Available online at www.sciencedirect.com
SCIENCE DIRECT
Hearing Research 218 (2006) 89–97
Hearing Research
www.elsevier.com/locate/heares

Research paper

A comparison of dehydration effects of V_2-antagonist (OPC-31260) on the inner ear between systemic and round window applications

Taizo Takeda [a,*], Setsuko Takeda [a], Akinobu Kakigi [a], Teruhiko Okada [b], Rie Nishioka [a], Daizo Taguchi [a]

[a] Department of Otolaryngology, Kochi Medical School, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan
[b] Department of Anatomy, Kochi Medical School, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan

Received 16 December 2005; received in revised form 28 April 2006; accepted 1 May 2006
Available online 14 June 2006

Abstract

V_2-antagonist (OPC-31260 (OPC)) application to the scala tympani reduced endolymphatic hydrops. In the present study, we investigated whether systemic administration or local infusion via the round window (RW application) of OPC would be more suitable for clinical use. In Experiment 1, the increase ratios of the cross-sectional area of the scala media of experimentally induced endolymphatic hydrops were quantitatively assessed among four groups of non-OPC application, RW application of xanthan gum, systemic application of OPC and RW application of OPC. In Experiment 2, the effects of systemic and RW applications of OPC on plasma vasopressin (p-VP) concentrations and plasma osmolality (p-OSM) were investigated. In Experiment 3, endocochlear DC potential (EP) was measured in guinea pigs with the RW application of OPC. Electron microscopic observations of the stria vascularis and the hair cells were also made. Both systemic and RW applications of OPC significantly reduced endolymphatic hydrops. However, systemic application resulted in the distension of the Reissner's membrane in the non-operated ear, which seemed to be caused by elevated p-VP levels resulting from the systemic application of OPC. In contrast, RW application of OPC produced no apparent toxic effects in the inner ear, as indicated electrophysiological or morphological changes. Thus, drug delivery via the round window is more useful for the clinical application of OPC for medical decompression.

Keywords: Vasopressin; V_2-antagonist; OPC-31260; Endolymphatic hydrops; Meniere's disease

1. Introduction

Water is the major component of the body, and especially of the inner ear. The homeostasis of water in the inner ear is essential for maintaining the function of hearing and equilibrium. After aquaporin (AQP) water channels were discovered (Agre et al., 1993), it became clear that these channels likely play a crucial role in inner ear fluid homeostasis. Recently, proteins or mRNAs of AQP1 (Stankovic et al., 1995; Sawada et al., 2002), AQP2 (Kumagami et al., 1998; Beitz et al., 1999; Merves et al., 2000; Sawada et al., 2002; Fukushima et al., 2005), AQP3 (Beitz et al., 1999), AQP4 (Takumi et al., 1998; Minami et al., 1998), AQP5 (Beitz et al., 1999; Mhatre et al., 1999), AQP7 and AQP9 (Huang et al., 2002) were reported to be expressed in the inner ear. Regarding the vasopressin (VP)-mediated AQP2 system (VP-AQP2 system) in the inner ear, we reported that AQP2 mRNA expression was upregulated by the acute application of VP (Sawada et al., 2002), and downregulated by the local application of V_2-antagonist (OPC-31260) in the cochlea

Abbreviations: AQP, aquaporin; EH, endolymphatic hydrops; EP, endocochlear DC potential; IR-L, increase ratios of the length of Reissner's membrane; IR-S, cross-sectional area of the scala media; non-OPC application, animal group without any treatment except for the sac obliteration; p-OSM, plasma osmolarity; p-VP, plasma vasopressin; RW, round window; RW application, local infusion via the round window; S_{max}, maximally expanded cross-sectional area of the scala media, enclosed by the arc-shaped extended Reissner's membrane; SR-S, shrink ratio of the scala media; V_2-R, vasopressin type 2 receptor; OPC, OPC-31260, a selective V_2-R antagonist; VP, vasopressin; VP-AQP2 system, vasopressin-mediated AQP2 system

* Corresponding author. Tel: +81 88 880 2393; fax: +81 88 880 2395.
E-mail address: takedat@med.kochi-u.ac.jp (T. Takeda).

0378-5955/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2006.05.001

as well as in the endolymphatic sac (Takeda et al., 2003), and that V_2-receptor (V_2-R) mRNA expression was down-regulated by chronic application of VP (Kitano et al., 1999). The VP-AQP2 system in the kidney is well known to be regulated via cyclic AMP (Nielsen et al., 1999). We confirmed that the VP-AQP2 system in the inner ear was also regulated via cyclic AMP, as indicated by the fact that systemic application of lithium, an inhibitor of adenylate cyclase, reduced AQP2 protein expression both in the stria vascularis and in the endolymphatic sac (Fukushima et al., 2005). Since the stria vascularis and endolymphatic sac are thought to be the main sites of the secretion and/or absorption of endolymph (Sterkers et al., 1988), the homeostasis of endolymph is thought to be, in part at least, under the control of the AQP2 water regulation system mediated by vasopressin (VP-AQP2 system).

We reported that p-VP levels were elevated in cases of endolymphatic hydrops, including Meniere's disease (Takeda et al., 1995), and that chronic application of VP produced endolymphatic hydrops (EH) in guinea pigs (Takeda et al., 2000). Such clinical and experimental lines of evidence suggest that VP-induced over-accumulation of endolymph is one of the causative factors of the formation of EH, one of the characteristic morphological findings of Meniere's disease. EH formation, if due to the mal-regulation of the VP-AQP2 system in inner ear fluid, could be prevented by inhibitors of the VP-AQP2 system. Actually, the infusion of OPC-31260, a competitive antagonist of V_2-R (Yamamura et al., 1992), into the scala tympani reduced EH remarkably (Takeda et al., 2003). These experimental results indicate that the application of OPC will provide a new treatment strategy for Meniere's disease. However, direct infusion of OPC into the inner ear is not suitable for clinical application. In the present study, we investigated whether or not the same effect was also obtained by systemic administration or local infusion via the round window (RW application), which are better suited for clinical use. Furthermore, the presence of inner ear injuries resulting from the RW application of OPC was studied electrophysiologically and morphologically.

2. Materials and methods

Three experiments were performed. Experiment 1 was designed to morphologically investigate the effects of systemic and RW application of OPC on experimentally induced endolymphatic hydrops. Experiment 2 was designed to investigate the effects of systemic application of OPC on p-VP concentrations. In Experiment 3, the influence of RW application of OPC on endocochlear d-c potential (EP) was examined. Electron microscopic findings of the stria vascularis and the hair cells were also studied. These experiments were approved by the Kochi Medical School Animal Care and Use Committee, which conform to The Animal Welfare Act and the guiding principles for animal care formulated by the Ministry of Education, Culture, Sports and Technology, Japan.

2.1. Experiment 1

Thirty-five guinea pigs were used in this experiment. All animals received the surgical obliteration of the endolymphatic duct in the left ear and were maintained undisturbed and freely moving in individual cages with free access to water and standard chow in a quiet room. Four weeks after the obliteration, the animals were divided into four groups of non-OPC application ($n = 10$), RW application of xanthan gum ($n = 5$), systemic application of OPC ($n = 10$) and RW application of OPC ($n = 10$). In the non-OPC application group, animals were sacrificed without any treatment. In the systemic application group, OPC was given transorally four times at a dose of 100 mg/kg/8 h. Oral administration was performed via a rubber catheter introduced into the esophagus. Animals were sacrificed 6 h after the last administration. In the RW application groups, the round window was exposed via a retroauricular approach under general anesthesia with an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). Xanthan gum dissolved in distilled water and a gelatinform of OPC (1 mg/animal) mixed with xanthan gum and distilled water were placed on the round window in RW application of xanthan gum and OPC, respectively, and then the retroauricular wound was closed with sutures. Thereafter, the animals were maintained for 30 h and then were sacrificed. All animals were subjected to quantitative assessment of volumetric changes of the endolymphatic space.

All animals were transcardially perfused with physiological saline solution under deep anesthesia by a peritoneal injection of pentobarbital, and fixation was performed with 10% formalin. The temporal bones of both sides were removed and postfixed in 10% formalin solution for 10 days or more. Thereafter, they were decalcified with 5% trichloroacetic acid and dehydrated in a graded ethanol series. They were embedded in paraffin and celloidin. The prepared blocks were cut horizontally into 6 μm sections. The sections were stained in hematoxylin and eosin, and observed under a light microscope.

2.1.1. Measurement procedure

For the quantitative assessment of changes of the endolymphatic space, the change ratios of the length of Reissner's membrane and the cross-sectional area of the scala media were measured from the mid-modiolar sections of the cochlea. Fundamentally, the measurement was performed as previously described (Takeda et al., 2000). In general, one specimen was used for the analysis of one cochlea. When the plane of the sections slightly deviated from the mid-modiolar axis, the sections that were cut closest to the mid-modiolar plane in individual turns were used. For this analysis, the following 4 parameters (Fig. 1) were measured in the basal, 2nd, 3rd and apical turns, not including the hook portion: (1) the length of