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Inner ear histopathology in "nervous Pointer dogs" with severe hearing loss

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Abstract

Ten puppy dogs (82, 131 or 148 days-old) from a Pointer cross-colony, exhibiting a juvenile severe hearing loss transmitted as an autosomal recessive trait, were used for histopathological characterization of the inner ear lesion. Immunostaining with calbindin, Na,K-ATPase, cytokeratins, S100, S100A1 and S100A6 antisera were helpful in identifying the different cell types in the degenerated cochleae.

Lesions, restricted to the Corti's organ and spiral ganglion, were bilateral but sometimes slightly asymmetrical. Mild to severe lesions of the Corti's organ were unevenly distributed among the different parts of the middle and basal cochlear turns while the apical turn remained unaffected at 148 days.

In 82 day-old puppies ($n = 2$), severe lesions of the Corti's organ, meaning that it was replaced by a layer of unidentifiable cells, involved the lower middle and upper basal turns junction area, extending in the upper basal turn. Mild lesions of the Corti's organ, with both hair and supporting cells abnormalities, involved the lower middle turn and extended from the rest of upper basal turn into the lower basal turn. The outer hair cells (ohc) were more affected than the inner hair cell (ihc). The lesions extended towards the basal end of the cochlea in the 131 ($n = 5$) and 148 ($n = 3$) day-old puppies. Additionally, the number of spiral ganglion neurons was reduced in the 131 and 148 day-old puppies; it is earlier than observed in most other canine hereditary deafness. These lesions were interpreted as a degeneration of the neuroepithelial type. This possible animal model might provide information about progressive juvenile hereditary deafness and neuronal retrograde degeneration investigations in human.

Keywords: Dog; Hearing loss; Hereditary; Neuroepithelial degeneration

1. Introduction

Deafness has been reported in 53 dog breeds and hereditary factors are suspected in dog breeds with high prevalence of congenital deafness (Strain, 1996). Inner ears pathological changes have been described in 19 dog breeds only and deafness is usually associated with a cochleosaccular (the most frequently described) or neuroepithelial type of degeneration (Hiraide and

Abbreviations: BAER, brainstem auditory evoked potentials; BSA, bovine serum albumin; NHS, normal horse serum; PD, postnatal days; SPL, sound pressure level; TBS, Tris buffer saline

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Paparella, 1988; Strain, 1996; Coppens et al., 2000b, 2001a, 2003b).

In cochleosaccular type of degeneration, lesions are characterized by atrophy of the stria vascularis, collapse of the cochlear duct, degeneration of the Corti's organ, abnormal tectorial membrane, and collapse of the sacculi (Steel and Bock, 1983; Strain, 1996). The degeneration of the spiral ganglion is delayed by months to years (Mair, 1976; Strain, 1996; Niparko and Finger, 1997). Cochleosaccular degeneration has been commonly described in Dalmatian in which the inherited nature of deafness has been well documented although the mode of inheritance remains controversial (Greibrockk, 1994; Famula et al., 1996, 2000, 2001; Muhle et al., 2002).

Neuroepithelial type of degeneration has been described in a family of Dobermann and in Shropshire terrier dogs (Igarashi et al., 1972; Wilkes and Palmer, 1992). Lesions are characterized by degeneration of Corti's organ with normal stria vascularis, normal tectorial membrane, no collapse of the cochlear duct and normal sacculi (Steel and Bock, 1983; Wilkes and Palmer, 1992; Strain, 1996). The degeneration of the spiral ganglion is largely delayed too (Igarashi et al., 1972; Steel and Bock, 1983; Wilkes and Palmer, 1992). An additional type of neuroepithelial degeneration, characterized by Corti's organ degeneration and early spiral ganglion neurons loss, has been described in a congenitally deaf Rottweiler puppy (Coppens et al., 2001a).

Beside information about dog deafness, detailed descriptions of inner ear lesions in this species may also be useful as dogs have been proposed as a suitable animal model to study human inner ear diseases and cochlear implant evaluation (Niparko et al., 1993; Lalwani et al., 1997; Niparko and Finger, 1997; Harvey et al., 2001; Sockalingam et al., 2002; Rak et al., 2002). Progress in gene localization is now under way in the canine species and Myo XVA gene, known to be involved in human deafness, has been recently evidenced in this species, opening prospects for its use as an animal model in heritable deafness studies (Rak et al., 2002).

A high incidence of juvenile bilateral deafness has been described in a colony of Pointer dogs selectively bred for excessive nervous behaviour (Klein et al., 1988; Steinberg et al., 1994). Although 74% of nervous dogs have a bilateral hearing loss (no brainstem auditory-evoked response (BAER) could be detected), this study has pointed out that hearing defect does not contribute to the nervous behaviour, as deafness is not always a shared defect among nervous dogs. Excepted for the hearing defect and the stereotypical behaviour, no other pathological change or vestibular defect has been observed in this colony (Klein et al., 1988; Steinberg et al., 1994). Brainstem auditory evoked response testing have revealed that hearing deficit may appear as soon as 21 days of age, progressively leading to profound deafness during the first half of the second month of life (Steinberg et al., 1994). Breeding experiments have indicated that deafness in this canine colony is an autosomal recessive trait that is fully penetrant (Steinberg et al., 1994).

Precise morphological descriptions are needed to classify hearing losses and the purpose of the present report is to detail the histopathological changes found in this readily available possible animal model.

Immunohistochemistry using calbindin, cytokeratins, Na,K-ATPase, S100, S100A1 and S100A6 antibodies were also undertaken in this study as they make it possible to recognize specific cell types or structure in the degenerated cochleae (Coppens et al., 2000a, 2001a,b, 2003a).

2. Materials and methods

2.1. Animals

Inner ears were collected from 10 Beagle-Pointer cross-puppies (males and females) from three different litters (Table 1). Pointer dogs came from a family of dogs selectively bred for excessive nervous behaviour, but showing also a high prevalence of deafness among the behavioural nervous dogs. This deafness is an autosomal recessive trait that is fully penetrant (Steinberg et al., 1994). No clinical vestibular involvement was detected in any dogs in this family. Subsequently, a colony was established by outcross and F1 backcross breeding to hearing beagle and keeshond Pointer dogs. The 10 puppies in this study are derived from that colony. The dogs were cared for according to the principles of the NIH Guide for the Care and Use of Laboratory Animals (NHI Grant RR02512).

For hearing by BAER tests, the puppies were sedated with morphine (1.5 mg/kg) and acepromazine maleate (0.5–1.2 mg/kg) intramuscularly. They also received atropine (0.02 mg/kg) by the same route. Averaged brainstem auditory evoked potentials were recorded with an electrodiagnostic apparatus (Dantec Cantata, Medtronic, Skovlunde, Denmark). Rarefaction click stimuli, (100 ms in duration, 10 Hz repetition rate) were delivered to the tested ear through an insert phone, the untested ear receiving a masking noise, 40 dB below the click level (Steinberg et al., 1994). Both ears were successively tested. Thresholds were looked for by decreasing the click stimuli from 132 dB SPL (sound pressure level, maximum output of the stimulator) by 3 dB steps and threshold was defined as the highest stimulus where no BAEP could be recorded (gain setting 2 μv/div). The dogs were considered as suffering severe hearing loss when the threshold was 102 dB SPL or above, i.e. 70 dB above average threshold in normal