HEPARIN DELAYS TOOTH MORPHOGENESIS AND CELL DIFFERENTIATION

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The embryonic tooth is an excellent tool to analyze epithelial-mesenchymal interactions governing morphogenesis and cytodifferentiation. The extracellular matrix between the inner epithelium and the dental mesenchyme is a natural reservoir for diffusible molecules such as growth factors (Ruch, 1987; Thesleff et al., 1992). A number of observations in different experimental systems suggest that circulating matrix components and tissue-derived autocrine or paracrine factors are important for tooth morphogenesis and cell differentiation. Heparin is a highly sulphated glycosaminoglycan structurally related to heparan sulphate that binds various extracellular matrix components to cells. Some of these components are growth factors, such as fibroblast growth factors (FGF), midkine (MK), pleiotrophin (PTN) and hepatocyte growth factor (HGF). These growth factors have been described as molecules that affect tooth morphogenesis and cell differentiation.

In the present work, we have studied the effect of heparin on molar and incisor histomorphogenesis and cell differentiation using 14 day-old mouse embryos (E14) first molars and incisors cultured in a serumless, chemically-defined medium. We demonstrated that heparin alters molar cusp formation and delays odontogenesis in molar and incisor explants.

Organ culture: Swiss 14-day pregnant mice (vaginal plug=day 0) were sacrificed and the embryos (E14) were removed from uterus. First lower embryonic molars and incisors were isolated and cultured for 4, 6 or 8 days on a semisolid medium, as previously described (Mark et al., 1990). Heparin (Sigma Chemical, Co.) was added to the culture medium to a final concentration of 50µg/ml. Controls were cultured in the absence of heparin. Culture medium was changed every two days. Then, molar and incisor explants were fixed in Bouin-Hollande solution, dehydrated, embedded in paraffin and cut in 4µm thick sections. Staining of sections was performed with hematoxylin and eosin.

Effect of heparin on tooth development: The stage of development of E14 first molars (cap stage) is characterized by the presence of a primitive enamel organ, mesenchymal aggregation and dental sac cells surrounding the outer dental epithelium (Figure 1A). E14 molars cultured for 4 days on agar-medium stayed at early bell stage of development and dental cusp morphogenesis was initiated. The cells of the dental papilla and inner dental epithelium remained undifferentiated (Figure 2A). After 6 days of culture, dental cusps developed and pre-odontoblasts were alligned and polarized, but were still undifferentiated. Inner dental epithelium resembled a pseudostratum with tall columnar cells (Figure 2C). After 8 days, mesenchymal cells differentiated and deposited pre-dentin. Inner epithelium contained polarized ameloblasts and nuclei came near stratum intermedium (Figure 2E).

Significant changes in tooth development were observed after addition of exogenous heparin in tooth germ cultures. When dental organs were cultured in the presence of heparin we observed an anomalous development with a clear inhibition of odontogenesis and a significant delay in tooth development when compared to controls. E14 organ molar explants treated with heparin for 4 days showed an odd morphogenesis: inner dental epithelium remained flat with cuboidal and undifferentiated cells, and cervical loop and dental cusp formation were incomplete (Figure 2B). First molars treated for 6 days presented a similar aspect, but cervical loops were further developed (Figure 2D). After 8 days of culture in medium with heparin, mesenchymal and epithelial cells remained undifferentiated; however, dental cusps progressed.

Odontogenesis of rodent incisors follows a specific and asymmetric pattern of development (lingual versus labial), the lingual surface being enamel free. The morphology of E14 incisor is shown in Figure 1B. E14 incisors cultured in the presence of heparin preserved tooth morphogenesis, but we observed a delay in odontoblast and ameloblast differentiation respect to control incisors. Thus, E14 incisors treated for 4 days had undifferentiated mesenchymal cells and inner dental epithelium was disorganized (Figure 3B) whereas in control experiments, odontoblasts were alligned and polarized in labial side, and inner dental epithelium remained as a fence (Figure 3A). Incisors treated for 6 days had preodontoblasts (Figure 3D), but in controls we observed a continuous gradient of odontoblast differentiation from distal to proximal region of incisor, with secretion of predentin followed by a gradient of preameloblats polarization (Figure 3C). After 8 days of treatment with heparin, incisors had functional odontoblasts and polarized preameloblasts appeared in the inner dental epithelium (Figure 3F); however, controls had functional ameloblasts (Figure 3E).

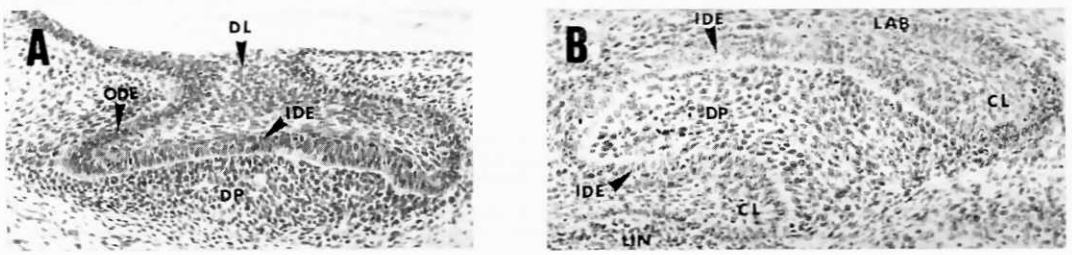

Figure 1. First molar (A) and incisor (B) of a 14-day-old mouse embryo. DL: dental lamina; IDE: inner dental epithelium; DP: dental papillae; ODE: outer dental epithelium; LAB: labial side; LIN: lingual side; CL: cervical loop. Magnification: x200

In the tooth organ formation, a coordinated interaction between epithelia and mesenchyme occurs. In these proccesses molecules such as growth factors and extracellular matrix interact as tooth development progresses. Some recent experiments indicate that the association of heparin and specific growth factors (TGFß1, BMP2, IGF1) induced functional odontoblast differentiation in dental papillae cultures (Bègue-Kirn et al., 1994). The addition of heparin to our cultures had a significant inhibitory effect on odontogenesis: incomplete dental cusp development and retardation of cell polarization and differentiation were observed. This inhibition of tooth development was similar to that observed in experiments performed by Sato et al. (1993). These authors indicated that exogenous heparin did not affect basement membrane formation or the interactions with specific growth factors of the tooth germ in vitro. However, we suggest that the exogenous administration of heparin, exceeding its physiological concentration, could immobilize heparin-binding growth factors and prevent their diffusion from the extracellular matrix toward membrane receptors and their signal transduction, both essential for normal tooth germ development.

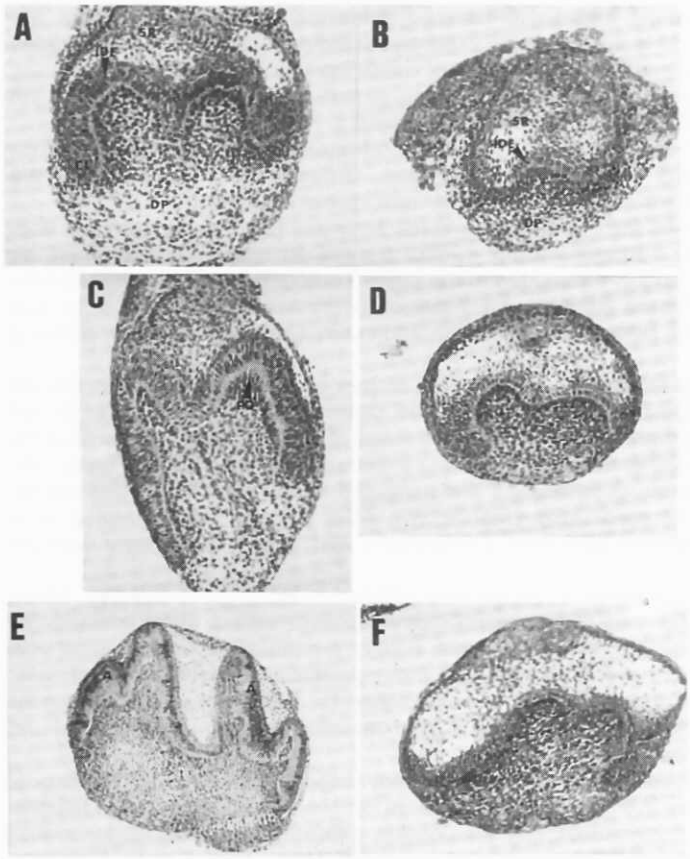

Figure 2. E-14 first molar cultured in the absence (A, C, E) or presence (B, D, E) of heparin (50µg/ml) for 4 (A, B), 6 (C, D) or 8 (E, F) days. SR: stellate reticulum; IDE: inner dental epithelium; DP: dental papillae; CL: cervical loop; PO: pre-odontoblasts; A: ameloblasts. Magnification: x200; (E) x100

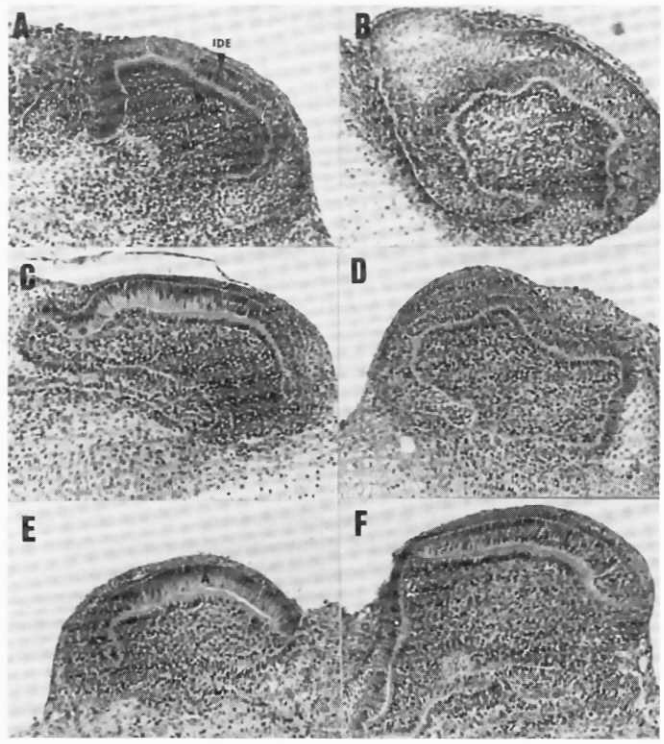

Figure 3. E-14 incisor cultured in the absence (A, C, E) or presence (B, D, F) of heparin (50µg/ml) for 4 (A, B), 6 (C, D) or 8 (E, F) days. IDE: inner dental epithelium; PO: pre-odontoblasts; DP: dental papillae. Magnification: x200

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