Hydrogen peroxide at a concentration used during neurosurgery disrupts ciliary function and causes extensive damage to the ciliated ependyma of the brain

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ABSTRACT

Objectives: Hydrogen peroxide (H_2O_2 : 3% w/v (1.1M)) has been used as a haemostatic agent during neurosurgery applied to both the external and ventricular surface of the brain. We hypothesised that H_2O_2 would be toxic to the ciliated ependyma, a single layer of cells, that separates cerebrospinal fluid from the neuronal tissue of the brain.

Methods: The effect of H_2O_2 was assessed by determining ependymal ciliary beat frequency (CBF) using high speed video analysis and ultrastructure by electron microscopy (EM).

Results: Brief exposure to H_2O_2 caused cessation of ciliary beat frequency and extensive damage of the ependyma.

Conclusions: Damage to the ciliated ependyma is of concern as regeneration following damage is very poor and if breached underlying neuronal tissue and a population of neuronal progenitor cells that lie immediately beneath may also be exposed to H_2O_2 .

Keywords: Hydrogen peroxide; haemostasis; ependyma; cilia; neurosurgery.

INTRODUCTION

A single layer of ciliated ependymal cells line the ventricles of the brain and the cerebral aqueducts separating cerebrospinal fluid from the neuronal tissue and underlying neuronal stem cell populations [13]. The role of the ependymal cells has been reviewed in detail [3] but the importance of their cilia, that beat continuously at approximately 37 Hz [2, 18] is just emerging. Recent work suggests that CSF movement by ependymal cilia establishes a concentration gradient of CSF guidance molecules that direct the migration of neuroblasts to various regions of the brain [15]. Ependymal cilia are also thought to play a role in host defence keeping the surface of the brain clear of debris. Abnormal ciliary function is associated with the development of ventricular dilatation and hydrocephalus [1, 6, 9, 12]. For example, intracerebroventricular antisense knockdown of *G_i2* results in ciliary stasis and ventricular dilatation in rats [12]. Metavanadate, an inhibitor of ciliary movement, also induces hydrocephalus in rats [14]. Small animals with primary ciliary dyskinesia (PCD) commonly develop hydrocephalus and hydrocephalus is also occasionally seen in humans with PCD [9, 10]. It is known that bacteria and their toxins adversely affect ciliary function [4, 5, 11] and it has been shown that viral or bacterial infection of the CSF, in animal models, may cause ependymal damage and hydrocephalus [7, 8].

Hydrogen peroxide (3% w/v) has been applied to the ventricular surface of the brain to achieve haemostasis during the neurosurgical removal of tumours. We have previously studied in detail the effects of *S. pneumoniae* on the ciliated brain ependyma and found that H_2O_2 released from the bacteria caused damage to the ependymal layer. The level of H_2O_2 produced by 10^8 cfu/ml *S. pneumoniae* (severe pneumococcal infection) was approximately 100 μ M. A 15 minute exposure to this concentration caused an a reduction in ependymal ciliary beat frequency [17]. By measuring both ciliary beat frequency and ultrastructure using scanning electron microscopy, we tested the hypothesis that a brief exposure to 3% w/v H_2O_2 , would cause damage to the brain ependyma from the 4th ventricle and inhibit ciliary function.

METHODS

The brains of Wistar rats (14-17 days old) were removed following cervical dislocation. Their cerebellum was mounted on a vibratome under ice-cold M199 medium containing penicillin (100IU/ml), streptomycin (100 μ g/ml). The brain was sliced (250 μ m) through the medulla oblongata and pons into the floor of the fourth ventricle so that the ciliated V shaped floor was visible. The slices were mounted in 2ml of pre-warmed (37°C) M199 medium prior to use. To determine CBF the brain slices were placed in a humidified (80-90%) incubation (37°C) chamber and observed using an inverted microscope system (Diphot, Nikon, UK). Tissue was allowed to equilibrate for 30 minutes prior to exchange of pre-warmed (37°C) M199 or M199 containing 3% w/v H_2O_2 . The media were exchanged once again after 1 minute. Beating cilia were recorded using a digital high-speed video camera (Kodak Ektapro Motion Analyser, Model 1012) at a rate of 400 frames per second, using a shutter speed of 1 in 2000. The camera allowed video sequences to be recorded and played back at reduced frame rate or frame by frame. CBF was determined by timing a given number of individual cilia beat cycles. Each time point represents the measurement of 4 individual cilia from each slice. Calculation of CBF (Hz): $400 \text{ (Number of frames per second)} / 5 \text{ (frames elapsed for 5 ciliary beat cycles)} \times 5 \text{ (conversion per beat)}$

cycle). For scanning electron microscopy, the brain slices were fixed in Sorensens phosphate buffered (pH 7.4) glutaraldehyde (4% w/v). After post-fixation in osmium tetroxide (1% w/v), samples were dehydrated through graded ethanols and immersed in hexamethyldisilazane (HMDS). The HMDS evaporated leaving dry tissue with no phase boundary damage (damage caused to the tissue by pressure differences of the air/liquid interface).

RESULTS

Prior to addition of H_2O_2 the basal ciliary beat frequency, of the control and treatment group, was 36.2 ± 2.2 Hz and 36.7 ± 1.1 Hz respectively (Figure 1). After the addition of the H_2O_2 the ciliary beat frequency decreased rapidly and no cilia were still beating after one minute (Figure 1). No change in the ciliary beat frequency of the control was seen during this time (Figure 1). On electron microscopy widespread destruction of the ependymal layer was seen compared to control brain slices (Figure 2A and 2B). It was difficult to identify cilia and gaps in the ependymal were seen, exposing underlying neuronal tissue. Removal of H_2O_2 by washing the tissue failed to restore ciliary beat frequency of the very few remaining cilia that could be identified.

CONCLUSION

We have shown for the first time that very short exposure to concentrations of H_2O_2 that have been used during neurosurgery to achieve haemostasis, causes profound damage to the ciliated brain ependyma and that the effect on cilia was not reversible. Significant gaps were also seen in the ependyma exposing underlying neuronal tissue. It is highly likely that the damage to the ependyma during its use in patients also exposes underlying neuronal and stem cell populations [13] to H_2O_2 . Vallfors and colleagues have shown that 3% w/v H_2O_2 can cause damage to the brain, including, thrombosis of leptomeningeal vessels, blood brain barrier disturbance and injury to mesothelial cells of the subdural and arachnoid surfaces [16, 17].

Hydrogen peroxide has not been used by all neurosurgeons to achieve homeostasis, therefore, given the damage seen in this study and that seen by Vallfors and colleagues, the use of H_2O_2 is used as a haemostatic agent during neurosurgery should be questioned. Ideally the functional *in-vivo* significance of our findings should be examined in a clinical study designed to examine the neurological sequelae of patients who have been exposed to H_2O_2 during neurosurgery.

FIGURE LEGENDS

Figure 1

Rapid inhibition of ependymal CBF caused by brief exposure to H_2O_2 . Control (■) and H_2O_2 (3% □) had media exchange after 1 minute (arrow). Data are mean $\pm$ s. e. mean of 4 independent experiments.

Figure 2A and 2B

The effect of 3% H_2O_2 on the floor of the 4th ventricle. The ciliated ependyma is clearly seen in control slice (A). However, when treated with 3% H_2O_2 for 1 minute (B), the ordered ultrastructure of the ciliated ependymal layer is grossly disturbed. No individual cilia were seen and large spaces were present between the cells exposing underlying neuronal tissue. The bar is 6.5 μm . Images are representative of the four independent experiments.

Competing interests

None

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