An investigation into the effects of midazolam and propofol on human respiratory cilia beat frequency in vitro

Abstract

Objective: Patients in intensive care are known to be prone to both upper and lower respiratory tract infection. Respiratory mucus forms a barrier to infection. Mucus transport rate (MTR) depends upon both the physical properties of mucus and the action of respiratory cilia. Patients undergoing anaesthesia are known to have a reduced MTR that may be related to a depressant effect on cilia beat frequency (CBF) by anaesthetic drugs. The aim of this study was to investigate the effects of two commonly used intensive care sedative agents, midazolam and propofol, on CBF using human nasal turbinate explants in vitro.

Design: We exposed ciliated tissue from human nasal turbinate explants to midazolam and propofol in supra-clinical concentrations (20 μM midazolam and 70 μM propofol) in a controlled and blinded manner for 90 min and measured CBF by the transmitted light technique.

Results: After 90 min, mean (SEM) CBF in the group exposed to midazolam and its control group were 13.0 (0.2) Hz and 12.9 (0.3) Hz, respectively. Mean (SEM) CBF in the group exposed to propofol was 13.6 (0.4) Hz and in the control group the value was 12.0 (0.6) Hz. There was no significant change in CBF (midazolam: p = 0.21, propofol: p = 0.31, MANOVA for repeated measures).

Conclusions: We have found no effect of midazolam or propofol in supra-clinical concentrations upon CBF in human turbinate explants after a 90-min exposure. This contrasts with previous work that has shown a depressant effect of inhalational anaesthetic agents on CBF.

Key words Lung, pathophysiology · Hypnotic benzodiazepines, midazolam · Anaesthetics i.v., propofol

Introduction

Basal atelectasis and chest infection are recognized complications of general anaesthesia [1] and intensive care [2]. In the intensive care unit (ICU), nosocomial respiratory infection is particularly problematic. Acute bacterial sinusitis may also occur and has been reported following searches for the cause of occult sepsis in the orally intubated ventilated patient [3]. While impaired coughing contributes significantly to the incidence of lower respiratory tract infection, other factors may also prove important. The major physical barrier to airway infection is the continuous flow of mucus, which traps organisms and debris. Mucus passes from the distal pulmonary tree proximally and from the nasal sinuses and nasal cavity posteriorly, both routes terminating in the pharynx where the mucus is swallowed. Some inhalational and intravenous anaesthetics have been shown to reduce mucus transport rate (MTR) [4, 5]. MTR has been shown to be reduced in the post-operative period [1]. MTR is dependent upon the physical properties of the mucus (amount, viscosity and elasticity) and on the
function of cilia of the epithelial cells. The cilia beat in a coordinated fashion sweeping the mucus along their surface. In our laboratory, it has been demonstrated using nasal turbinate explants that ciliary activity can be maintained in vitro for up to 10 days [6]. It has also been shown that volatile anaesthetic agents reduce ciliary beat frequency (CBF), the effect persisting for 60–90 min after the agent is washed out, eventually reverting to basal levels [6]. To facilitate intermittent positive pressure ventilation in intensive care it is often necessary to administer sedative drugs. The aim of this study was to examine the effects of midazolam and propofol on human respiratory CBF in vitro. Any depressant effect may contribute to the increased incidence of respiratory tract infection in sedated patients.

Materials and methods

Tissue preparation

Following ethical committee approval and informed patient consent, nasal turbinates were obtained from 16 healthy patients undergoing routine turbinectomy for a primary diagnosis of tissue hypertrophy, with no history of allergy. The method of ciliated disc preparation has been previously described by Raphael and colleagues [6]. The turbinates were transferred to the laboratory in sterile 199 media (GIBCO BRL, Life Technologies, Paisley, UK) within 1 h of collection. Ciliated discs were cut from the turbinate using a sterile 4-mm biopsy punch and gently agitated with a sterile pipette to remove any mucus. Areas of biopsy were chosen from flat clean surfaces that appeared healthy to the naked eye, it was assumed that a representative sample was taken. The discs were stored overnight at 37°C in a humid atmosphere of 5% CO₂ in air; previous work in our laboratory has shown that ciliary function is maintained under these conditions [6]. Discs with visible cilia were then mounted into specially designed, thermostatically controlled, twin perfusion chambers [7]. The chamber allows microscopic examination under controlled conditions and was connected to a reservoir containing 300 ml of Hank’s Balanced Salt Solution (HBSS, Sigma Chemical, Dorset, UK) which flowed through the chamber using a gravity feed system.

Drug exposure

Two chambers were used at any one time, one for drug exposure and the other acted as a matched control. The investigator was blinded to which chamber received the study drug. Four ciliated discs were selected and mounted into the perfusion chambers. Two discs were placed into a chamber, which was to be perfused with the drug-containing media and the remaining two discs were mounted into the second chamber. Initially both chambers were connected to the control reservoir containing HBSS and the media was maintained at a flow rate of 0.5 ml min⁻¹. The chambers were maintained at 37±0.1°C by use of an integral heating element. After a 30-min equilibration period, a set of baseline CBF readings was made on each chamber (t₀). Subsequently, one chamber was disconnected from the control reservoir and connected to a reservoir containing the drug being investigated. A concentration of 20 μM midazolam was made by the addition of midazolam maleate powder (Roche Products, Hertfordshire, UK) to HBSS. A solution of 70 μM propofol was made by solubilizing the pure drug (Zeneca Pharmaceuticals, Cheshire, UK) in dimethyl sulphoxide (DMSO, Sigma Chemical, Dorset, UK), which was then diluted 2500-fold in HBSS. Glass containers were used throughout. The propofol control solution contained an equal concentration of DMSO. The chambers were then perfused from the control reservoir for a final period of 30 min and a final set of CBF readings was taken (tₙ). The timings of the readings and the experimental conditions during each set of readings are summarized in Table 1.

Table 1 Summary of the time course of experimental readings and perfusion conditions present

<table>
<thead>
<tr>
<th>Reading number</th>
<th>Time point (min)</th>
<th>Perfusion conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>t₃₀</td>
<td>Baseline readings. Both chambers are perfused from the same drug-free reservoir</td>
</tr>
<tr>
<td>2</td>
<td>t₆₀</td>
<td>One chamber has been perfused with the drug for 30 min</td>
</tr>
<tr>
<td>3</td>
<td>t₁₂₀</td>
<td>The chamber receiving the drug has now been perfused with the drug for 90 min</td>
</tr>
<tr>
<td>4</td>
<td>t₁₅₀</td>
<td>Washout readings. Both chambers are again perfused from the same drug-free reservoir. The chamber which received the drug has been washing out for 30 min</td>
</tr>
</tbody>
</table>

CBF measurement

CBF measurement using a photosensitive cell was first described in 1962 [8]. We have used television-video modification of the transmitted light technique [9], the apparatus used in our laboratory has been validated and described previously [7, 10]. Using a Nikon Diaphot 300 microscope (Nikon UK, Surrey, UK) with a × 60 objective lens and a × 10 eyepiece lens, a ciliated edge was located using a Panasonic VW-CL 110-AE video camera (Quadrant Video Systems, Nottingham, UK). The ciliated epithelium was displayed on a high resolution Sony KX-14CP video monitor (Quadrant Video Systems). A pinhole photodiode was placed against the image of the ciliated edge and the signal obtained was displayed on a Gould 20 MHz cathode ray oscilloscope (Gould Instruments Systems, Essex, UK). Once a suitable signal had been obtained, this was downloaded to a Dell 386sx computer (Dell Computer, Berkshire, UK), after having been subjected to low pass filtering with 25 Hz cut-off, to reduce interference from the television monitor. The signal was then sampled for 15 s at 200 Hz by an analogue-to-digital converter, stored and displayed. The signals were then transferred to a RM centra V466 computer (Research Machines, Oxon, UK), where they were analysed using Mathematica 2.2, which provided a power spectrum using fast Fourier transformations. The CBF was taken to be the peak of the power spectrum, which represented a mean value derived from the signal [10].

All edges used for CBF measurement were continuous, at least 60 μm long and free of mucus. A simple diagram of the location of the ciliated edges on the discs was constructed to ensure that the same edges were analysed at all time points during the experiments – this was to reduce any error due to inter-edge variation in