Intratympanic Dexamethasone on Cochlear Blood Flow in Guinea Pigs

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Abstract To determine the effect of intratympanically delivered dexamethasone on cochlear blood flow. 29 white Hartly guinea pigs were divided into 2 groups. By laser Doppler flowmeter, baseline data were recorded in the first 15–20 min, followed by 20 min' recording after applying dexamethasone or normal saline, with a record of flow every 5 min in 2 groups. Friedman Test was used to test the change of blood flow in these four timing. There were no significant changes of blood flow after injecting dexamethasone ($p = .18 > .05$) and normal saline ($p = .93 > .05$). The effects of dexamethasone on cochlear blood flow were variable and not significant. The following research would be designed under pathological conditions, such as cochlear ischemia, to show the possible mechanism of dexamethasone for specific inner ear disease. Level of evidence: 3a (SR of case–control studies).

Keywords Steroids · Microcirculation · Round window · Laser Doppler flowmeter

Introduction

This research is to determine the effect of intratympanic (IT) dexamethasone (DEX) on the cochlear blood flow (CoBF). Many inner ear diseases, such as sudden sensorineural hearing loss (SSNHL), are idiopathic although several hypotheses were proposed. There are two commonly accepted theories for the etiology of SSNHL: (A) inflammatory theory (B) circulation theory.

High dose systemic steroids combined with vessel dilators or blood circulation promoters are the mainstream of the therapy for SSNHL. However, long-term use of high dose systemic steroids often causes side effects. Besides, the effective treatment can't be achieved until the blood

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concentration of the drug is high enough to cross the blood-perilymph barrier [1]. Direct administration of the drug to inner ear can avoid the above problems. However, the inner ear lies deep in the skull, making it difficult for the medications to enter.

The round window membrane lies between middle and inner ear. Some medications could infiltrate through it to the perilymph of the inner ear. Clinically, intra-tympanic injection of steroids has been proposed with good results [2, 3]. By this route, there are two potential advantages: (1) Achieving the same concentration in the perilymph with lower dose of steroid than that by systemic administration [2]. (2) Avoiding the side effects from high dose systemic steroid.

The mechanisms of intra-tympanic administration of DEX might include the followings: (A) The semipermeability of the round window [4]. (B) The low molecular weight and high fat-solubility of DEX [5]. (C) DEX receptors in the inner ear [6]. (D) The anti-inflammatory effect of DEX. (E) The effect of DEX on the CoBF. The former four mechanisms have been accepted already. Our research focused on the last.

Materials and Methods

29 White Hartly guinea pigs with good Preyer's reflex were used, including one expired during experiment and two had unstable baseline of CoBF. Valid data were from 25 guinea pigs, 20 received DEX (Oradexon[®], 4 mg/ml) and 5 normal saline.

All guinea pigs were left hungry for 2–3 h. Intraperitoneal pentobarbital sodium of 30 mg/kg was used for deep anesthesia. Supine position on the Harvard[®] hemothermic blanket control unit (Harvard Apparatus, Massachusetts, U.S.) with body warmed and head fixed with head holder on the vibration-proof table under constant indirect illumination. Anal thermometer connected to this unit was used to monitor the body temperature which was maintained between 37.8 and 38.2°C in each animals.

Tracheostomy was performed with a 15# blade. A Harvard[®] rodent ventilator, model 683 (Harvard Apparatus, Massachusetts, United States) was connected to the tracheal tube. The tidal volume was set as 5 ml per kilogram of body weight and respiratory rate 42–46 counts per minute. For systemic blood pressure monitoring, an Intramedic[®] polyethylene tube (PE-10, ID = 0.28 mm, OD = 0.61 mm) was inserted into the left femoral artery. 0.1~0.2 c.c. heparin was infused to avoid occlusion. Then a 3-way plug was connected and systemic blood pressure was monitored through the whole procedures. To monitor the CoBF, we made an incision at ventral medial side of left lower jaw. Under surgical microscope, mastoidectomy

was performed to open the temporal bulla and to expose the cochlea and the round window. The mucosa on the surface of the cochlea to be measured was scratched to eliminate the artifacts from the blood flow of capillary of the mucosa. The probe of Laser Doppler perfusion monitor was connected to micromanipulator. The tip of the probe was put on the surface of the basal turn of cochlea and was micro-adjusted to about 1 mm apart from the surface of cochlea. The baseline CoBF was continuously measured for 20 min. For avoidance of capillarity, we applied DEX or normal saline soaked pledgets on the round window membrane followed by administration of the same agent of 0.1~0.15 c.c. to fill up the middle ear cavity.

After administration of DEX or NS, we then continuously recorded the CoBF for 20–70 min with the sampling frequency of 5 min. The average of the maximal and minimal flow at each time point was recorded as the CoBF value, and the change of CoBF in each time point was calculated by (CoBF in each time point – average CoBF in baseline)/average CoBF in baseline.

We used SPSS 18.0 to do the Friedman Test to compare the change of CoBF in DEX and NS groups. For the relationship of CoBF and Physical conditions, such as body weight, blood pressure, body temperature, the Pearson's correlation analysis was also used.

Results

The valid data were from 29 guinea pigs with the average body weight 363.57 gm, blood pressure 70.84 mmHg, body temperature 38.0°C, which didn't change significantly during the whole process of recording in each animal. There were 24 guinea pigs in DEX group. The average change of CoBF after applying DEX and NS in 4 different timing (5, 10, 15, 20 min) was showed in Table 1 and Fig. 1. There were no significant changes of blood flow after applying dexamethasone ($p = .18 > .05$) and normal saline ($p = .93 > .05$), also see Table 2.

Ten of the guinea pigs in the DEX group were continuously recorded the CoBF for 70 min. The result of their CoBF also showed no significant changes ($p = .86 > .05$), indicating that there was still no change of CoBF after applying dexamethasone even for 70 min, also see Table 3.

Table 1 Average change (%) of blood flow in NS and DEX groups at each time point

	5 min	10 min	15 min	20 min
DEX	0.38%	9.08%	2.42%	−5.08%
NS	0.60%	−2.20%	1.40%	−0.40%

Fig. 1 Average change of blood flow in NS and DEX

Table 2 No statistical significance in change of CoBF in NS and DEX groups. Friedman test was used for the statistical analysis

DEX	N	24
	χ^2	4.911
	Freedom	3
	sig.	0.178
NS	N	5
	χ^2	0.429
	Freedom	3
	sig.	0.934

a. Friedman Test

Table 3 No statistical significance in change of CoBF in DEX after 70 minutes of application. Friedman test was used for the statistical analysis

DEX	N	10
	χ^2	7.79
	Freedom	13
	sig.	0.857

a. Friedman Test

The result of Pearson's correlation analysis showed no significant correlation between body weight, blood pressure, body temperature and CoBF before and after medication.

Discussion

The result of our study showed the IT administration of DEX yielded no immediate or short-term effect on the CoBF of healthy guinea pigs. The CoBF would be affected by the systemic blood pressure, body temperature, subtle movement of the investigated animals, and vibration, noise, room temperature and illumination from the environment.

We controlled every parameter mentioned above and kept them constant during the whole process of recording. It is well accepted that glucocorticoid has anti-inflammation function. However, its effect on blood circulation remains controversial.

In physiological status, 3 different effects of glucocorticoids on blood circulation have been proposed. First, glucocorticoid decreased the blood flow by inhibiting the production of prostaglandin I₂ which is a vasodilator [7]. It also maintained the vascular tone in forearm artery by potentiating vascular responses to noradrenaline [8, 9]. Some researches indicated that glucocorticoids control the blood pressure in rats through increasing the sensitivity of alpha 1-adrenergic receptors to norepinephrine [10]. On the other hand, there were evidences supporting that glucocorticoids increase blood flow by increasing the sensitivity of beta-adrenergic receptors of cultured rat arterial smooth muscle cells through increasing the isoproterenol-stimulated cAMP levels [11]. In the researches of CoBF, Shirwany et al. [12] reported that IT DEX increased CoBF in 6 guinea pigs within 30 min and the effect sustained more than 1 h. The mean increase of CoBF was 29.26%. Lastly, Fukusaki et al. [13] showed that perineural injection of DEX to lumbar nerve root did not change the radicular blood flow. Lamm and colleague [14] revealed no significant change of CoBF by intravenous high (25 mg) and low (2.5 mg) dose prednisolone in normal guinea pigs.

In the pathological status, glucocorticoid effects on the blood flow also showed 3 different ways. First, Hydrocortisone and DEX were proved to inhibit the inducible nitric oxide synthase (iNOS) and reduce the production of NO, with subsequent inhibition of inflammatory reaction and vessel dilatation [15]. Kunz et al. [16] indicated administration of DEX in the mesangial cells stimulated by interleukin-1 would inhibit the performance of iNOS. Mollace et al. [17] showed DEX would inhibit the process of translation from glyceryl trinitrate to NO by lipopolysaccharide. The above reports yielded that DEX decreased blood flow in the various pathologic conditions. On the contrary, Hafezi-Moghadam et al. [18] claimed that vessel dilatation and subsequent blood flow increase were caused by high dose DEX through activating endothelial nitric oxide synthase (eNOS) with subsequent production of nitric oxide (NO) in acute myocardial infarction. Nagura et al. [19] proposed that intravenous hydrocortisone could prevent photochemically induced CoBF decrease with more prominent effect than vasodilators, such as amidotrizoate and ATP (adenosine triphosphate), due to its anti-oxidative effects. Lastly, Lamm and colleague [14] revealed no significant change of CoBF by intravenous high and low dose prednisolone in noise-damaged guinea pigs.

According to the researches and literature review, there are three possibilities of steroid effect on the blood

circulation. First, steroids do not affect the blood circulation. Second, the effect of steroids on the blood circulation is not as obvious as its anti-inflammation effect. Third, steroids only affect the blood circulation in specific conditions, such as tissue inflammation or ischemic state. In addition, we'll have to take the size of vessels into consideration.

IT injection of steroids has been used worldwide to treat sudden deafness. Mammalian and human cochleas have glucocorticoid receptors [6, 20], thus specific reaction is expected when local steroids is given. However, our research didn't show consistent effect of DEX on CoBF, which meant DEX might not affect the cochlea by changing the blood flow. However, our experiment was under physiological condition.

Conclusion

The effect of steroid to blood circulation is still unclear. In order to explore its direct effect in blood circulation, we used healthy mouse to verify this effect. Result indicated no direct effect of steroid on the blood circulation, and the way steroids affect blood circulation may be more complicated than we thought, or through an indirect way. In the future, the effect of DEX on the pathologic CoBF could be explored to further verify the mechanism of how steroids affecting CoBF.

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Conflict of interest None.

References

1. Parnes LS, Sun AH, Freeman DJ (1999) Corticosteroid pharmacokinetics in the inner ear fluids: an animal study followed by clinical application. *Laryngoscope* 109:1–17
2. Chandrasekhar SS (2001) Intratympanic dexamethasone for sudden sensorineural hearing loss: clinical and laboratory evaluation. *Otolaryngol Neurotol* 22(1):18–23
3. Haynes DS, O'Malley M, Cohen S, Watford K, Labadie RF (2007) Intratympanic dexamethasone for sudden sensorineural hearing loss after failure of systemic therapy. *Laryngoscope* 117(1):3–15
4. Goycoolea MV (1992) The round window membrane under normal and pathological conditions. *Acta Otolaryngol Suppl* 493: 43–55
5. Chandrasekhar SS, Rubinstein RY, Kwartler JA, Gatz M, Connelly PE, Huang E (2000) Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents. *Otolaryngol Head Neck Surg* 122(4):521–528
6. Pitovski DZ, Drescher MJ, Drescher DG (1994) Glucocorticoid receptors in the mammalian inner ear: RU 28362 binding sites. *Hear Res* 77(1–2):216–220
7. Axelrod L (1983) Inhibition of prostacyclin production mediates permissive effect of glucocorticoids on vascular tone. Perturbations of this mechanism contribute to pathogenesis of Cushing's syndrome and Addison's disease. *Lancet* 1(8330):904–906
8. Walker BR, Connacher AA, Webb DJ, Edwards CR (1992) Glucocorticoids and blood pressure: a role for the cortisol/cortisone shuttle in the control of vascular tone in man. *Clin Sci (Lond)* 83(2):171–178
9. Walker BR, Williams BC (1992) Corticosteroids and vascular tone: mapping the messenger maze. *Clin Sci (Lond)* 32(6): 597–605
10. Haigh RM, Jones CT (1990) Effect of glucocorticoids on alpha 1-adrenergic receptor binding in rat vascular smooth muscle. *J Mol Endocrinol* 5(1):41–48
11. Jazayeri A, Meyer WJ 3rd (1988) Glucocorticoid modulation of beta-adrenergic receptors of cultured rat arterial smooth muscle cells. *Hypertension* 12(4):393–398
12. Shirwany NA, Seidman MD, Tang W (1998) Effect of trans-tympanic injection of steroids on cochlear blood flow, auditory sensitivity, and histology in the guinea pig. *Am J Otolaryngol* 19(2):230–235
13. Fukusaki M, Miyako M, Nakamura T, Miyoshi H, Sumikawa K, Konishi H et al (2001) Perineural injection to nerve root and radicular blood flow: a clinical study during spinal surgery. *Euro J Anaesthesiol* 18(2):70–74
14. Lamm K, Arnold W (1998) The effect of prednisolone and non-steroidal anti-inflammatory agents on the normal and noise-damaged guinea pig inner ear. *Hear Res* 115(1–2):149–161
15. Radomski MW, Palmer RM, Moncada S (1990) Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 87(24):10043–10047
16. Kunz D, Walker G, Eberhardt W, Pfeilschifter J (1996) Molecular mechanisms of dexamethasone inhibition of nitric oxide synthase expression in interleukin 1 beta-stimulated mesangial cells: evidence for the involvement of transcriptional and post-transcriptional regulation. *Proc Natl Acad Sci USA* 93(1): 255–259
17. Mollace V, Muscoli C, Iannone M, Palma E, Rotiroti D, Romeo F et al (2002) Dexamethasone inhibits the inducible bioconversion of glyceryl trinitrate to nitric oxide. *J Cardiovasc Pharmacol* 39(4):544–551
18. Hafezi-Moghadam A, Simoncini T, Yang Z, Limbourg FP, Plumier JC, Rebsamen MC et al (2002) Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nat Med* 8(5):473–479
19. Nagura M, Iwasaki S, Wu R, Mizuta K, Umemura K, Hoshino T (1999) Effects of corticosteroid, contrast medium and ATP on focal microcirculatory disorders of the cochlea. *Eur J Pharmacol* 366(1):47–53
20. Rarey KE, Curtis LM (1996) Receptors for glucocorticoids in the human inner ear. *Otolaryngol Head Neck Surg* 115(1):38–41