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A comparative study of intratympanic steroid and NO synthase inhibitor for treatment of cochlear lateral wall damage due to acute otitis media

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Abstract

We studied the damage to the cochlear lateral wall induced by otitis media and the therapeutic effects of intratympanic administration of steroid and nitric oxide (NO) synthase inhibitor. In Sprague–Dawley rats, right middle ear cavities were inoculated with lipopolysaccharide, followed after 30 min by intratympanic administration of dexamethasone, NOS-inhibitor or PBS. Twenty-four hours after lipopolysaccharide inoculation, cochlear blood flow was measured by laser-Doppler flowmetry. Prostaglandin E_1 was topically applied to the round window membrane of the right ear and changes in cochlear blood flow were calculated. Changes of cochlear blood flow were significantly different among the three groups. Increases in cochlear blood flow following PGE_1 application were higher in the group that received NOS-inhibitor. Electron microscopic examination revealed that changes in the stria vascularis were less severe in rats treated with dexamethasone or NOS-inhibitor. Our results show the effectiveness of intratympanic dexamethasone or NOS-inhibitor in treating cochlear lateral wall damage caused by otitis media.

Keywords: Otitis media; Cochlear disturbance; Nitric oxide (NO) synthase inhibitor; Dexamethasone

1. Introduction

Disturbances of cochlear function due to otitis media have been demonstrated in clinical (Paparella et al., 1984; Ryding et al., 2002) and experimental studies (Morizono et al., 1985; Spandow et al., 1989). The round window membrane may allow bacterial toxins to pass from the middle ear and cause damage in the inner ear. A disrupted round window membrane is thought to facilitate passage of harmful substances from the middle to the inner ear (Engel et al., 1998). Hair cells in the cochlea can be damaged following endotoxin-induced otitis media and the cochlear lateral wall has been proposed as a target site of cochlear damage following endotoxin-induced otitis media (Watanabe et al., 1995; Sone et al., 1999; Ichimiya et al., 1999). Disturbances at this site have been examined morphologically, but functional evaluation of the damaged cochlear lateral wall has yet to be studied.

In a study using a model of endotoxin-induced otitis media, we evaluated the influence of otitis media on the cochlear lateral wall (Sone et al., 2003). At 24 h after inoculation of endotoxin into the middle ear cavity, cochlear blood flow was significantly decreased and vacuolar changes were seen in the stria vascularis. After 14 days, cochlear blood flow returned to normal and the cochlear lateral wall had a histologically normal appearance. In patients with acute otitis media, clinical audiometry (≤ 8 kHz) indicates that inner ear damage may be reversible in most cases. Recurrent acute otitis media, however, could cause permanent cochlear damage (Ryding et al., 2002). From these clinical findings, it should be possible to establish efficient strategies for preventing cochlear damage following otitis media.

Glucocorticoids have been shown to control effectively the severity of otitis media (Baggett et al., 1997; Park and Yeo, 2001), and to prevent subsequent cochlear damage (Takeuchi and Anniko, 2000). Glucocorticoids inhibit, at the transcriptional level, the expression of many cytokines and immunomodulatory genes, including inducible nitric oxide synthase (iNOS) (Matsumura et al., 2001). Cochlear damage following acute otitis media has also been studied in relation to NOS (Hess et al., 1999; Watanabe et al., 2000a; Takumida et al., 2000). NO has several functions, including regulation of blood flow (Brechtelsbauer et al., 1994), and, in large quantities, it can mediate endotoxin-induced damage to the cochlea. As a result, NOS-inhibitors have been introduced to prevent cochlear damage

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following otitis media (Watanabe et al., 2000b; Popa et al., 2000).

We now examined changes in the cochlear lateral wall induced by otitis media, and the therapeutic efficacy of intratympanic administration of dexamethasone and NOS-inhibitor. In the preliminary study, we confirmed that application of prostaglandin E_1 to the round window membrane increased cochlear blood flow (Tominaga et al., 2002). Prostaglandins are known to inhibit platelet aggregation and to have vasodilator activity. Since changes in the cochlear lateral wall can affect its blood flow, the response of cochlear blood flow to prostaglandin E_1 may be influenced by cochlear lateral wall damage. We have examined the influence of the drugs on changes in cochlear blood flow following prostaglandin E_1 application and also studied changes in the cochlear lateral wall by electron microscopic examination.

2. Materials and methods

2.1. Animals and inoculation with endotoxin

We used 16 female Sprague–Dawley rats weighing 150–225 g each. During surgery and when they were killed, the animals were anesthetized using a mixture of ketamine hydrochloride (40 mg/kg), xylazine hydrochloride (8 mg/kg), and acepromazine maleate (1 mg/kg), administered intramuscularly. All animals were free of middle ear infection prior to treatments. Endotoxin inoculation was performed under a microscope, by instilling 30 μl of lipopolysaccharide (5 mg/ml) from *Escherichia coli* (Sigma, St. Louis, MO, USA) into the right middle ear cavity of each animal through the tympanic membrane, using a 25-gauge needle. Twenty-four hours before middle ear inoculation, 0.2 ml of lipopolysaccharide (0.2 mg/ml) was injected intraperitoneally to enhance the effects of the endotoxin. No treatment was performed on the left ears.

Those animals inoculated with lipopolysaccharide were divided into three groups of four rats each. Thirty minutes after middle ear inoculation of lipopolysaccharide, the first group received 30 μl of phosphate-buffered saline (PBS) in the right middle ear cavity, the second group received 30 μl of 1% dexamethasone dissolved in PBS (Sigma), and the third group received 30 μl of NOS-inhibitor dissolved in PBS (1.8×10^{-7} M/kg) (ONO-1714, (1*S*,5*S*,6*R*,7*R*)-7-chloro-3-imino-5-methyl-2-azabicyclo [4.1.0] heptane hydrochloride) (Ono Pharmaceutical, Osaka, Japan). Four untreated rats served as control group. Experimental protocols were approved by the Nagoya University Committee on the Use and Care of Animals.

2.2. Measurement of cochlear blood flow

Treated animals were examined at 24 h after inoculation. Under general anesthesia, left femoral arteries were canulated to monitor systemic blood pressure (BP). Body temperature was maintained at approximately 38 °C with a servo-regulated heating blanket. Each right bulla was identified and opened carefully through a ventrolateral approach. After fluid, mucosa, and periosteum overlying the bone were removed, a 1.0-mm probe from the laser-Doppler flowmeter (ALF21, Advance, Tokyo, Japan) was placed over the basal turn of the cochlea where maximum output of cochlear blood flow was measured. The time constant of the flowmeter was 1.0 s.

The ratio of cochlear blood flow to BP (normalized cochlear blood flow, Ohlsén et al., 1992) was monitored, and the average ratio (average for 5 min after stabilization of cochlear blood flow) was calculated. Cochlear blood flow was measured similarly in the left ears. The ratio of normalized cochlear blood flow in the right ear to that in the left ear was calculated and analyzed. Values of normalized cochlear blood flow varied among the rats studied, so normalized cochlear blood flow in the untreated ear was used as a baseline value for each rat. Cochlear blood flow and BP were monitored continuously throughout the experiment, using a computer-based chart recorder.

2.3. Topical application of prostaglandin E_1

After complete removal of any fluid overlying the round window membrane, 3 μl of 1.4×10^{-2} M prostaglandin E_1 α-cyclodextrine, molecular weight 354.49 (Ono Pharmaceutical), was topically applied to the round window membrane of each right ear. Cochlear blood flow was monitored for 30 min after application of prostaglandin E_1. Changes in normalized cochlear blood flow were calculated as the ratio of normalized cochlear blood flow before and after application of prostaglandin E_1. Changes in normalized cochlear blood flow from four normal rats were used as control values.

2.4. Electron microscopic examination

After deep anesthesia, right ears of the rats were resected and examined. The ears were fixed by perilymphatic perfusion with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde, post-fixed in 1% osmium, dehydrated, and embedded in Epon. Ultrathin sections of the cochlear lateral walls were stained with uranyl acetate and lead citrate, and examined using a Hitachi H-7100 transmission electron microscope.

2.5. Statistical analysis

Data from measurements of cochlear blood flow were analyzed using the Kruskal–Wallis test and the Mann–Whitney test.

3. Results

When the tympanic bullae were opened, we found middle ear cavities filled with fluid in all rats inoculated with

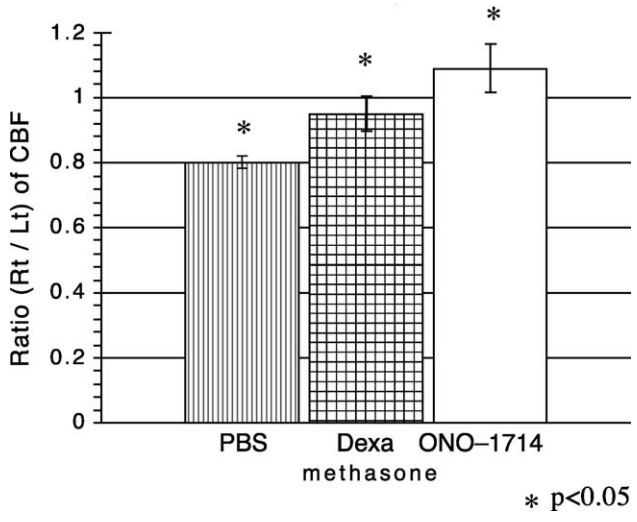

Fig. 1. The ratio of the value of normalized cochlear blood (CBF) in treated ears to that in an untreated ear for the three groups (Average ± S.E., $n=4$ in each group).

lipopolysaccharide, however, the volume of fluid was less in rats treated with dexamethasone or ONO-1714. Mucosa overlying the promontory was edematous, but after complete removal of the fluid, the round window membrane appeared to be normal in all rats that received lipopolysaccharide.

3.1. Changes of normalized cochlear blood flow

In rats that received PBS after lipopolysaccharide inoculation, the ratio (normalized cochlear blood flow in the right ear to that in the left) was 0.80 on average. The average of the ratios in rats that received dexamethasone was 0.95 and that in rats that received ONO-1714 was 1.09. There was a significant difference among these three groups (Fig. 1). These results indicate that cochlear blood flow in ears treated with ONO-1714 was higher than in untreated ears.

3.2. Changes in normalized cochlear blood flow after application of prostaglandin E_1

After application of prostaglandin E_1 to the round window membrane of normal rats, cochlear blood flow increased within minutes and remained at an increased level for >30 min. The increased normalized cochlear blood flow in rats treated with lipopolysaccharide showed a different pattern (Fig. 2). Normalized cochlear blood flow in rats receiving PBS after treatment with lipopolysaccharide showed the least increase compared to other rats. In rats treated with dexamethasone or ONO-1714, the cochlear blood flow increased within minutes, although to a lesser extent than in control rats.

The average of changes in normalized cochlear blood flow 30 min after application of prostaglandin E_1 was 1.23 in control rats. In rats treated with PBS, dexamethasone or ONO-1714 after middle ear inoculation of lipopolysaccharide, the average of changes in normalized cochlear blood flow 30 min after application of prostaglandin E_1 was 1.06, 1.11 and 1.18, respectively. The increase in rats treated with PBS or dexamethasone was significantly lower than in control rats. Rats treated with ONO-1714 showed no sig-

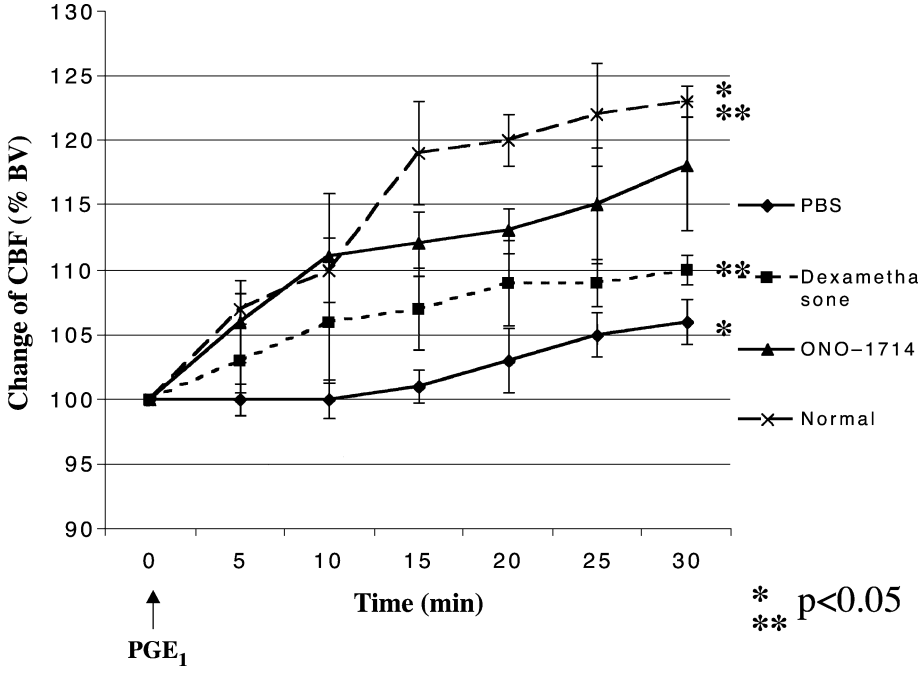

Fig. 2. Time curves of normalized cochlear blood (CBF) for 30 min after application of prostaglandin E_1 (PGE_1) in three lipopolysaccharide inoculated groups of rats and a control group. All values are expressed as percentage changes from the baseline value (BV). (Average ± S.E., $n=4$ in each group).

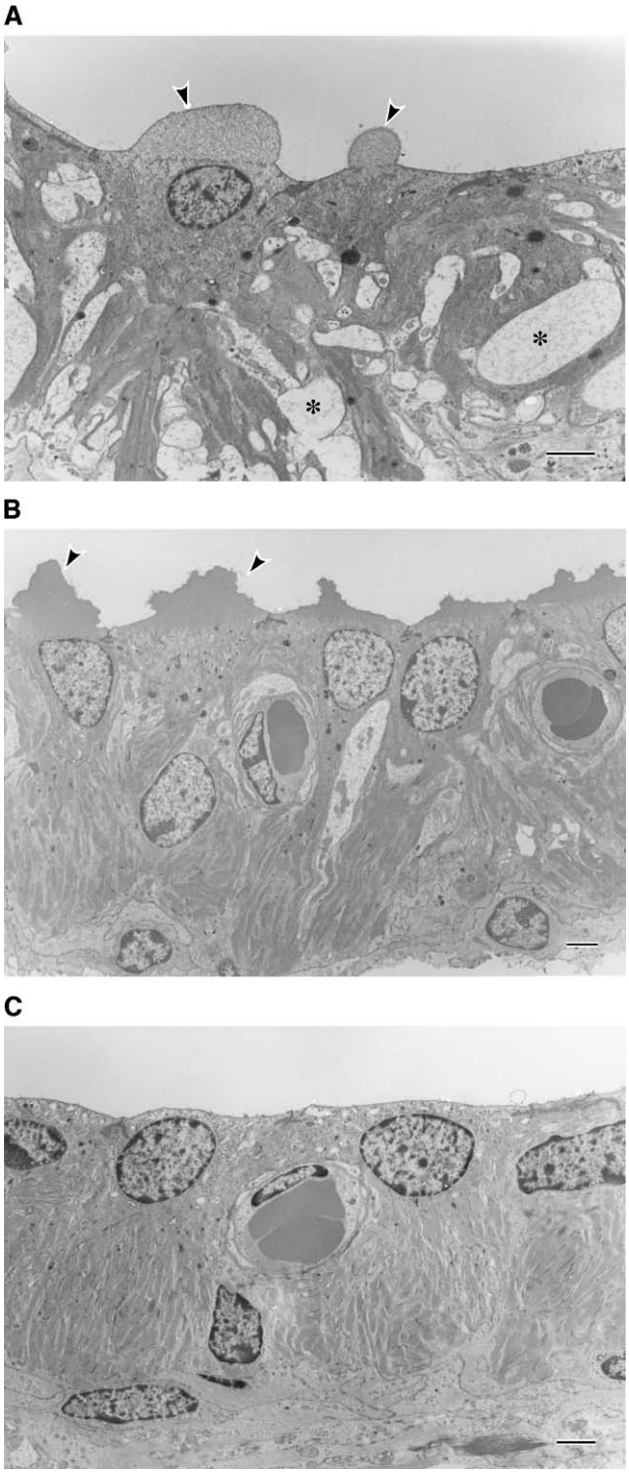

Fig. 3. Electron microscopic findings for the stria vascularis in rats treated with PBS (A), dexamethasone (B), or ONO-1714 (C). (A) Intercellular spaces in the intermediate cells are markedly enlarged (asterisk) and the marginal cells project towards the endolymphatic space (arrow heads). Scale bar = 2 μm (B) Pronounced changes of the marginal cells (arrow heads), but only slight enlargement of intercellular spaces. Scale bar = 2 μm (C) Almost normal appearance of the stria vascularis. Scale bar = 2 μm.

nificant difference in response to prostaglandin E_1 application compared to controls.

3.3. Electron microscopic findings

The stria vascularis of rats treated with PBS after inoculation of lipopolysaccharide showed very marked enlargement of intercellular spaces in the intermediate cell area (Fig. 3A). Projection of the marginal cells towards the endolymphatic space was also observed. The stria vascularis of rats treated with dexamethasone after lipopolysaccharide showed slight enlargement of intercellular spaces and projection of the marginal cells towards the endolymphatic space (B). Rats treated with ONO-1714 following lipopolysaccharide showed the stria vascularis of almost normal appearance (C). There were no abnormal findings in the spiral ligament of any rats.

4. Discussion

The inner ear disorders could have been caused by inflammation in the middle ear. Clinical audiometry (≤ 8 kHz) of the inner ear in patients with acute otitis media has indicated that the damage is mostly reversible. However, a recent study demonstrated that hearing levels at higher frequencies (8–16 kHz) were elevated in children with recurrent acute otitis media (Ryding et al., 2002). Failure of early treatment could lead to permanent inner ear damage. Previous studies have demonstrated that acute otitis media may have an influence on the cochlear lateral wall (Watanabe et al., 1995; Sone et al., 1999; Ichimiya et al., 1999). In the present study, we focused on morphological and functional changes in the cochlear lateral wall induced by otitis media, and studied the effects of intratympanic administration of dexamethasone and NOS-inhibitor.

Lipopolysaccharide is purified endotoxin from Gram-negative bacteria and is known to cause systemic vasodilation. Our microscopic observations revealed that administration of lipopolysaccharide was followed by marked enlargement of intercellular spaces in the intermediate cells of the stria vascularis. This may have been caused by elevated microvascular pressure and increased vascular permeability induced by lipopolysaccharide, as suggested by Okamoto et al. (1998). From a pathophysiological viewpoint, lipopolysaccharide induced vasodilation could produce intercellular spaces in the stria vascularis, leading to disruption of the cochlear lateral wall.

Intratympanic administration of glucocorticoids has been shown to increase perilymph steroid levels within one hour of administration, and increase endolymph steroid levels to a substantially higher level than perilymph levels (Parnes et al., 1999; Chandrasekhar et al., 2000). Intratympanic glucocorticoid is considered to be an effective therapy for inner ear disorders, avoiding side effects. High levels of glucocorticoid receptors have been found in the spiral ligament of

the inner ear in rats and humans (Rarey et al., 1993; Rarey and Curtis, 1996). Topical application of dexamethasone to the guinea pig round window membrane led to a significant increase in blood flow in the cochlear lateral wall (Shirwany et al., 1998). This may indicate a direct effect of dexamethasone on the cochlear lateral wall.

We found that dexamethasone administration following lipopolysaccharide attenuated cochlear lateral wall damage, to some extent, as shown by the morphology and by an increased response to prostaglandin E_1. This may have been due to attenuation of the otitis media, as reported previously (Baggett et al., 1997; Park and Yeo, 2001), or been a direct effect on glucocorticoid receptors on the cochlear lateral wall. Glucocorticoids inhibit the expression of many cytokines and immunomodulatory genes, including iNOS, at the transcriptional level (Matsumura et al., 2001). In experimental glioma, dexamethasone significantly alters iNOS mRNA production within the tumor (Swaroop et al., 2001). The present study demonstrated that NOS-inhibitor is much more effective than dexamethasone against lipopolysaccharide induced disorders of the cochlear wall. Our results support the hypothesis that the effects of dexamethasone on the inner ear disorders caused by bacterial otitis media are mainly exerted through suppression of NOS production. Protection of disturbances of cochlear blood flow observed in rats that received dexamethasone or the NOS-inhibitor is considered to be relevant to an electrophysiological result, which showed that a NOS-inhibitor protected from the sensorineural hearing loss caused by exotoxin inoculation (Popa et al., 2000).

NO has several functions, including regulation of blood flow (Brechtelsbauer et al., 1994; Nakashima et al., 2003). Both NO from constitutive NOS (cNOS) and iNOS play an important role in cochlear tissue damage (Fessenden and Schacht, 1998). NO, soluble guanylate cyclase and cyclic guanosine monophosphate have been detected in the lateral wall of the cochlea, lending support to the idea that NO is involved in the regulation of cochlear blood flow (Michel et al., 1999). *N*(G)-nitro-L-arginine methyl ester (L-NAME), a NOS-inhibitor, has been shown to protect against cochlear damage caused by endotoxin-induced otitis media (Watanabe et al., 2000b; Popa et al., 2000). ONO-1714, the NOS-inhibitor used in the present study, has a stronger inhibitory activity against iNOS than dose L-NAME (Naka et al., 2000).

The spiral ligament of the cochlear wall is considered to have an important role in cochlear blood flow (Konishi et al., 1998). We found that normalized cochlear blood flow in rats receiving dexamethasone or ONO-1714 showed a greater increase following prostaglandin E_1 application, than did that in rats receiving PBS. Prostaglandin E_1 applied to the round window membrane may exert an effect on the spiral ligament via the perilymph. Prostaglandin E_1 prevents inner ear disturbances caused by microvascular thrombosis (Umemura and Nakashima, 1997). Prostaglandin E_2 and prostaglandin I_2 both appear to increase cochlear blood flow (Rhee et al., 1999). Although the blood flow of the cochlear lateral wall might be regulated in the spiral ligament, the source of cochlear blood flow now measured with the laser-Doppler flowmeter is considered as the stria vascularis, which has an abundant blood flow. No abnormal findings were observed in the spiral ligament, however, its function may have been disturbed as shown by different responses of normalized cochlear blood following application of prostaglandin E_1. Our findings might suggest that the drugs could protect the function of the spiral ligament against acute otitis media.

The mechanism of NOS inhibition now proposed remains only a hypothesis and has not been proven directly, however, the present study did show that intratympanic administration of dexamethasone and NOS-inhibitor were effective to treat for disturbances in the cochlear lateral wall induced by otitis media.

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