DEVELOPMENT OF INNERVATION OF HUMAN PERMANENT SUCCESSOR TOOTH GERMS

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Introduction

The importance of innervation in tooth development has been a dispute until Jakobsen et al., (1991) reported a case of unilateral absence of the teeth in the mandible where the mandibular canal was absent. Luukko et al., (2008) reported that navigation of trigeminal nerve and tooth patterning take place in a spatio-temporally directed manner.

Several studies have been conducted on animals and few studies have been carried out using human foetuses where innervation of deciduous teeth has been reported (Christensen et al., 1993; Zmijewska et al., 2003). There is no information available on the innervation of permanent tooth germs. The aim of the present study was to study the development of innervation of human permanent successor tooth germs at different developmental stages.

Materials and methods

The study material comprised of spontaneously aborted human foetuses and dead neonates which were collected after obtaining the consent from the parents and the hospital authority. Four dead foetuses aged 24-33 weeks and a 4 days old dead neonate who were free from visible physical deformities were included in the study sample. Upper and lower jaws were dissected and fixed in buffered 4% paraformaldehyde for 48 hours at 4°C followed by decalcification in 8% ethylene-diamine-tetraacetic acid (EDTA) for 2-3 weeks. Once decalcified, the jaws were processed to obtain 4 µm thick paraffin and 15 µm thick frozen sections. All the sections were made in a bucco-lingual direction of the jaw. Paraffin sections were used to prepare haematoxylin and eosin staining, and immunohistochemistry for PGP 9.5 (protein gene product 9.5) and S-100 protein. Dilutions of PGP 9.5 (Ultracone, UK) and S-100 protein (Sigma, USA) were 1:5000 and 1:1000 respectively. Frozen sections were stained for non-specific cholinesterase by Karnovsky and Roots (1964) method.

Results

Haematoxylin and eosin staining

The sections demonstrated permanent tooth germs at "bud", "cap" and "early bell" stages. They were seen lingual to the deciduous predecessor tooth germs at "late bell" stage. All tooth germs depicted normal histological appearance described elsewhere (Ten Cate et al., 2003).

Cholinesterase (ChE) cytohistochemistry

Cholinesterase stains the Schwann sheet of immature nerves. ChE positive nerve fibers were observed in the ectomesenchyme which was seen surrounding the epithelial growth of permanent tooth germs at "bud" stage (Fig.1). Tooth germs at the "cap" and "early bell" stages showed the presence of ChE stained nerve fibers in the ectomesenchyme surrounding the enamel organ (Fig 2). In addition, thick nerve bundles were seen emerging toward the base of permanent tooth germs at "cap" (Fig 3) and "early bell" stages.

Immunohistochemistry of PGP 9.5 and S-100

Protein gene product 9.5 and S-100 are specific markers to identify nerve axons and Schwann sheets respectively. PGP 9.5 immunoreactive nerve fibers were identified in the ectomensenchyme in close proximity to the enamel organ of tooth germs similar to nerves depicted by ChE reactions. Some PGP 9.5 positive nerves were found among cells of the dental follicle and in the condensed ectomensenchyme inside the follicle of tooth germs at "cap" stage (Fig 4). S-100 positive nerve fibers also showed a staining pattern similar to that of PGP 9.5.

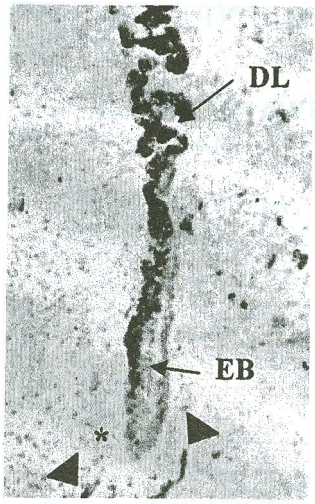

Figure 1. ChE reactive nerve fibers (arrowheads) are seen in the ectomesenchyme (*) in close proximity to the tooth germ at "bud" stage. EB; epithelial bud, DL; dental lamina X100

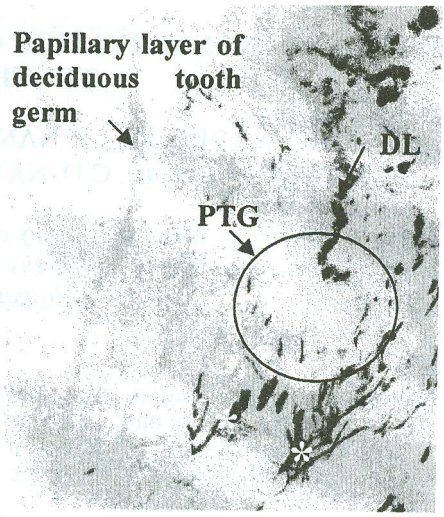

Figure 3. ChE reactive nerve bundle (*) is seen emerging toward the base of a tooth germ at "early cap" stage. DL; dental lamina, PTG; permanent tooth germ X40

Figure 2. ChE reactive nerve fibers (arrows) are seen in the condensed ectomesenchyme surrounding the enamel organ (EO) of a tooth germ at "cap" stage. DL; dental lamina X100

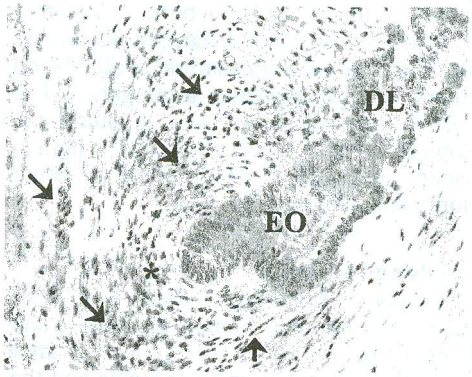

Figure 4. PGP 9.5 positive nerve fibers (arrows) are seen in the ectomesenchyme in close proximity to the tooth germ at "early cap" stage. Some nerve fibers are seen in the condensed ectomesenchyme (*). EO; enamel organ, DL; dental lamina X100.

Discussion

Since histological features of tooth germs conformed to the normal structure described elsewhere (Ten Cate et al., 2003) it is reasonable to extrapolate the present findings to events in normal human tooth development.

The present finding of appearance of nerves in the ectomesenchyme in close proximity to tooth germs at "bud", "cap" and "early bell"

stages corresponds to the reported evidence of innervation of deciduous tooth germs (Christensen et al., 1993; Zmijewska et al., 2003). Moreover, the presence of nerves among cells of the dental follicle in tooth germs at "cap" stage is similar to that of deciduous tooth germs. However, the occurrence of thin nerves within the condensed ectomensenchyme inside the dental follicle of tooth germs at "cap" stage has not been reported for deciduous teeth.

Conclusion

It is reasonable to state that development of innervation pattern of tooth germs is relatively same in both dentitions.

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