td>In-hospital death (%)</td>
<td>4.9</td>
<td>1.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Next-generation sequencing for malignant hyperthermia
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DNA testing for mutations in the RYR1 gene is an important part of diagnostics for susceptibility to malignant hyperthermia (MH). Several methods based on polymerase chain reaction (PCR) such as RFLP (restriction fragments length polymorphism) and ARMS (amplification refractory mutation system) have been used to screen for known causative mutations. Sanger sequencing has been applied to discover new mutations or to confirm findings obtained by other methods.

However, for some MH families, no variant in RYR1 has been demonstrated. There are other families where a RYR1 variant has been found, but there is discordance between the MH phenotype and RYR1 genotype and other genes may therefore contribute to the susceptible phenotype. Several candidate genes have been proposed, but other than CACNA1S, none has been confirmed. In collaboration with colleagues at the University of Washington, we have embarked on exome sequencing using next-generation sequencing (NGS) technology. Preliminary analyses revealed variants in RYR1 and CACNA1S that had not been detected using Sanger sequencing of the entire coding regions of these genes. NGS, known also as clonal sequencing, can generate massive read depth, giving a high sensitivity and the possibility of conducting parallel analysis of multiple patient samples with cost savings.
We applied NGS to sequence the whole coding sequence and flanking intronic regions of RYR1 and CACNA1S for 10 MH-susceptible patients. In nine of the patients, diagnostic mutations had been previously excluded, while the 10th patient was known to carry an uncharacterized RYR1 variant and acted as a positive control. Long PCR was performed on good quality genomic DNA samples to amplify the whole coding sequence of RYR1 and CACNA1S. The PCR products were pooled for each patient and barcoding tags were attached for indexing samples. All of the samples were loaded on one lane of the flow cell for cluster generation and sequencing on an Illumina GAIIx. Sequence analysis was performed using Illuminator software (a program developed in-house and available at http://dna.leeds.ac.uk/illuminator/).

Using NGS, we were able to obtain an average read depth of 500 for each nucleotide. In the 10 patients, we localized four single-nucleotide changes in RYR1 (including the positive control and one diagnostic mutation) and seven in CACNA1S. We were also able to identify insertions and deletions in amplified DNA fragments.

Our experience suggests that NGS improves the sensitivity of RYR1 and CACNA1S screening compared with conventional methods. This method offers substantial reduction in analysis time and cost reduction and we envisage using it diagnosis in the near future.

**Electronic Personal Assessment Questionnaire PreOperative: patient experience and face validity of an interactive, electronic questionnaire for the preoperative assessment of patients due to undergo general anaesthesia**

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Anaesthetic preoperative assessment is vital to ensure safe surgery, especially as efficiency drives within the NHS have led to more patients being admitted on the same day as surgery. Electronic preoperative assessment questionnaires may facilitate better use of resources. Electronic Personal Assessment Questionnaire PreOperative (ePAQ-PO) is a web-based instrument designed for use in routine clinical practice. The system provides a comprehensive assessment of patients’ medical and anaesthetic history. Similar systems have been used safely and effectively in other specialties.1, 2

Ethical approval was obtained from the national research ethics service, and written informed consent obtained from 300 patients who then completed the ePAQ-PO in a study designed to evaluate its psychometric properties. As a measure of face validity and clinical utility, subsequent to completion of the ePAQ-PO, patients completed the QQ-10; a 10-item questionnaire that explores patient views on the use of the questionnaire in the context of their clinical episode. Each item includes a statement followed by a five-point response scale from ‘strongly disagree’ to ‘strongly agree’. The value and burden scales of QQ-10 were computed.

The mean age was 54 yr (SD =15). 11.6% were aged >70 yr. Forty-five per cent were ASA I, 43.7% were ASA II, and 11.3% were ASA III. Sixty-five per cent were female and 35% male. A total of 297 patients (99%) also completed QQ-10. The majority of patients ‘mostly’ or ‘strongly’ agreed with statements relating to improved communication (77%) and ease of use (98.7%). 82.9% enjoyed completing the questionnaire and 98.4% said that they would be happy to complete it again in the future as part of their standard care. No patients found it embarrassing or upsetting and only 0.7% found it too complicated. 3.4% of patients felt that the questionnaire was too long. The mean value and burden score for the cohort were 81 and 9, respectively (SD =13.2 and 11.5). There was a non-significant trend towards increased value scores for patients with higher ASA scores. These data suggest high patient acceptability and value attributable to ePAQ-PO. The burden of the questionnaire is low. The web-based questionnaire is likely to be accepted and used by the majority of patients undergoing elective preoperative assessment.

**Funding**

Sheffield Hospitals Charity provided financial support for this research study. Sheffield PCT and Connecting for Health (via Yorks and Humber SHA) provided a grant for the initial development of ePAQ-PO.

**References**


**Methods and interim results of a validation study of the covariates model for target-controlled infusion of propofol**

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Using the Marsh pharmacokinetic (PK) model to deliver a target-controlled infusion (TCI) of propofol, White and
colleagues derived a covariates model which adjusts for age and sex and also body weight. Modifications were made to reflect reduced central compartment volume and clearance with increasing age, especially in females. The original model was developed using venous blood samples. We describe a validation study comparing venous with arterial samples.

This is an ongoing study with a target recruitment of 30 patients. Prior consent is obtained from patients of ASA class I–II undergoing elective surgery. TCI propofol is delivered according to the covariates model. Patients are randomized to a low–high–low (2–5–2) schedule with plasma targets set at 0, 15, and 30 min. Venous blood samples for propofol analysis are drawn at 1.5, 5, 16.5, 20, 31.5, 35, and 45–60 min. Arterial samples are drawn at 1.5, 5, 16.5, and 20 min. Performance of the model is assessed by the calculation of median performance error (MDPE) as a measure of bias and median absolute performance error (MDAPE) as a measure of inaccuracy.

Data from 10 male patients are presented here, with five allocated to the low–high–low schedule and five to the high–low–high schedule. The age range was 28–58 yr and weight range 79–117 kg. The MDPE and MDAPE for venous and arterial samples at 0–20 min and venous samples at 0–60 min are illustrated in Table 2.

These interim results indicate an acceptable predictive performance of the covariates model across multiple step changes in plasma target. There is a trend towards increased model under-prediction based on arterial sampling, which probably represents measurement of the bolus delivered during a step increase in plasma target concentration. It will be important to use the complete data set to compare the performance of alternative PK models for propofol.

### Sodium hydrosulphide alleviates remote lung injury after blast limb trauma

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Blast limb trauma can induce remote organ injury including lungs and other vital organs. Hydrogen sulphide has recently been indicated as a cellular signal molecule involved in many physiological and pathological processes. Previous studies have demonstrated that endogenous hydrogen sulphide donor (sodium hydrosulphide, NaHS, or sodium sulphide, Na2S) can alleviate ventilator-induced lung injury and ischaemia–reperfusion lung injury. The aim of the present study was to investigate the effects of NaHS on remote lung injury after blast limb trauma in rats.

In accordance with the laboratory guidelines of the Third Military Medical University, Chongqing, China, a blast limb trauma animal model was established by using chartaceous electricity detonators in adult male Sprague–Dawley rats under surgical anaesthesia induced by pentobarbital i.p. injection (50 mg kg⁻¹ of body weight). The blast limb trauma induced blood vessel damage, comminuted fractures, soft tissue injury, and minor burns. The rats undergoing blast limb trauma were randomly treated with either NaHS (5 mg kg⁻¹ i.p.) or the same volume of normal saline (n=8). Another eight rats without any intervention or injury served as sham controls. The blood and lung tissue sample were harvested at 6 h after injury. Lung water content ([(wet lung weight−dry lung weight)/wet lung weight]×100%) was used to reflect the severity of lung injury. Tumour necrosis factor α (TNFα) and interleukin 6 (IL-6) in plasma and lung tissue homogenates were measured with enzyme-linked immunosorbent assays (R&D system, USA). Malondialdehyde (MDA) concentration in plasma and lung tissue was measured with a commercially available kit (Jiancheng Biotechnology Company, China).

Blast limb trauma-induced lung injury was demonstrated by an increase in lung water content together with inflammatory and oxidative stress responses. NaHS treatment attenuated lung water content and partially reversed the up-regulation of TNFα and IL-6 induced by blast limb trauma in plasma and lung tissue. NaHS treatment also significantly decreased the MDA concentration (Table 3).

Our data indicate that NaHS, an exogenous H2S donor, can alleviate the lung injury after blast limb trauma which is likely to be due to the suppression of the inflammatory response and the inhibition of oxidative stress.

### Table 2 MDPE and MDAPE expressed as median [IQR (range)]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Venous</th>
<th>Arterial</th>
<th>Venous</th>
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<tr>
<th>Time (min)</th>
<th>Venous</th>
<th>Arterial</th>
<th>Venous</th>
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</table>

References

This study was supported by a grant (06Z034) from the 11th five-year scheme, China.

**Funding**

This study was supported by a grant (06Z034) from the 11th five-year scheme, China.

### Table 3 The changes of lung injury, pro-inflammatory cytokines, and oxidative product [mean (sd), n=8]. **P<0.01 vs Sham; +++P<0.001 vs Blast**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Blast</th>
<th>Blast+NaHS</th>
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</thead>
<tbody>
<tr>
<td>Lung water content (%)</td>
<td>77 (2)</td>
<td>85 (2)**</td>
<td>81 (1)**</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα (pg ml⁻¹)</td>
<td>455 (185)</td>
<td>1026 (221)**</td>
<td>417 (77)**</td>
</tr>
<tr>
<td>IL-6 (pg ml⁻¹)</td>
<td>303 (60)</td>
<td>2327 (597)**</td>
<td>1378 (240)**</td>
</tr>
<tr>
<td>MDA (nmol ml⁻¹)</td>
<td>4.24 (1.20)</td>
<td>10.11 (2.85)**</td>
<td>3.41 (0.81)**</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα (pg mg⁻¹ protein)</td>
<td>38 (11)</td>
<td>165 (45)**</td>
<td>66 (16)**</td>
</tr>
<tr>
<td>IL-6 (pg mg⁻¹ protein)</td>
<td>41 (11)</td>
<td>240 (59)**</td>
<td>102 (30)**</td>
</tr>
<tr>
<td>MDA (nmol mg⁻¹ protein)</td>
<td>3.43 (0.37)</td>
<td>10.97 (4.32)**</td>
<td>6.33 (2.54)**</td>
</tr>
</tbody>
</table>

### Evaluation of three non-rebreathing oxygen delivery masks

**M. Kumar**, N. Saxena*, A. Wilkes and I. Hodzovic

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Oxygen masks are used to deliver oxygen to patients, mountaineers, and aviators. The TopOut Oxygen mask is currently used by mountaineers. It is claimed that since the introduction of the mask, the mortality rate has decreased as a result of improvement in economy and efficiency of the oxygen delivery. The claimed improvement in oxygen delivery may also be of benefit to patients. Intersurgical and Flexicare are commercially available non-rebreathing oxygen delivery masks. In this study, we compared the performance of the three masks in a lung model.

The test arrangement consisted of a ventilator connected to a lung model in turn connected to a manikin head via its trachea. The manikin breathed with tidal volumes (TV) of 300 or 500 ml and respiratory rates (RR) from 10 to 30 at 5 bpm intervals, with oxygen flows of 1, 2, 4, 6, 8, and 10 litre min⁻¹ delivered to the mask. In order to simulate gas exchange in the lungs, setting-specific flows of N₂ and CO₂ were added to the test lung. The gas was sampled at the tracheal level and inspired and expired concentration of O₂ and CO₂ were recorded using an AS/3 Datex monitor.

The primary outcome was the inspired oxygen concentration. Overall, the TopOut is more efficient in delivering oxygen at all flows for all tested RR (P<0.0001). The performances of the three masks at two settings (TV 300 ml and RR 10 and 30 bpm) are shown in Figure 2.

The results show that TopOut is more efficient in oxygen delivery in comparison with the other two medical masks currently in use. However, further testing will be required in human volunteers to determine if this improvement is clinically significant.

### Funding

Supported by MoD grant and manufacturers of TopOut.

### References


**Fig 2** FIO₂ (%) at different O₂ flows for the three masks.
Effect of apocynin on endothelial cells under conditions of sepsis

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Sepsis is the most common cause of mortality in the intensive care unit. Overproduction of inflammatory mediators and reactive oxygen (ROS) and nitrogen (RNS) species have been linked to mitochondrial dysfunction and reduced cellular ATP production during sepsis. NADPH oxidase enzymes (NOX 1–5 and Duox 1–2) are membrane-associated enzymes that catalyse molecular oxygen to superoxide. Although NADPH oxidase is better known as the source of the phagocyte respiratory burst, NOX proteins modulate cell signalling pathways for transcription factor activation and cytokine production via intracellular superoxide and hydrogen peroxide production. We assessed the effect of apocynin, a naturally occurring vanillin-like NOX inhibitor, on mitochondrial function, oxidative stress, and inflammation in human endothelial cells under conditions mimicking sepsis.

Human umbilical vein endothelial cells (HUVECs) were cultured for up to 7 days with 2 μg ml⁻¹ lipopolysaccharide (LPS) and 20 μg ml⁻¹ peptidoglycan G (PepG), plus 0–1 mM apocynin. Constitutive NOX 1–3 mRNA expression was confirmed in HUVEC using RT–PCR. Mitochondrial membrane potential was analysed using the fluorescent probe JC-1 and metabolic activity was determined using Alamar Blue. Total glutathione was measured as a marker of oxidative stress and total radical production was measured using carboxy-dichloro-dihydro-fluorescein diacetate. Interleukin-6 (IL-6) concentrations were measured in medium as an index of inflammation using enzyme immunoassay. Cell viability was assessed using acid phosphatase.

Apocynin had no effect on cell viability at any concentration. Under conditions of sepsis, apocynin inhibited ROS production in a dose-dependent manner (P<0.05); associated mitochondrial membrane potential and glutathione were higher at all concentrations (P<0.05). However, apocynin had no effect on metabolic activity at any concentration.

Apocynin protects against ROS production and oxidative stress and inhibits IL-6 secretion (Fig. 3), but had mixed effects on mitochondrial function.

Acknowledgement

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Modulation of oxidative stress and mitochondrial health by mitochondrial-targeted antioxidants in an in vitro model of chemotherapy-induced neuropathic pain

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² Department of Anaesthesia and Pain Medicine, University of Edinburgh, Edinburgh, UK

Neuropathic pain is a common side-effect of chemotherapy which can necessitate dose reduction. Current treatments are ineffective and although the precise mechanisms involved are unclear, oxidative stress seems to be important. We have previously reported that paclitaxel causes mitochondrial damage which is prevented by antioxidants targeted to mitochondria. To further investigate the cause of this damage as a mechanism of neuropathic pain, we investigated reactive oxygen species (ROS) production and glutathione levels in cells exposed to paclitaxel, and the effects of MitoVitE and melatonin.

Dorsal root ganglion (DRG) neuronal cells (50B11) were treated with 0–100 μM paclitaxel, with or without 1 μM MitoVitE, melatonin, or the untargeted vitamin E analogue,
Trolox. The rate of ROS production was measured using a fluorescent probe and glutathione was measured using monobromobimane. Mitochondrial function was also measured as metabolic activity and membrane potential using Alamar Blue and JC-1, respectively.

The rate of ROS production was increased by paclitaxel (P<0.001, Fig. 4). In cells also exposed to MitoVitE or melatonin, the rate of ROS production was reduced (both P<0.0001, Fig. 4), while Trolox had no effect. In paclitaxel-treated cells without antioxidants, glutathione levels decreased; this was not seen when cells were also exposed to MitoVitE or melatonin. Similarly, mitochondrial dysfunction occurred in paclitaxel-treated cells and was prevented by MitoVitE or melatonin.

We have shown that paclitaxel induces production of ROS, reduces endogenous antioxidants, and causes mitochondrial damage in DRG cells. This was ameliorated by antioxidants which protect mitochondria. Future work will relate this modulation of oxidative stress to clinical measures of neuropathic pain.

Funding
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Estimating the number of tracheostomies performed in critical care in England
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The number of tracheostomies managed in England’s intensive care units (ICUs) is unknown. We aimed to determine the numbers of percutaneous and surgical tracheostomies managed in critical care units in the North West of England and extrapolate from the Greater Manchester critical care network admissions database (‘MIDAS’) to estimate approximate national numbers.

The MIDAS database was interrogated for all admissions between January 1, 2010, and January 25, 2012. Pivot tables were created using Microsoft Excel and exported into SPSS Statistics to determine frequencies of percutaneous and surgical tracheostomies, along with bed days. Hospital Episode Statistics (HES) is a data warehouse containing details of all admissions to NHS hospitals in England. The Intensive Care National Audit & Research Centre (ICNARC) collects data submitted from units participating in its Case Mix Programme (CMP). HES and ICNARC data in the public domain were used to determine the numbers of critical care units, beds, and admissions in England. Neither HES nor ICNARC collect data concerning the patient’s airway.

Eight Trusts (17 ICUs, 11 hospitals, 154 beds) submitted data to MIDAS for 16 589 admissions covering 99 037 bed days (Table 4). There are 241 ICUs in England. By extrapolating the MIDAS figures, we can estimate that 14 200 (95% CI 6800–21 600) tracheostomies were managed annually in England’s ICUs.

We have estimated approximate numbers of tracheostomies managed in England’s ICUs using two different methods and the results are similar. HES recorded around 5700 surgical tracheostomies performed in theatres during 2009/2010 and our estimates suggest that the majority of tracheostomies in England are performed and managed in critical care by intensivists, rather than surgeons. The profile of the ICUs within the MIDAS data set is similar to the national profile, although the patient populations may be different. A national tracheostomy register would allow an accurate determination of these numbers and allow appropriate follow-up.

References
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2 Available from www.icnarc.org (accessed 15 April 12)
Functional analysis of the p.D1056H RYR1 variant associated with malignant hyperthermia and exertional heat stroke

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It has long been suggested that there is a link between malignant hyperthermia (MH) and exertional heat stroke (EHS), primarily based on the similarity of the symptoms of both disorders and data obtained through animal models. Porcine and, more recently, murine models of MH can be induced into the hypermetabolic reaction through exposure to stress and heat, respectively. Abnormal in vitro contracture test (IVCT) results in survivors of EHS have been reported by several groups. In the UK, cases of EHS in the military are referred for IVCT, if they are found to have persistently abnormal responses to heat tolerance tests.

Missense variants in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel (RYR1) are associated with the majority of MH cases and reports of RYR1 variants in patients with EHS are emerging. The role of the great majority of these RYR1 variants is yet to be functionally verified.

In this study, we present the functional analysis of the p.D1056H RYR1 variant that has been identified in an MH family through exome sequencing. On subsequent screening of the UK MH population, the variant was also found in an EHS patient whose IVCT results, after undergoing the IVCT, tested negative for MH. The p.D1056H variant was introduced into a subclone of human RYR1 before full-length reconstruction into a pTUNE-inducible expression vector. Wild-type and p.D1056H RYR1 cDNA clones were transiently transfected into HEK293 cells and expression was induced at a standardized level for both constructs using IPTG. Forty-eight hours post-transfection, cells were loaded with a fluorescent calcium indicator and challenged with incremental doses of caffeine. Subsequent calcium release was visualized using confocal microscopy. Area under the curve measurements indicated that cells expressing the p.D1056H RYR1 variant exhibited a trend for greater calcium release than wild-type at low doses of caffeine. Furthermore, the p.D1056H RYR1 variant exhibited an increased sensitivity to caffeine as demonstrated by a statistically significant decrease in EC50 (1.834 mM) when compared with wild-type (3.804 mM; P<0.01).

The data presented are consistent with the p.D1056H RYR1 variant having a functional role in EHS and possibly MH. These findings further support the link between MH and EHS.

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Zinc in inflammation and sepsis
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Sepsis is a major cause of mortality in ICUs, affecting about 18 million people every year. It has a mortality rate of around 25% for uncomplicated sepsis, increasing to 80% in those patients who go on to develop multiple organ failure. Zinc is a micronutrient, essential for many biological functions, and has anti-inflammatory and anti-oxidative properties. A relationship has been established between zinc deficiency and the severity of sepsis, in which zinc deficiency further augments the pathological processes of sepsis. This relationship is of importance, as zinc deficiency is a common phenomenon in the elderly, alcoholics, and critically ill, who are also at increased risk of sepsis.

In order to study the precise mechanisms of effects of zinc in sepsis, we aimed to establish a cell culture model of sepsis with differing zinc environments: zinc deficiency (ZD, 1 μM), zinc adequacy (ZA, 5 μM), and zinc excess (ZE, 20 μM). For this purpose, the chelating resin Chelex 100 was used to remove zinc from culture medium. The effects of altered zinc status on a human endothelial cell line (HUVEC-C) were confirmed by identifying changes in zinc-regulated genes and cellular zinc concentration (Fig. 5A). Under conditions mimicking sepsis: 2 μg ml−1 LPS plus 20 μg ml−1 peptidoglycan G (PepG), significant increases in interleukin (IL)-6 and IL-8 were seen, but the zinc environment had no effect on these measures, despite the difference in cell zinc content (Fig. 5) and observed differences in expression of zinc-regulated genes.

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Effect of ranolazine on mitochondrial function in an in vitro model of sepsis
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The development of mitochondrial dysfunction through oxidative stress is a common pathology shared by many conditions, including sepsis. Ranolazine, an anti-anginal agent, protects cardiac myocytes from oxidative damage via

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numerous mechanisms: the closure of late voltage-gated $\text{Na}^+$ channels, enhanced glucose metabolism, protection of mitochondrial complex I, and enhanced $\text{Ca}^{2+}$ buffering. We hypothesized that ranolazine may also protect mitochondria from oxidative damage under conditions of sepsis.

Cultured human umbilical vein endothelial cells (HUVEC-C cell line) were treated with ranolazine at concentrations of $0–150 \text{ mM}$, with and without $2 \text{ mg ml}^{-1}$ lipopolysaccharide (LPS) and $20 \text{ mg ml}^{-1}$ peptidoglycan (PepG) to mimic sepsis. Treated cells were cultured for both $24 \text{ h}$ and $7 \text{ days}$. Acid phosphatase assay was used to assess the effect of ranolazine on cell health. Investigations into mitochondrial function in intact cells included: membrane potential, endogenous glutathione levels, volume, permeability transition pore opening, and metabolic activity.

At concentrations above $100 \text{ mM}$, ranolazine had detrimental effects on cell viability. Mitochondrial membrane potential (measured using JC-1) was significantly and dose dependently higher in cells treated with ranolazine ($0–90 \text{ mM}$) only for $7 \text{ days}$ (Fig. 6A) but not in the combined presence of LPS/PepG (Fig. 6B). Similar effects were also seen on mitochondrial permeability pore opening. Ranolazine did not affect total glutathione levels, metabolic activity, or mitochondrial volume.

These data suggest that ranolazine offers no protection to mitochondria under conditions of sepsis in endothelial cells in vitro.

Oxygen consumption before and after vascular surgery

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Preoperative cardiopulmonary exercise testing (CPET) is used to identify patients at increased risk of postoperative morbidity and mortality. A study in patients having abdominal surgery calculated oxygen consumption from measurements...
of cardiac output and of oxygen tension in arterial and mixed venous blood samples and concluded that oxygen consumption was 44% higher after surgery than before surgery.3 It has been suggested that CPET identifies patients with limited physiological reserve who cannot sustain this increase in postoperative $\dot{V}O_2$.4 However, other studies did not find an increase in $\dot{V}O_2$ after surgery.3,4

We measured $\dot{V}O_2$ in 23 patients at rest on the evening before major vascular surgery and about 24 h after surgery. Thirteen patients underwent open abdominal aortic aneurysm (AAA) repair and 10 patients femoral-popliteal bypass surgery. Patients breathed air through a non-rebreathing valve attached to a tight-fitting face mask (Hans Rudolph, Shawnee, KS). Expired gases were mixed, dried, and passed through a dry gas meter. Mixed expired $O_2$ and $CO_2$ fractions were measured at steady state (Servomex paramagnetic $O_2$ analyser, Datex-Ohmeda Normocap capnograph). Expired gas volume was measured over 5 min. $\dot{V}O_2$ was calculated:

$$\dot{V}O_2 = \dot{VE} \cdot \{(1 - \overline{FE}_O_2 - \overline{FE}_CO_2)/(1 - \overline{FI}_O_2) \cdot \overline{FI}_O_2 - \overline{FE}_O_2\}.5$$

Figure 7 shows $\dot{V}O_2$ measurements in individual patients.

In patients undergoing AAA repair, there was a mean change of $\dot{V}O_2$ after surgery of $-9.5\%$ (95% CI: $-22.4\%$ to $3.4\%$) (median $-13.2\%$; Q1: $-25.3\%$; Q3: $1.5\%$). In patients having femoral-popliteal bypass, the mean $\dot{V}O_2$ increased by $1.8\%$ (95% CI: $-10.2\%$ to $13.8\%$) (median: $-2.6\%$; Q1: $-14.2\%$; Q3: $16.2\%$). Overall, we did not observe an increase in $\dot{V}O_2$ after major vascular surgery.

Comparing performance of three non-rebreathing oxygen masks: Topout, Intersurgical™, and Flexicare™, a randomized, cross-over volunteer study

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Flexicare (Hudson type mask) and Intersurgical oxygen masks are commercially available non-rebreathing oxygen delivery masks. Topout mask is a mountaineering mask claimed to have high efficiency of oxygen use. This may be beneficial in the pre-hospital and combat environments where supply of oxygen may be limited. The oxygen flows of 2–4 litre min$^{-1}$ are deliverable by currently available oxygen concentrators. Our laboratory study showed that the TopOut mask was more efficient in delivering oxygen. We decided to compare oxygen delivery function of the three masks at low oxygen flows in a volunteer-based randomized cross-over study.

After local research ethics committee approval, 15 volunteers were invited to take part. Measurements were taken with subjects on a CPET bike. Oxygen was administered by one of the three oxygen delivery masks in a randomized order. Inspired ($Fi$) and expired ($Et$) $O_2$ and $CO_2$ were sampled using nasal cannulae.1 Volunteer’s perception of mask comfort was assessed using visual analogue scale (VAS=0 mm: very uncomfortable, 100 mm: very comfortable). This study was powered to detect a difference of $\overline{EO}_2$ of 0.1 (10%). SPSS v 16 was used to analyse the data.

The main findings of our study are shown in Table 5. The volunteer’s (11 males and four females) mean (range) age was 38 (23–52) yr. There was no difference in heart rate, arterial pressure, respiratory rate, tidal volume, and oxygen saturation between the three masks, before or during the tests.

Although the TopOut mask favours the efficient use of oxygen when used at high altitude, the current TopOut mask design does not seem to offer significant advantage over the Hudson and Intersurgical oxygen delivery masks.

This study found no difference in oxygen delivery and comfort of use between the TopOut, Intersurgical, and Flexicare masks at $O_2$ flow of 4 litre min$^{-1}$ in exercising volunteers. The design of the current oxygen delivery masks

### References

2 Biccard BM. Anaesthesia 2004; 59: 60–8

### Table 5. Results. Values are mean (sd) (n=15)

<table>
<thead>
<tr>
<th></th>
<th>TopOut</th>
<th>Flexicare</th>
<th>Intersurgical</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\overline{EO}_2$ (%)</td>
<td>26 (3)</td>
<td>25 (3)</td>
<td>26 (4)</td>
<td>0.8</td>
</tr>
<tr>
<td>$\overline{FI}_O_2$ (%)</td>
<td>37 (7)</td>
<td>34 (7)</td>
<td>34 (3)</td>
<td>0.1</td>
</tr>
<tr>
<td>VAS (mm)</td>
<td>56 (23)</td>
<td>59 (30)</td>
<td>41 (25)</td>
<td>0.2</td>
</tr>
</tbody>
</table>
needs to be adjusted so they can be used with low oxygen flows.

**Funding**
Supported by a Ministry of Defence grant.

**Reference**
1 Wagstaff TA, Soni N. *Anaesthesia* 2007; 62: 492–503

**Lipopolysaccharide stimulation modifies nociceptin/orphanin FQ mRNA expression in immunocytes**

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The nociceptin/orphanin FQ (N/OFQ) system comprises a 17 amino acid peptide N/OFQ, its precursor pre-pro N/OFQ, and its receptor NOP.¹ There is growing evidence that the N/OFQ system has an immunomodulatory role in inflammation, but the mechanisms remain unclear.² We have measured changes in mRNA expression for NOP and ppN/OFQ in polymorphonuclear cells (PMNs), key mediators of innate immunity, in response to a standard in vitro septic stimulus.

With ethics committee approval and informed consent, we took 30 ml of whole blood from healthy volunteers into pre-treated EDTA-containing tubes (Sarstedt Monovette®). After separation through the density medium Polymorphprep™ (Axis-Shield), isolated PMNs were incubated with increasing concentrations of lipopolysaccharide (LPS: 0, 1, 2.5, and 5 µg ml⁻¹) at 37°C, 5% CO² for 20 h. PMNs were subsequently lysed and mRNA extracted using the mirVana™ extraction kit, quantified via the Nanodrop 2000™ spectrophotometer, before proceeding to reverse transcription (Multiscribe™ Applied Biosystems). We performed quantitative polymerase chain reaction (qPCR) using commercial TaqMan™ probes for NOP, pp-N/OFQ, and the housekeeper gene B2M. Further experiments were performed incubating whole blood with LPS for 5 h at concentrations of 0, 0.01, 0.1, and 1 µg ml⁻¹. We then extracted PMNs and peripheral blood mononuclear cells (PBMCs) and performed qPCR as described above. Results are expressed as change in cycle threshold (Ct) relative to B2M (ΔCt). Data were analysed using Friedman’s analysis of variance (ANOVA) test with Dunn’s post hoc test compared with control values.

Increasing concentrations of LPS reduced NOP mRNA expression in PMN and PBMCs (Table 6). Despite there being a shorter incubation time difference (5 vs 20 h), these changes were greater in whole blood vs isolated PMNs. ppN/OFQ was not reliably detected.

The effect of LPS on NOP expression is more pronounced in whole blood suggesting that (i) there is an interaction between cell types and/or (ii) an interaction of LPS with other mediators in whole blood.

**Funding**
Funded by ARS Heath Family Fund Project Grant.

**References**
1 Lambert DG. *Nat Rev Drug Discov* 2008; 7: 694–710

**Jet (wet) nebulizer and metered dose inhaler delivery of drugs**

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Disposable jet or wet nebulizer use and effectiveness is not evidence-based; there is a shortage of clinical trials and a lack of quality.¹ Studies of nebulizer performance show only 5.7–12% of solutions reached the lungs in volunteers inhaling radioactive labelled sodium chloride or albumin in saline,²,³ and observations in one paper raised doubts as to whether therapeutic agents in aqueous solutions can be delivered by aerosol in significant amounts beyond the larynx. 0.5–10 µm spheres of drug and water in 0.9% NaCl produced by nebulizers are more likely to deposit drug during normal humidification of inspired air high in the respiratory tract and spheres pass no further. This suggests that absorption of drugs such as steroid, salbutamol, or ipratropium occurs in the nasal cavity, mouth, pharynx, and from the gut when swallowed; drugs do not descend and pass

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**Table 6** Immunocyte NOP expression. ΔCt values; high numbers equate to low expression of NOP. *P<0.05; —, not measured

<table>
<thead>
<tr>
<th>LPS (µg ml⁻¹)</th>
<th>Isolated PMN [median (IQR), n=9]</th>
<th>Whole blood-PMN [median (IQR), n=5]</th>
<th>Whole blood-PBMC [median (IQR), n=5]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>11.0 (10.2–12.4)</td>
<td>8.9 (6.6–10.4)</td>
<td>11.0 (6.4–12.5)</td>
</tr>
<tr>
<td>0.01</td>
<td>—</td>
<td>15.1 (12.1–15.7)</td>
<td>15.7 (12.8–17.5)</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>16.0 (13.7–18.0)*</td>
<td>15.1 (13.0–18.0)</td>
</tr>
<tr>
<td>1.0</td>
<td>12.7 (11.8–13.2)</td>
<td>16.2 (15.5–17.1)*</td>
<td>16.6 (13.6–18.8)*</td>
</tr>
<tr>
<td>2.5</td>
<td>12.5 (11.6–14.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5.0</td>
<td>14.6 (12.8–15.5)*</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>
across bronchial walls to smooth muscle but arrive and exert their effect via a vascular route.

Metered dose inhaler (MDI) propellant is an anaesthetic agent norflurane (C\textsubscript{2}F\textsubscript{4}H\textsubscript{2}) which will produce a partial pressure in the airway helping to carry drugs during inspiration. An inhalation agent effect of smooth muscle relaxation (bronchodilation) and a sedative effect are likely.

Salbutamol MDIs (Salamol\textsuperscript{®} IVAX and Ventolin\textsuperscript{®} A&H) were placed inside a 0.5 litre reservoir bag, the bag was evacuated, and 70 actuations of the smaller IVAX filled and cooled the bag, equalling 7 ml volume per actuation; 30 actuations of the larger A&H filled the same volume equaling 16 ml per actuation, 99% of which is propellant.\textsuperscript{5} A sealed 1 litre reservoir bag containing a salamol MDI was filled with 100% oxygen, 10 actuations reduced the oxygen concentration to 89%, measured with a Datex anaesthetic machine oxygen analyser. These results could represent a partial pressure of norflurane per Salamol\textsuperscript{®} MDI puff of ~1% or 1 kPa at room temperature. A Ventolin\textsuperscript{®} MDI activated twice into four different asthma spacers reduced oxygen levels from 21% to 17% in Ablespacer\textsuperscript{TM} (volume 126 ml), 18% in Vortex\textsuperscript{®} (180 ml), to 19% in Babyhaler\textsuperscript{®} (393 ml), and to 19% in Volumatic (788 ml); 10 puffs into Ablespacer\textsuperscript{TM} reduced oxygen to 11%. A mixture of norflurane and drug will be delivered first to the lungs during a deep inspiration, addicts use purloined MDIs and reservoirs to obtain a ‘high’.\textsuperscript{5} A study of nine healthy volunteers breathing either norflurane or chlorofluorocarbon (CFC) propellant carrying radioactive labelled beclosmethosone showed 53% and 4% lung deposition, respectively. A smaller particle size of norflurane of 1.1 µm, to CFC of 3.5 µm was thought to account for the difference,\textsuperscript{6} a carrier effect of norflurane and a reduction in resting bronchial tone were not considered. If asthmatic patients are able to inspire, MDI delivery and a reduction in resting bronchial tone were not consid-
ered. If asthmatic patients are able to inspire, MDI delivery and a reduction in resting bronchial tone were not consid-
ered. If asthmatic patients are able to inspire, MDI delivery and a reduction in resting bronchial tone were not consid-
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**References**


**Plasma dimethylarginines in a surgical model of the acute inflammatory response**

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Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOS) and has attracted considerable interest as a marker, and possibly mediator of endothelial dysfunction. In critically ill patients, plasma ADMA concentration is associated with the severity of organ failure and predicts mortality.

After ethical approval and with written informed consent, we collected plasma samples pre-, immediately post-, and 24 h after operation from 33 patients undergoing open thoracotomy for lung resection. Plasma was immediately centrifuged and stored at –60°C for subsequent analysis. ADMA and symmetric dimethylarginine (SDMA) were measured by high-performance liquid chromatography (HPLC). C-reactive protein (CRP) (measured as a surrogate indicator of the severity of the inflammatory response) was processed as a routine sample in the hospital biochemistry laboratories. Analysis was performed using SPSS v19 and Confidence Interval Analysis (CIA) software (University of Southampton).

The median age of the study group was 69 yr (IQR 61–74). Patients underwent pneumonectomy (n=5), lobectomy (n=24), and wedge/sub-lobar resection (n=4). Preoperative ADMA levels in this cohort were higher than in previously published controls\textsuperscript{1} [mean (sd), 0.52 (0.09) vs 0.46 (0.08) µmol litre\textsuperscript{–1}, P=0.02 (95% CI 0.02–0.09), Student’s t-test]. Thoracotomy was associated with a significant inflammatory response evidenced by an increase in CRP 24 h after operation. ADMA was seen to decrease significantly after operation. SDMA levels were unchanged through the perioperative period (Table 7). Twenty-four hours after operation, the decrease in plasma ADMA (ADMA\textsubscript{24 hpostop–preop}) was associated with the increase in CRP (CRP\textsubscript{24 hpostop–preop}) [r=−0.55, P=0.001 (95% CI −0.75 to −0.25), Pearson’s correlation].

We previously demonstrated a decrease in plasma ADMA but not SDMA during the acute inflammatory response.\textsuperscript{2} This study confirms this finding with the novel observation that the decrease in ADMA is proportionate to the severity of the inflammatory response. Our previous work suggests that ADMA metabolism is not increased during such a response;\textsuperscript{2} we hypothesize that the observed decrease represents increased partitioning of ADMA within cells, a physiological response serving to regulate NOS activity. Mechanisms by which this response becomes disordered

| Table 7 | Perioperative plasma ADMA, SDMA, and CRP. Data presented as median (IQR) or mean (sd). \textsuperscript{*}Friedman test. \textsuperscript{a}Repeated-measures ANOVA |
|---------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
|         | Preop | Postop | 24 h postop | P-value |
| CRP (mg| 5.1 (1.6–9.9) | 5.1 (1.3–13.1) | 118.7 (81.1–134.9) | <0.001\textsuperscript{*} |
| litre\textsuperscript{–1}) | | | | |
| ADMA (µmol| 0.52 (0.09) | 0.53 (0.09) | 0.37 (0.07) | <0.001\textsuperscript{a} |
| litre\textsuperscript{–1}) | | | | |
| SDMA (µmol| 0.49 (0.14) | 0.52 (0.14) | 0.49 (0.19) | 0.88\textsuperscript{a} |
| litre\textsuperscript{–1}) | | | | |
could provide important insights into the pathophysiology of septic shock.

**Funding**

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**References**


**Isoflurane enhances the proliferation and angiogenesis of ovarian cancer cells**

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Ovarian cancer remains the leading cause of death among all gynaecological tumours worldwide.1 Clinical retrospective analyses have indicated that general anaesthesia during surgical removal of ovarian cancer might promote tumour metastasis.2 The aim of the study was to determine the effect of the anaesthetic agent isoflurane on proliferation and angiogenesis of human SK-OV-3 cancer cells. Human SK-OV-3 cancer cells were cultured in a normal cell culture incubator and exposed to 2% of isoflurane in 20% O₂ and 5% CO₂ balanced with 73% nitrogen or 75% nitrogen in 20% O₂ and 5% CO₂ for 2 h in a specially designed chamber. Cultured cells in the normal incubator without any treatment served as controls. Subsequently, cell proliferation and cell cycle changes were determined with an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and flow cytometry, respectively, from 24 to 72 h after gas exposure. The expression of proliferation marker Ki67, cell cycle synthesis marker cyclin D1, and angiogenesis marker VEGF were assessed with immunofluorescence *in situ* staining. Data are from three experiments. Compared with control or nitrogen exposure, SK-OV-3 cell proliferation was significantly increased at any time point after gas exposure (Fig. 8). Cells entering into the synthesis phase of the cell cycle were increased by 14% (*P*<0.05) and 18% (*P*<0.05) at 48 and 72 h, respectively, after isoflurane exposure when compared with control at the corresponding time point. Ki67, cyclin D1, and VEGF expression were increased by 12% (*P*<0.05), 19% (*P*<0.001), and 11% (*P*<0.001), respectively, at 24 h post-exposure. Our data suggest that, at a clinically relevant concentration, isoflurane increased the proliferation and angiogenesis of ovarian cancer cells. Our study suggests that inhalation agents such as isoflurane may contribute to cancer recurrence after surgery under general anaesthesia.

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**References**