

# Therapeutic efficacy of topical application of dexamethasone to the round window niche after acoustic trauma caused by intensive impulse noise in guinea pigs

F-L CHI<sup>1</sup>, M-Q YANG<sup>1</sup>, Y-D ZHOU<sup>2</sup>, B WANG<sup>1</sup>

<sup>1</sup>Department of Otorhinolaryngology, Eye Ear Nose and Throat Hospital of Fudan University, and <sup>2</sup>Department of Otorhinolaryngology, Changhai Hospital, Second Military Medical University, Shanghai, China

## Abstract

**Objective:** To assess the therapeutic efficacy of dexamethasone administered topically to the round window niche, following acoustic trauma induced by intensive impulse noise, in guinea pigs.

**Methods:** Adult, male, albino guinea pigs with a normal Preyer's reflex were exposed to 80 impulse noises (peak value 167 dB, duration 0.5 ms, interval 2 s). Dexamethasone (40 mg/ml) or saline was then topically applied to the round window niche. Each animal's auditory brainstem response was measured before and one day after exposure, and three weeks after topical treatment. Cochlear morphology was examined to assess hair cell loss and spiral ganglion cell damage. To assess oxidative activity, cochlear malondialdehyde and superoxide dismutase concentrations were determined three weeks post-treatment. Following topical application, the pharmacokinetic characteristics of dexamethasone in cochlear perilymph were analysed using high-performance liquid chromatography.

**Results:** Animals receiving dexamethasone showed reduced noise-induced outer hair cell loss (three weeks post-treatment), and significant attenuation of noise-induced auditory brainstem response threshold shifts (one day post-exposure and three weeks post-treatment), compared with controls. There was no difference in spiral ganglion morphology. Animals receiving dexamethasone also showed a significantly lower malondialdehyde concentration and a higher superoxide dismutase concentration, post-exposure. Following topical application, the perilymph dexamethasone level peaked at 5330.522 µg/ml (15 minutes post-treatment), and was 299.797 µg/ml 360 minutes later.

**Conclusion:** Topical application of dexamethasone to the round window niche has protective effects against intensive impulse noise induced trauma in the guinea pig cochlea. This drug can diffuse into the inner ear through the round window membrane and persist in the perilymph for a relatively long period. The mechanism of protection may involve an anti-oxidant effect.

**Key words:** Hearing Loss, Noise-Induced; Therapeutics; Dexamethasone; Model, Animal

## Introduction

Blast deafness is acute acoustic trauma induced by discontinuous impulse noise or an intense explosion. The inner ear is injured due to the large change in the apical peak of the frequency spectrum induced by the intense noise.<sup>1</sup> The auditory brainstem response (ABR) threshold shift shows a process of slow-moving recovery after such trauma, which may be related to noise-induced cochlear oedema. In the early phase following such injury, scanning electron microscopy has identified oedema of the cuticular plate, collapse of the stereocilia of the inner and outer hair cells, and significant oedema of the hair cells; in the late phase, ciliary loss and hair cell death are seen.

Blast deafness can occur due to warfare, military training, mine explosions and powerful thunder. Research into the mechanisms, prevention and treatment of blast deafness is important in order to facilitate improved hearing protection.

Two general mechanisms are responsible for blast deafness: mechanical<sup>2</sup> and metabolic.<sup>3</sup> Mechanical damage occurs when movement of the basilar membrane is excessive, inducing detachment of the tectorial membrane, disconnection of the interciliary bridges and even rupture of the basilar membrane. Metabolic disorders have multiple origins: (1) ionic, involving an intracellular influx of potassium or calcium; (2) ischaemic; (3) excitotoxic, following an excessive

release of glutamate; and (4) via the production of cochlear free radicals, which cause cell damage.

An important factor in blast deafness is the increased concentration of reactive oxygen species (such as aldehyde groups (e.g. malondialdehyde), ketones and hydroxide radicals), which result in damage to hair cells and spiral ganglion cells.<sup>4–6</sup> Although it is unclear how noise induces the accumulation of reactive oxygen species, this may be related to a reduction in cochlear anti-oxidase activity. The concentration of malondialdehyde may reflect the degree of lipid peroxidation and thus indirectly indicate the extent of cell injury. The intra-cochlear malondialdehyde level increases after blast injury and decreases with prednisolone treatment in guinea pigs *in vivo*.<sup>7–9</sup> Superoxide dismutase is the body's first line of defence against free radicals, and its concentration reflects the body's ability to clear reactive oxygen species.<sup>10</sup>

In humans, glucocorticoids are used extensively in the management of inner ear pathology, such as sudden sensorineural hearing loss,<sup>11</sup> autoimmune inner ear disease<sup>12</sup> and Ménière's disease.<sup>13</sup> Endogenous glucocorticoids have protective effects against noise-induced deafness.<sup>14</sup> Prednisolone has been shown to have a protective effect against blast injury in guinea pigs.<sup>15</sup> When used to treat inner ear disorders, glucocorticoids are typically administered systemically.<sup>16,17</sup>

Intratympanic administration<sup>18</sup> allows a higher local concentration of medication.

Animal studies have involved continuous injection of drugs via a miniature osmotic pump.<sup>15,19</sup> In the current study, we administered dexamethasone via the post-aural route through the round window niche. Although dexamethasone is commonly used clinically at a concentration of 4 mg/ml, our study used 10 times that concentration. The pharmacokinetics of dexamethasone were assessed and its therapeutic effect on blast deafness evaluated, in order to investigate its mechanism of action against lipid peroxidation in this clinical setting.

## Materials and methods

### Animals and materials

This study used 74 adult, male, albino guinea pigs (supplied by the Experimental Animal Center, Shanghai, China) weighing approximately 250 g, with a normal Preyer's reflex.

All experimental procedures, including the care and use of animals, were approved by the laboratory animals department, Shanghai Medical College, Fudan University.

Dexamethasone 21-phosphate disodium salt (Sigma–Aldrich, Santa Clara, California, USA) was prepared at a concentration of 40 mg/ml in sterile water.

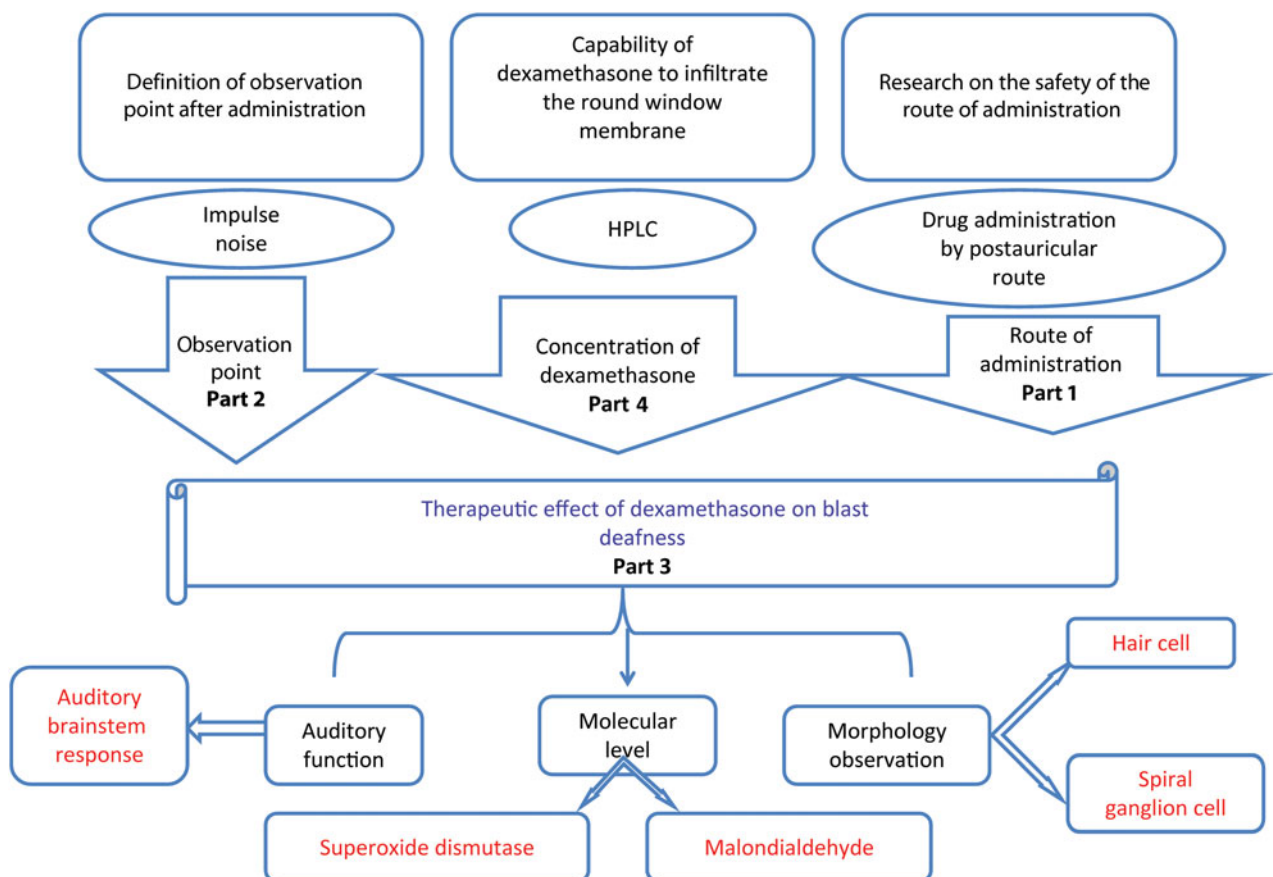


FIG. 1

Outline of the experimental design. HPLC = high-performance liquid chromatography



FIG. 2  
Apparatus used for impulse noise exposure.

### Experimental protocol

The experimental protocol for this study is shown in Figure 1. The experiment was divided into four parts.

In part one (i.e. preliminary experiment one), 10 guinea pigs were randomly divided into two groups: a saline group ( $n = 5$ ) and a dexamethasone group (40 mg/ml,  $n = 5$ ).

In part two (i.e. preliminary experiment two), another 10 animals were randomly divided into two groups: a noise plus saline group ( $n = 5$ ) and a noise plus dexamethasone group ( $n = 5$ ).

In part three (i.e. experiment one), 30 guinea pigs were randomly divided into two groups: a noise plus saline group ( $n = 15$ ) and a noise plus dexamethasone group ( $n = 15$ ).

In part four (i.e. experiment two), 24 guinea pigs were divided into eight groups of three each. Dexamethasone was administered via the round window niche; perilymph was then sampled 15, 30, 60, 120, 180, 240, 300 and 360 minutes afterwards.

### Noise exposure

Within an anechoic room, the animals were exposed to acoustic trauma comprising 80 intense impulse noise events created using an electric spark generator (D-86: Jiangwan 1st Factory, Shanghai, China; Figure 2). Guinea pigs were placed in a custom-made frame 10 cm from the noise source. Impulse noise

events had a peak value of 167 dB SPL, with a 2 second pulse interval and 0.5 millisecond pulse width.

### Surgical procedure

The surgical procedure was performed one day after noise exposure, and is illustrated in Figure 3.

Before surgery, animals were anaesthetised with an intramuscular injection of Xylazine (25 mg; Pharma Chemical Plant, Nanjing, China) and ketamine (2 ml, 0.0008 ml/g). In addition, they were injected subcutaneously around the incision site with 0.5 ml of 0.5 per cent lidocaine hydrochloride for local anaesthesia.

The bulla was exposed and opened under sterile conditions. A gelatin sponge soaked with dexamethasone (40 mg/ml) or saline was gently placed on the round window niche of each left cochlea. Dental cement was used to close the bulla. The animals were left in quiet conditions for at least 30 minutes.

### Auditory brainstem response measurement

In preliminary experiment one, ABR measurements were obtained before and one week after drug administration, to assess whether dexamethasone affected normal hearing. In preliminary experiment two, ABR was measured 6 hours after noise exposure and one, two, three and four weeks after drug administration, to determine the best point at which to assess the therapeutic effect of dexamethasone on blast deafness.

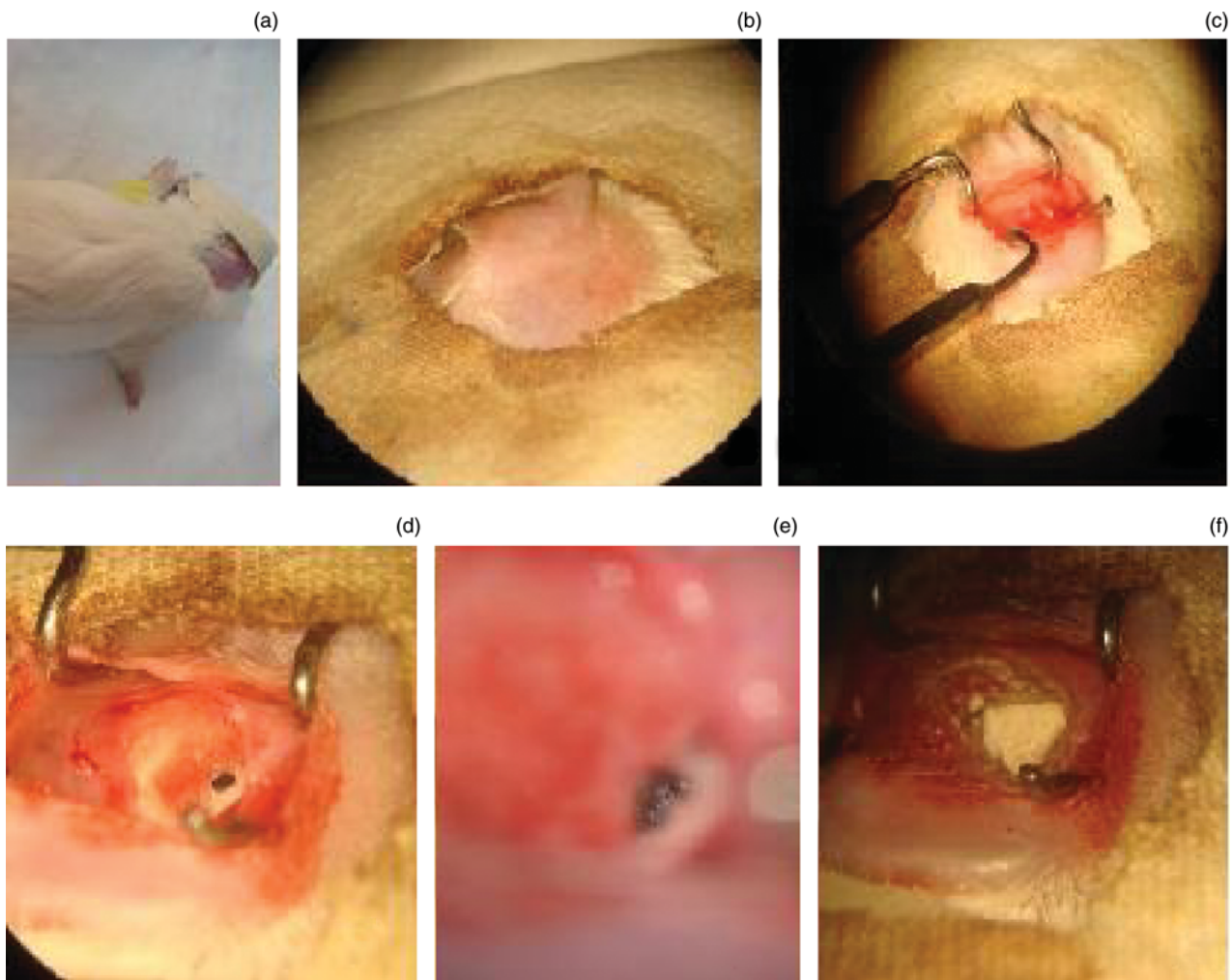


FIG. 3

The surgical procedure. (a, b) The guinea pig was anaesthetised generally and locally. (c, d) The bulla was exposed and opened under sterile conditions. (e) A gelatin sponge soaked with dexamethasone or saline was gently placed against the round window niche of each left cochlea. (f) Dental cement was used to close the bulla.

In experiment one, ABR measurements were obtained: before noise exposure (day 0), after noise exposure and before drug administration (day 1), and three weeks following drug administration (day 21). The first ABR measurement was performed to confirm normal hearing and to define baseline hearing for each animal. The threshold shift following drug administration was determined by comparing the ABR thresholds measured on day 21 versus day 1.

For ABR measurement, animals were anaesthetised using Xylazine 25 mg and ketamine 2 ml, 0.0008 ml/g. Stainless steel needle electrodes were placed subcutaneously at the vertex (the recording electrode), the left post-aurem (the ground electrode) and the right post-aurem (the reference electrode; see Figure 4). Animals were placed in an anechoic room and tones (0.1 millisecond click tone bursts, 13.3 times per second for repeated speed rate) were delivered to the left external auditory canal via tubes with adapters. Auditory brainstem responses were recorded with high and low pass filter settings of 150–1500 Hz. An average response was based on 500 sweeps, obtained

at 5 dB intervals. Thresholds were determined by visual inspection of wave III, at appropriate latency, verified three times (see Figure 5).

#### *Histological examination*

Histological examinations were performed in experiment one. Six animals were sacrificed after the third ABR measurement. After injection of pentobarbital sodium (2 mg/ml), the cochleae were quickly removed. A solution of succinate dehydrogenase (containing 0.272 g of 0.2 M potassium dihydrogen phosphate, 6.447 g of 0.2 M dibasic sodium phosphate, 5.403 g of 0.2 M sodium succinate and 0.2 g of 0.1 per cent nitroblue tetrazolium) was infused through each cochlear window. The cochleae were placed in this solution at 37°C for 50 to 55 minutes and then fixed in 10 per cent formaldehyde for more than 8 hours at 4°C. They were then dissected under a binocular optical microscope to remove the organ of Corti from the apex to the base. The samples, mounted on a histological slide in glycerin, were observed under an optical microscope (magnification  $\times 400$ ). The



FIG. 4

Electrode placement for auditory brainstem response measurement.

prevalence of missing outer hair cells and inner hair cells was calculated.

Four animals from both the noise plus saline group and the noise plus dexamethasone group in experiment one were sacrificed, after ABR measurement. After deep anaesthesia by injection of pentobarbital sodium (2 mg/ml), the cochleae were quickly removed. Ten per cent formaldehyde was infused through the cochlear windows. The cochleae were fixed in the formaldehyde for over 8 hours and then decalcified in 10 per cent ethylene diamine tetra-acetic acid for seven days with one change per day. The cochleae were subsequently trimmed, oriented in agar and embedded in paraffin. Cochleae were sectioned at 10 µm thickness, and mid-modiolar sections were mounted on slides and stained with haematoxylin and eosin. The final specimens were observed under an optical microscope (see Figure 6).

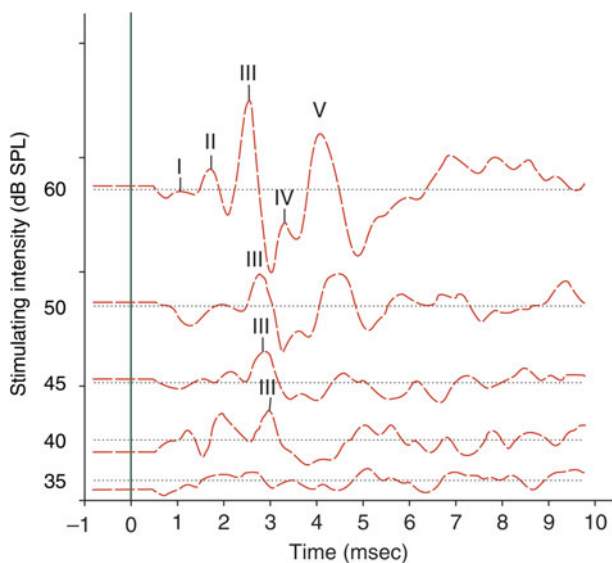


FIG. 5

Determination of auditory brainstem response thresholds by visual inspection of wave III.

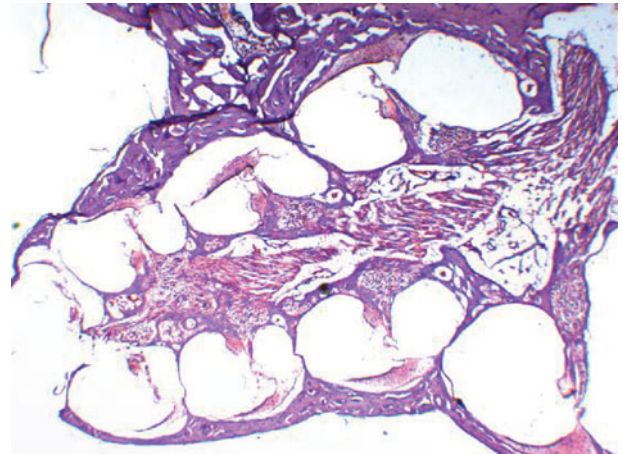


FIG. 6

Photomicrograph of spiral ganglion cells from experimental group one animal. (H&E; ×400)

*Malondialdehyde and superoxide dismutase concentration*

Fourteen animals from both the noise plus saline group and the noise plus dexamethasone group in experiment one were sacrificed after deep anaesthesia by injection of pentobarbital sodium (2 mg/ml), as were seven normal, control animals. The cochleae were quickly removed and placed into pre-cooled phosphate-buffered saline. After removing the cochlear wall, the basement membrane and central axis of the left cochlea in each group were weighed, placed in a 1.5 ml centrifuge tube and ground into a cochlear homogenate in a quantity of saline nine times the weight of the tissue. The homogenate was then centrifuged at 4°C and 2000 revolutions/minute for 10 minutes; the supernatant represented the 10 per cent cochlear homogenate.

The malondialdehyde and superoxide dismutase concentrations in the homogenate were determined as instructed by the Kid directions (Jiancheng, Nanjing, China).

*High-performance liquid chromatography*

Dexamethasone was dissolved in 100 ml artificial perilymph<sup>15</sup> to yield a 120 µg/ml stock solution. A comparator curve was calculated by diluting the dexamethasone stock solution in mobile phase to final concentrations of 60, 30, 15, 7.5 and 6 µg/ml dexamethasone.

Twenty-four animals were anaesthetised with pentobarbital sodium (2 mg/ml) and 40 mg/ml of dexamethasone was administered via middle-ear injection. After 15, 30, 60, 120, 180, 240, 300 and 360 minutes, the cochleae were washed three times with a total amount of approximately 1 ml water. The animals were then sacrificed, and perilymph samples were collected by surgically removing the cochlea, opening the ear vesicle and extracting the cochlear perilymph fluid using a micro-injector via the round window membrane. Samples were immediately frozen at -20°C.

Each cochlear perilymph fluid sample was taken from three cochleae.

High-performance liquid chromatography separation was conducted on a C18 column (4.6 × 150 mm, internal diameter 5 μm) at 20°C. The mobile phase was acetonitrile–ammonium sulphate (27:73 volume/volume) at a flow rate of 1.0 ml/minute, and the detection wavelength was 245 nm. The injection volume was 20 μl.

### Statistical analysis

Auditory brainstem response threshold shifts for the experimental and control groups were compared using the chi-square test. The chi-square test was also used to compare the prevalence of missing outer hair cells in each animal, and the cochlear malondialdehyde and superoxide dismutase concentration in the experimental versus control groups. Differences were considered statistically significant when the *p* value was less than 0.05.

## Results and analysis

### Auditory brainstem response

In preliminary experiment one, there was no significant difference in the ABR click threshold shift between the two groups, either before ( $t = 0$ ,  $p = 1.00$ ) or after ( $t = 0.63$ ,  $p = 0.54$ ) drug administration. This suggests that dexamethasone at a concentration of 40 mg/ml had no effect on normal guinea pig hearing (see Figure 7).

In preliminary experiment two, there was no significant difference in the ABR click threshold shift between the two groups one day after noise exposure.

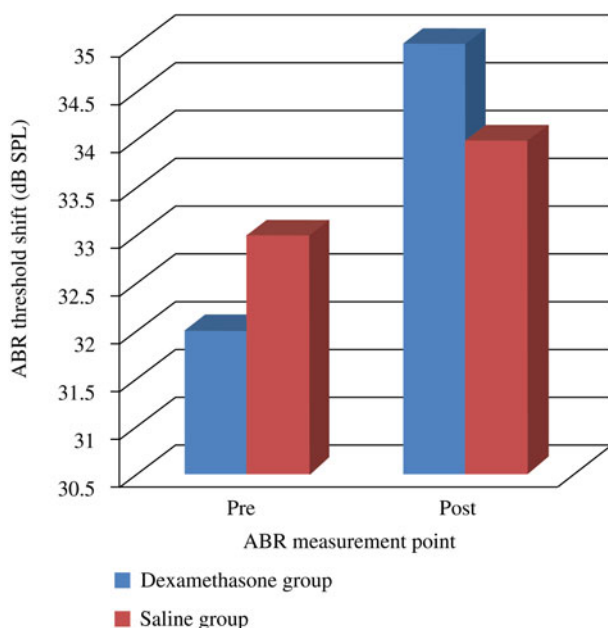


FIG. 7

Auditory brainstem response (ABR) click threshold shift observed in the dexamethasone and saline groups (preliminary experiment group one), before (pre) and after (post) treatment. No statistically significant difference was seen between the two groups at either time point.

The threshold shift of the ABR click threshold shift one week, two weeks, three weeks and four weeks after saline administration was not significant in the noise plus saline group. In the noise plus dexamethasone group, the ABR click threshold shift decreased gradually, comparing the one-, two- and three-week results; however, there was no obvious difference between results at three and four weeks. Therefore, three weeks post-administration was chosen as the observation point for the study (see Figure 8).

Figure 9 shows the ABR click threshold shift results for experimental group one before noise exposure, 1 day after noise exposure and three weeks after drug administration.

### Cochlear hair cell histology

In the experiment one animals, light microscopy revealed severe damage of the hair cells in the superior part of the basal turn and the inferior part of the second turn of the basal membrane; however, there was no damage or loss in the third and fourth turns. Basal membrane hair cell loss was more severe in the saline subgroup than in the dexamethasone subgroup. In the dexamethasone group, it was mainly the outer hair cells which were lost; few inner hair cells were lost (Figure 10a). In the saline group, few outer hair cells

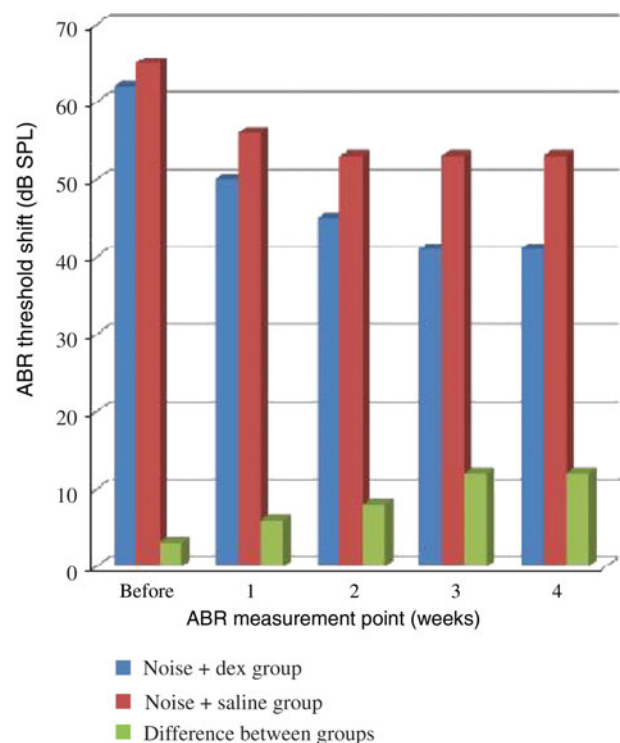


FIG. 8

Auditory brainstem response (ABR) threshold shifts for the noise plus dexamethasone (dex) and noise plus saline groups in pre-experiment group two, and the ABR threshold shift difference between these two groups, following treatment administration. The ABR threshold shift difference increased gradually over weeks one to three, but was no larger on week four. Therefore, week three was chosen as the experimental observation point.

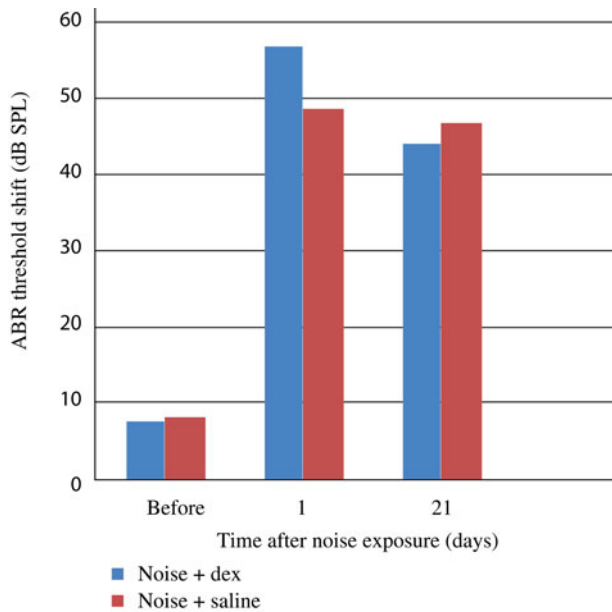


FIG. 9

Auditory brainstem response (ABR) click threshold shifts for the noise plus dexamethasone (dex) and noise plus saline groups in experimental group one, before and after noise exposure. Note that treatment was administered 1 day after noise exposure. A significant difference between the two groups' ABR threshold shifts was observed at both 1 and 21 days after noise exposure ( $p < 0.05$ ).

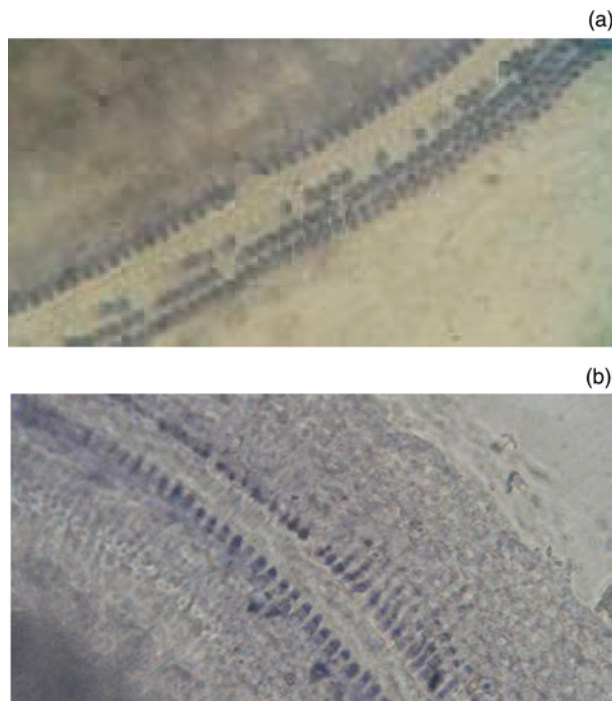


FIG. 10

Photomicrographs showing hair cell loss in the inferior part of the second cochlear turn. (a) In the dexamethasone group, it was mainly the outer hair cells which were lost; few inner hair cells were lost. (b) In the saline group, few outer hair cells remained. (Succinate dehydrogenase staining;  $\times 400$ )

remained (Figure 10b). Hair cell loss in the dexamethasone versus saline subgroups is shown in Figure 10.

In experiment one animals, hair cell loss in the basal turn, the second turn and overall was significantly greater in the saline group than in the dexamethasone group ( $p < 0.05$ ). However, there was no significant difference in hair cell loss in the third and apical turns, comparing these two groups ( $p > 0.05$ ; see Table I).

*Spiral ganglion morphology*

On light microscopy 21 days after drug administration in experiment one animals, there was no significant difference in spiral ganglion cell counts in the inferior part of the second turn and the superior part of the basal turn, comparing the dexamethasone and saline subgroups (Figure 11).

*Malondialdehyde and superoxide dismutase concentration*

Table II shows the malondialdehyde and superoxide dismutase concentrations in experiment one animals' cochleae 21 days after treatment, and also in seven untreated animals (used as controls).

Malondialdehyde and superoxide dismutase concentration were significantly greater in the dexamethasone group compared with the normal group ( $p < 0.05$ ). The difference in malondialdehyde and superoxide dismutase concentration between the saline and normal groups was not significant ( $p > 0.05$ ).

*High-performance liquid chromatography*

Following round window membrane dexamethasone administration in experiment two animals, high-performance liquid chromatography measurements indicated that the peak concentration of dexamethasone in cochlear perilymph was 5330.522  $\mu\text{g/ml}$ . The cochlear perilymph dexamethasone concentration decreased gradually over time; 360 minutes after drug administration, it was 299.7966  $\mu\text{g/ml}$ . The plotted curve for cochlear perilymph dexamethasone concentration versus time had an equation of  $Y = 0.00005C + 0.447$ , with  $R = 0.9998$  and a linearity range of 0.1–10 mg/ml (Table III). This curve is shown in Figure 12.

**Discussion**

*Determination of drug administration route and concentration*

Mini-osmotic pumps connected to a cannula are often used to infuse drugs into the scala tympani in animal research models, in order to observe the therapeutic effect. This administration route has the benefit of achieving high drug concentrations in the inner ear, without systemic effects.

A study on guinea pigs has investigated the effects of transtympanic dexamethasone injection on cochlear blood flow (assessed with laser Doppler flowmetry), auditory sensitivity (assessed by ABR) and histology.<sup>19</sup> A significant increase in cochlear blood flow was observed within 30 seconds of injection, to a mean of

TABLE I  
PERCENTAGE HAIR CELL LOSS IN EACH COCHLEAR TURN

Group	Ears ( <i>n</i> )	Turn				Total
		Apical	3rd	2nd	Basal	
Dex	6	17.5 ± 0.96	8.3 ± 0.3	27.4 ± 0.8	19.8 ± 0.7	67.4 ± 12.1
Saline	6	9.3 ± 0.4	17.8 ± 0.7	56.1 ± 0.8	61.5 ± 0.1	74.5 ± 15.3
<i>p</i>		>0.05	>0.05	<0.05	<0.05	<0.05

Data refer to experimental group one, and represent means ± standard deviations (%) unless otherwise specified. Dex = dexamethasone

129.26 per cent, without any significant change in auditory sensitivity. This increase in cochlear blood flow was sustained, and did not return to baseline until at least 1 hour after drug application. No histological changes were observed. The author concluded that transtympanic steroid application was not likely to be detrimental to the inner ear, and also that the increased blood flow may indicate a possible mechanism accounting for the pharmacological effects of steroids in the inner ear.

Our study findings suggest that dexamethasone has no effect on auditory function in normal guinea pigs. We chose to administer dexamethasone via the round window membrane, because of the ease and potential clinical application of this route. Other authors have used direct delivery routes other than the round window, sometimes with slow- or sustained-release formulations.<sup>20</sup> In these studies, the experimental drug was injected into the middle ear directly, to the required volume. In a preliminary experiment conducted prior to the current study, we compared the

round window and middle-ear administration routes, and found that the latter could result in more inflammation in the middle ear and even the inner ear.

When considering drug administration via the round window membrane, one major question is whether the drug is able to permeate the round window membrane and enter the perilymph to a sufficient degree to reach a therapeutic concentration. The round window membrane is considered the principal route of entry for transtympanically injected drugs. It is a semi-filtration membrane which has different filtration properties for different substances. The permeability of the round window membrane to different substances is affected by several factors, including the substance's molecular size, configuration, concentration, liposolubility and electric charge, and also the round window thickness. Previous studies have noted that both transforming growth factor- $\alpha$  and the *GJB2*<sub>R75W</sub> gene are able to pass through the mammalian round window membrane.<sup>21,22</sup> The relative molecular mass of dexamethasone is 516.41 Da, smaller

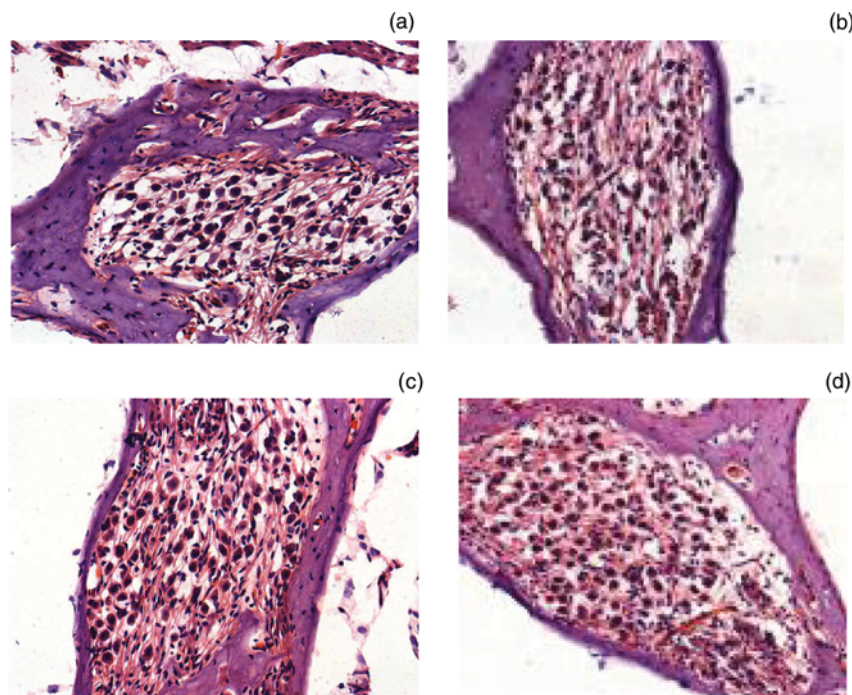


FIG. 11

Photomicrographs showing spiral ganglion cells in the (a) inferior second turn and (b) superior basal turn in the dexamethasone group; and in the (c) inferior second turn and (d) superior basal turn in the saline group. (H&E; ×400)



**TABLE II**  
MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE CONCENTRATION 21 DAYS AFTER TREATMENT

Group	Ears (n)	MDA (nmol/mg prot)	SOD (U/mg prot)
Dex	7	4.71 ± 0.30	3.75 ± 0.27
Saline	7	6.76 ± 1.02	4.59 ± 0.56
Normal*	7	4.23 ± 0.72	3.22 ± 0.88

Data refer to experimental group one, and represent means ± standard deviations unless otherwise specified. \*Seven normal, untreated animals used as controls. MDA = malondialdehyde; prot = protein; SOD = superoxide dismutase; dex = dexamethasone

than that of transforming growth factor-α and the *GJB2<sub>R75W</sub>* gene.

In Li and colleagues' study,<sup>23</sup> a Waters 600 high-pressure liquid chromatograph was used to investigate the pharmacokinetics of dexamethasone infiltration of the round window membrane. These authors verified that dexamethasone at a concentration of 100 g/l could pass through the round window membrane quickly, achieving a peak value of 310.6 g/l 60 minutes after administration. The concentration then decreased gradually to 35 mg/l 300 minutes later.

In our study, a gelatin sponge soaked with dexamethasone at a concentration of 40 mg/ml was placed on the round window niche. Because of the location of the gelatin sponge, dexamethasone had sufficient time to permeate into the cochlear perilymph, and achieved a high concentration as predicted. High-performance liquid chromatography indicated that the highest perilymph dexamethasone concentration was 5330.522 µg/ml, and was still 299.797 µg/ml 6 hours after administration.

Other authors have found that histamine facilitates permeation of dexamethasone into the cochlear perilymph.<sup>24</sup>

In the clinic, sudden sensorineural hearing loss is often treated with intratympanic administration of dexamethasone at a concentration of 24 mg/ml.<sup>25</sup>

The protective effect of dexamethasone against noise-induced trauma has been assessed in guinea pigs.<sup>18</sup> Animals were administered dexamethasone (at 1, 10, 100 and 1000 ng/ml) and artificial perilymph directly into the scala tympani, via a mini-osmotic

**TABLE III**  
PERILYMPH DEXAMETHASONE LEVEL OVER TIME\*

Time (min)	Dex (µg/ml)
15	5330.522
30	3809.258
60	2948.451
120	1268.339
180	678.549
240	524.259
300	376.516
360	299.796

\*After topical application of dexamethasone (dex) to round window niche.

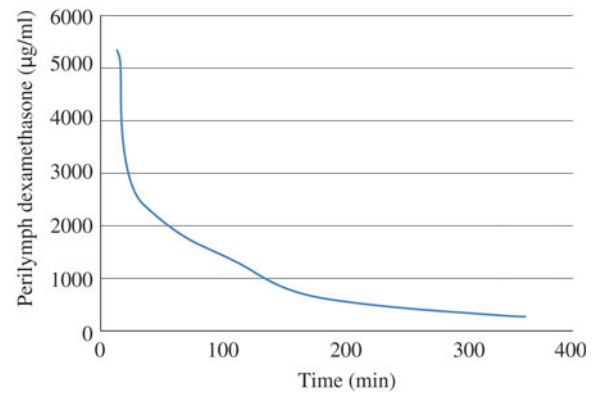


FIG. 12

Cochlear perilymph dexamethasone concentration versus time, in experiment two animals.

pump, and then exposed to 120 dB SPL octave band noise, centred at 4 kHz, for 24 hours. Seven days after noise exposure, results in dexamethasone-treated animals indicated a dose-dependent reduction in noise-induced outer hair cell loss (significant at 1, 10 and 100 ng/ml dexamethasone) and a similar attenuation of noise-induced ABR threshold shifts (significant at 100 ng/ml dexamethasone), compared with control animals receiving artificial perilymph.

In our study, we were able to achieve a higher peak dexamethasone concentration in the cochlear perilymph (i.e. 5330.522 µg/ml) than that reportedly achieved in previous studies (100 ng/ml).<sup>18</sup>

The cochlear perilymph dexamethasone concentration is also influenced by the timing of drug application to the round window membrane. A recent study<sup>26</sup> examined the dosage and timing of dexamethasone applied to the round window in order to optimise hearing protection. A reduced application time was needed for higher steroid concentrations: 20 per cent dexamethasone applied for 30 minutes achieved similar levels of hearing protection to 2 per cent dexamethasone applied for 60 minutes. Prior to cochlear implantation, a longer duration of dexamethasone application to the round window resulted in improved hearing protection; this waiting time could be reduced by increasing the steroid concentration. These results indicate that the diffusion of dexamethasone into the cochlea is the prime determinant of the degree of hearing protection achieved. In our study, a gelatin sponge was used to apply dexamethasone for a sufficient time period to allow permeation through the round window membrane into the cochlear perilymph.

*Therapeutic effect of dexamethasone on blast deafness*

Exposure to high intensity noise can cause irreversible hearing damage, as a result of direct mechanical or secondary metabolic effects. In China and various other countries, the recommended treatment for noise-induced hearing loss consists of nursing in a silent

environment, intravenous injection of glucocorticoid with or without vasodilator substances, and hyperbaric oxygen therapy. A protective effect of glucocorticoid has been demonstrated by Henry *et al.*<sup>27</sup> In the current study, guinea pigs were administered either dexamethasone or saline following noise exposure. Noise-induced hearing loss was reduced in the dexamethasone group compared with controls. This protective effect has also been observed for methylprednisolone (another glucocorticoid), by Zhou *et al.*,<sup>28</sup> following intratympanic administration of methylprednisolone or saline in guinea pigs. Furthermore, a protective effect has been observed for endogenous glucocorticoids: activation of the hypothalamus–pituitary–adrenal axis is associated with reduced threshold shifts after acoustic trauma.<sup>14</sup>

Current treatment for acute acoustic trauma is based on systematic or local glucocorticoid administration. A study by Abamrane *et al.*<sup>29</sup> compared three noise-induced hearing loss treatments applicable in humans: magnesium for seven days or one month, or conventional methylprednisolone therapy. Three months after impulse noise trauma, one-month magnesium treatment preserved more hair cells than the two other treatments. Although methylprednisolone accelerated functional recovery, compared with magnesium, its long-term advantage remained moderate. In this study, several exploratory methods were used to evaluate the efficacy of dexamethasone infiltration through the round window membrane, following impulse noise trauma.

In the current study, a particularly damaging noise trauma was applied: 80 impulses with 167 dB SPL peak value, sufficient to induce permanent hearing loss and intensive, irreversible hair cell loss in guinea pigs (as previously described by Zhou *et al.*).<sup>28</sup> Baseline auditory thresholds were determined in all animals before noise exposure and before treatment. In experimental group one, the study and control animals had comparable auditory function before noise exposure, and before treatment. After noise exposure, the ABR click threshold shift decreased by 50 to 80 dB in both the study and control subgroups, and light microscopy indicated hair cell loss especially in the superior part of the basal turn and the inferior part of the second turn. Outer hair cell loss was more severe than inner hair cell loss. These changes in hearing function and hair cell morphology concur with the formation of a permanent threshold shift. These effects have already been observed in guinea pigs with noise-induced hearing loss treated with intracochlear methylprednisolone administration.<sup>15</sup>

#### *Mechanism of glucocorticoid effect following blast deafness*

Glucocorticoids are used extensively in the treatment of disease, because of their intensive anti-inflammatory action and other therapeutic effects. Glucocorticoids such as dexamethasone not only protect normal hair cells but also inhibit further injury of damaged hair

cells. In our experimental group one, hair cell injury observed three weeks after treatment was significantly less in the dexamethasone group compared with the control group, indicating that dexamethasone has a therapeutic effect following intense acoustic trauma. In addition, we observed no significant difference in the corresponding spiral ganglion cells, comparing the dexamethasone and control groups, suggesting that noise may not damage the spiral ganglion cells.

The exact manner by which dexamethasone improves noise-induced hearing loss is still unknown, but several mechanisms may be possible. At the cellular level, glucocorticoids exert their effects by binding to the glucocorticoid receptor, a transcription factor which belongs to the nuclear receptor superfamily and is capable of regulating several genes. Glucocorticoid receptors have been demonstrated within the inner ear in rodents and humans, in the spiral ligament, spiral ganglion cells and spiral limbus, and to a lesser extent in the stria vascularis and the inner and outer hair cells of the organ of Corti, amongst other sites.<sup>30</sup> The administration of RU486, a glucocorticoid receptor antagonist, to noise-traumatised mice antagonised the protective effect of methylprednisolone. Thus, the protective effect of this drug in the inner ear appears to be mediated by the binding of glutathione reductase.<sup>31</sup> Tahera *et al.*<sup>32</sup> demonstrated that glucocorticoid can directly modulate hearing sensitivity following moderate acoustic trauma resulting in a 10–30 dB hearing loss. Importantly, this study demonstrated that nuclear factor- $\kappa$ B (NF- $\kappa$ B) is involved in the glutathione reductase mediated signalling pathway in the inner ear. These authors concluded that down-regulation of endogenous glucocorticoid production and increased nuclear translocation within the cochlea resulted in reduced acoustic trauma. Mifepristone, another glucocorticoid receptor antagonist, may influence the inner hair cells and the afferent nerve fibres beneath the inner hair cells, without affecting the outer hair cells.<sup>33</sup> Thus, the evidence suggests that glucocorticoids can play an important therapeutic role in treating hearing impairment following acoustic trauma.

Karlidag *et al.* evaluated the efficacy of methylprednisolone administered (40 mg/kg intramuscular) to guinea pigs prior to noise exposure. Malondialdehyde levels within cochlear tissue were used as a marker of oxidative stress. However, in this study methylprednisolone was found to have no significant effect on the malondialdehyde concentration increase observed after noise exposure. This apparent discrepancy, compared with previous findings, may be explained by differing routes of methylprednisolone administration.

During lipid peroxidation, a significant quantity of aldehydes (e.g. malondialdehyde) are produced by  $\beta$ -schizolysis of the lipid group. The extracellular malondialdehyde concentration reflects the degree of injury caused by oxygen free radicals attacking cell membranes,<sup>34</sup> thus, malondialdehyde concentration

can be used as an indicator of the degree of lipid peroxidation. Uhler *et al.*<sup>35</sup> found that high-dose methylprednisolone could prevent membrane lipid peroxidation injury induced by free radicals *in vivo*, and that it had an anti-oxidant effect. Dexamethasone may decrease ischaemia reperfusion injury by lowering the malondialdehyde concentration.<sup>8</sup> Yamane *et al.* found that superoxide anions were formed in the luminal membranes of guinea pigs following exposure to noise at 120–125 dB for 3 hours, and were also expressed in the stria vascularis after noise exposure.<sup>36</sup> The greater the noise intensity, the greater the expression of hydroxide radicals<sup>37,38</sup> and malondialdehyde<sup>39</sup> in guinea pig cochleae. Dexamethasone prevents lipid peroxidation through activating anti-oxidases such as glutathione reductase and superoxide dismutase.<sup>33</sup>

In experiment two, the malondialdehyde concentration in the dexamethasone group was greater than that in the normal group but less than that in the saline group. This suggests that oxygen free radical activity in the dexamethasone group was lower than that in the saline group. We conclude that dexamethasone decreased the amount of oxygen free radicals induced by acoustic trauma, and also accelerated the metabolism and clearing of oxygen free radicals by reinforcing anti-oxidation capacity, thus reducing cellular injury and protecting the guinea pigs from hearing damage.

Superoxide dismutase is an effective oxygen free radical scavenger and can alleviate noise-induced hearing loss. It clears reactive oxygen species and thus helps balance oxidative conditions. Certain pathological conditions strengthen the lipid peroxidation process; this may trigger a compensatory increase in superoxide dismutase synthesis. We found greater superoxide dismutase activity in both the saline and dexamethasone groups, compared with the normal group; however, this value was greatest in the saline group. This suggests that dexamethasone may strengthen the anti-oxidative capability of the cochlea, and thus enable improved hearing following acoustic trauma. Further study is required to confirm this last point.

Methylprednisolone (delivered via intrathecal injection) has been found to be beneficial for the treatment of ischaemic spinal cord injuries. Intratympanic dexamethasone delivery increases the cochlear blood flow. These results suggest that glucocorticoids may protect hair cells from ischaemic and hypoxic injury through increasing the cochlear blood flow.<sup>19,34</sup>

Nagashima *et al.*<sup>40</sup> observed that systemic administration of dexamethasone led to a significant increase in glutathione concentration in the cochlear modiolus, 2 to 24 hours after injection.

Under normal conditions, the inner ear possesses remarkably stable homeostatic mechanisms enabling maintenance of the functional integrity of the inner ear fluid. Any disturbance that disrupts homeostasis may have short- and long-term effects on the cellular function of the auditory system. Traumatic noise exposure has been demonstrated histopathologically

to destroy auditory hair cells (especially outer hair cells), to contribute to collapse of the organ of Corti, and to impair cochlear circulation.<sup>41</sup>

- **Glucocorticoids are used extensively in the clinical management of inner ear pathology, including sudden sensorineural hearing loss**
- **The exact mechanism by which dexamethasone improves noise-induced hearing loss is still unknown**
- **Dexamethasone has therapeutic effects on blast deafness in guinea pigs by protecting hair cells and accelerating hearing recovery; one probable mechanism is action against oxygen free radical toxicity**

Recently, the general caspase inhibitor N-t-Boc-Asp(OMe)-fluoromethylketone has been found to prevent tumour necrosis factor  $\alpha$  induced auditory hair cell death. Tumour necrosis factor  $\alpha$  is a pro-inflammatory cytokine. A significant reduction in the efficacy of dexamethasone otoprotection against tumour necrosis factor  $\alpha$  ototoxicity has been observed when the following substances, structures and cellular events are blocked: (1) glucocorticoid receptors; (2) phosphatidylinositol 3-kinase; (3) activation of protein kinase B; and (4) NF- $\kappa$ B-1. Dexamethasone protects hair cells against tumour necrosis factor  $\alpha$  apoptosis *in vitro* via activation of phosphatidylinositol 3-kinase, protein kinase B and NF- $\kappa$ B-1 signalling.<sup>42</sup> The ototoxicity of tumour necrosis factor  $\alpha$  may be mediated via up-regulation of Bax and tumour necrosis factor receptor 1 expression, as well as via an increase in the Bax/Bcl-2 ratio. Dexamethasone protects the organ of Corti against tumour necrosis factor  $\alpha$  ototoxicity by up-regulating Bcl-2 and Bcl-xl expression, and by inhibiting tumour necrosis factor  $\alpha$  induced increases in Bax, tumour necrosis factor receptor 1, and the Bax/Bcl-2 ratio. These results support the use of local dexamethasone treatment to conserve hearing following acoustic trauma.

## Conclusion

From these results, we conclude firstly that dexamethasone applied topically to the round window niche has a therapeutic effect on guinea pigs with signs of blast deafness, by protecting hair cells and accelerating hearing recovery.

Secondly, impulse noise increases the concentration of malondialdehyde and superoxide dismutase within cochlear tissues, and the application of dexamethasone after noise exposure decreases this rise. This suggests that one of the mechanisms by which dexamethasone protects guinea pig hearing following blast deafness probably involves action against oxygen free radical toxicity.

Thirdly, we conclude that dexamethasone can permeate the round window membrane and reach the cochlear perilymph, where it can attain a high concentration for a relatively long period.

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Address for correspondence:

Dr F-L Chi,  
Department of Otorhinolaryngology,  
Eye Ear Nose and Throat Hospital of Fudan University,  
Shanghai 200031, China

E-mail: chifanglu@yahoo.com.cn

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