Comparison of the effects of four i.v. anaesthetic agents on polymorphonuclear leucocyte function

J. A. H. DAVIDSON, S. J. BOOM, F. J. PEARSALL, P. ZHANG AND G. RAMSAY

Summary

Initial resistance to bacterial infection is mediated primarily by polymorphonuclear leucocytes (PMN). Anaesthetic agents have been reported to impair various aspects of PMN function. It is possible that the use of these agents to sedate critically ill patients may further compromise an already depressed host defence mechanism. A flow cytometric technique with fresh whole blood from 10 healthy volunteers was used. Phagocytic and respiratory burst activity of PMN incubated for 1 h with either propofol, thiopentone, midazolam or ketamine at both clinical plasma concentrations and 100 times this concentration were determined. Thiopentone at the higher concentration reduced both respiratory burst activity (mean peak channel 50.7 compared with control value of 77.6 ($P < 0.0001$)) and phagocytosis (mean peak channel 47.5 compared with 79.9 ($P < 0.0001$)). Ketamine at 100 times the clinical plasma concentration also reduced respiratory burst and phagocytosis, but this failed to reach statistical significance ($P = 0.10$ and $P = 0.053$, respectively). No significant depression occurred in the other groups. The results suggest that these i.v. anaesthetic agents, at clinically relevant concentrations, have minimal effects on PMN phagocytosis and oxygen free radical production. At higher concentrations thiopentone and ketamine may affect phagocytic function and thiopentone may impair intracellular cytolytic activity. (Br. J. Anaesth. 1995; 74: 315-318)

Key words

Anaesthetic techniques, i.v. Blood, leucocytes.

Initial resistance to bacterial infection is mediated primarily by polymorphonuclear leucocytes (PMN). The PMN cellular response to microbial invasion may be classified into chemotaxis, adherence, phagocytosis and intracellular killing. A disorder of any of these PMN functions may allow bacterial infection to develop. Many normal functions of the immune system are depressed after exposure to the combination of anaesthesia and surgery. However, the contributory role of anaesthetic agents to impairment of the immune system is poorly understood. Although it would appear that many of the immune changes that occur in surgical patients are primarily the effect of surgical trauma and endocrine response, it is important to assess the influence of different anaesthetic agents on the immune response.

The inhibitory effect of some anaesthetic agents on PMN function has been well documented [1, 2]. This may result not only in perioperative immunosuppression, but if used to sedate critically ill patients, may potentially further compromise an already depressed host defence mechanism.

In this study we have compared the effect of four i.v. anaesthetic agents, propofol, thiopentone, midazolam and ketamine, on PMN phagocytosis and production of oxygen free radicals (respiratory burst activity) which is the main method of bacterial killing by PMN [3].

Materials and methods

Whole venous blood, obtained from 10 healthy volunteers, was incubated for 1 h with propofol, thiopentone, midazolam or ketamine at both clinical concentrations [4-7] and at 100 times this concentration (table 1). Using a whole blood technique, the phagocytic and respiratory burst activity of PMN from each sample were determined by flow cytometry [8, 9].

PREPARATION OF BACTERIA

*Staphylococcus aureus* (strain NCTC 8532) was grown in a tryptic soy broth (Difco, Detroit, MI, USA) at 37 °C and colonies were harvested at 24 h. The bacteria were heat-inactivated (65 °C for 30 min), washed three times in normal saline and labelled with a 5% solution of propidium iodide (Sigma, St Louis, MO, USA) for 30 min. The

<table>
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<th>Table 1</th>
<th>Concentrations of i.v. anaesthetics used in the study</th>
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<tr>
<td></td>
<td>Therapeutic plasma concentration</td>
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<tr>
<td>Propofol</td>
<td>5 µg ml⁻¹</td>
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<tr>
<td>Midazolam</td>
<td>150 ng ml⁻¹</td>
</tr>
<tr>
<td>Thiopentone</td>
<td>20 µg ml⁻¹</td>
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<tr>
<td>Ketamine</td>
<td>3 µg ml⁻¹</td>
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fluorescent bacteria were washed and resuspended in phosphate-buffered saline containing glucose 5 mmol litre⁻¹ and 0.1% gelatin (PBSg). Bacterial density was adjusted using a spectrophotometer to an absorbance value of 2.5 at 620 nm, equivalent to approximately 2.4 x 10⁵ colony forming units ml⁻¹. Aliquots were stored at −80°C and thawed immediately before use.

PREPARATION OF SAMPLE FOR MEASUREMENT OF PHAGOCYTOSIS

Phagocytic function was determined by measuring the ability of cells incubated for 1 h with each anaesthetic agent to phagocytose propidium iodide-labelled Staphylococcus aureus. Propidium iodide-labelled Staphylococcus aureus suspension (900 µl) was added to 100 µl of heparinized whole blood (10 u.ml⁻¹) that had been preincubated with the anaesthetic agent. Samples were then prewarmed at 37°C in a water bath and incubated with rotational agitation for 30 min. Ethylenediaminetetraacetic acid (EDTA) 3 mmol litre⁻¹ (2 ml) in PBSg was added to terminate phagocytosis. Samples were then centrifuged at 100 g for 5 min and the supernatant removed. Ice cold distilled water (2 ml) was added to the sample for 20 s to lyse the red cells. EDTA 6 mmol litre⁻¹ (2 ml) in PBSg was added to restore isotonicity and the sample again centrifuged (100 g for 5 min) and the supernatant removed. The sediment was resuspended in EDTA 3 mmol litre⁻¹ in PBSg in readiness for flow cytometry.

MEASUREMENT OF RESPIRATORY BURST ACTIVITY

During phagocytosis there is a marked increase in oxygen consumption and glucose oxidation. The biochemical basis of this respiratory burst is activation of a membrane-bound pyridine nucleotide-dependent oxidase that produces superoxide. The burst is followed by the formation of hydrogen peroxide via the action of the enzyme superoxide dismutase.

Respiratory burst activity may be determined by a quantitative assay of hydrogen peroxide produced by PMN, utilizing the fluorochrome 2',7'dichloro-fluorescein diacetate (DCFH-DA) and flow cytometric techniques [8]. DCFH-DA (a stable non-fluorescent compound) diffuses into the cells and is hydrolysed to 2',7'dichloro-fluorescein (DCFH). Intracellular DCFH in PMN is rapidly oxidized to highly fluorescent 2',7'dichloro-fluorescein (DCF) which emits a green fluorescence in the presence of hydrogen peroxide [10]. The reaction is quantitatively related to hydrogen peroxide concentration [8] and as DCF is a polar fluorescent compound, it remains trapped within the cell. Therefore, it is possible to detect DCFH oxidation in PMN which can be used as a measure of hydrogen peroxide concentration and flow rate being adjusted to achieve a sequential flow of single cells sufficiently spaced so that each cell is measured individually. In the sensing region the cells intersect the light beam originating from an argon laser, where the excitation wavelength can be varied [12].

As the focused light hits each cell it is scattered, absorbed or emitted as fluorescence. A fraction of the light is then collected and transmitted through filters to photomultipliers. The flow cytometer was calibrated daily to ensure standard laser intensity and alignment using commercially available products.
These trains of pulses of varying amplitudes are presented to the pulse height analysing circuit. Each pulse is measured individually and the total number at each digital measurement increment is stored in its appropriate channel. All intensities of cellular fluorescence were registered within the scale of the 128-channel resolution using the photomultiplier gain setting. This information was displayed as a histogram (fig. 1).

Lymphocytes, monocytes, a few contaminating erythrocytes, aggregated cells and debris were excluded from the analysis using combined and low angle forward light scatter, 90° angle light scatter and gate analysis methods.

When a single population of cells was present in the cyttofluorograph, mean DCF and propidium iodide fluorescence per cell was determined by the following equation: total (PMNL counts on each channel x channel number)/total PMNL count. Intracellular fluorescence was determined using 488 nm excitation. Red fluorescence from propidium iodide was collected through a 620-nm long pass filter and green fluorescence from DCF through a 530-nm band pass filter.

Red blood cell counts were within normal limits and therefore no correction was made for variation in erythrocyte count, which in previous studies had been shown to affect the interaction of bacteria and PMN.

Results are expressed as mean channel fluorescence per cell (minimum of 3000 cells), and groups were compared by t test.

Results
Thiopentone at the higher concentration significantly reduced both respiratory burst activity (mean peak channel 50.7 compared with control value of 77.6 (P < 0.0001)) and phagocytosis (mean peak channel 47.5 compared with control of 79.9 (P < 0.0001)). Ketamine at 100 times the clinical plasma concentration also reduced respiratory burst and phagocytosis, but this failed to reach statistical significance (P = 0.10 and P = 0.055, respectively). No significant depression was seen in the other groups (figs 2, 3).

Discussion
Neutrophils play a central role in the antibacterial host defence mechanism. Under normal circumstances the host response to bacteria that cross endothelial and epithelial barriers involves recruitment of neutrophils to the sites of bacterial colonization and invasion followed by microorganism ingestion and destruction. Interference with the normal equilibrium between bacteria, environment and host defence mechanisms may render the individual more susceptible to infection. This may be particularly relevant in the critically ill or intensive care patient where there may be an existing disequilibrium as a result of enhanced bacterial pathogenicity and deficiencies in host defences. Moreover, as anaesthetists treat increasing numbers of patients with AIDS and other immunodeficiency syndromes, careful clinical research is warranted to determine factors that may exaggerate postoperative immuno-suppression.

In this study we have demonstrated that at 100 times the clinical free plasma concentration, thiopentone and ketamine affected phagocytic function and thiopentone impaired intracellular cytolyis. Our results suggested that propofol, thiopentone, midazolam and ketamine, at clinically relevant concentrations, had minimal effects on PMN phagocytosis and oxygen free radical production.

There have been many in vivo and in vitro observations on the effect of anaesthetic agents on human immune systems. These often detail conflicting results. Several investigators have found an in vitro decrease in PMN free radical production [13–15] and a decrease in phagocytosis in vivo [16, 17] and in vitro [18–20] in PMN exposed to anaesthetic agents. Other studies have not confirmed these changes in PMN functional variables [21–26]. As a result of such contradictory data, useful conclusions have been difficult to obtain. These differences may be methodological.

In vitro studies would appear more relevant, in
that they represent events that actually occur in the human immune system. However, the primary difficulty of separating the effects of multiple intraoperative factors that may interfere with the immune response from the direct effects of anaesthetics themselves remains. In addition, counting the drug washout effect during cell preparation from anaesthetized patients is difficult. A variety of methodological and theoretical problems also affect in vitro work, including the questionable relevance of results from in vitro testing on purified cells to in vivo clinical events, the difficulty of purifying PMN without altering their native function and the difficulty in preserving PMN subsets in culture for prolonged periods. Indeed, when comparing the most recent in vitro data on the immune effects of anaesthetics with earlier studies, several areas of disagreement can be related to the dramatic increase in immunological in vitro technology in the past decade [1].

In this study we have endeavoured to use a relatively new technique to determine the effect of i.v. anaesthetic agents on PMN function. This whole blood technique allows PMN to be exposed to known concentrations of drug while remaining within the normal milieu, with PMN only being processed and separated for analysis by flow cytometry once phagocytosis—respiratory burst activity have been terminated. Consequently, we believe that this in vitro technique is more representative of an in vivo effect than methods which separate the PMN before exposure to anaesthetic agents.

In conclusion, in this study we have demonstrated that at clinically relevant concentrations, propofol, thiopentone, midazolam and ketamine had little effect on PMN phagocytosis and free radical production in PMN from ASA I individuals. The effect on patients who may have depressed neutrophil function, for example the septic patient [27], may be different. Moreover, although the cells were exposed to the anaesthetic agents for a period in excess of 90 min, the effect of prolonged exposure to these concentrations, for example during sedation in the intensive care unit, may also be different.

References