Original Article

Patterns of Glycoconjugate Distribution during Molar Tooth Germ Development in Mice

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Abstract:

Objective: The aim of the present study was to evaluate the structure and distribution of Glycoconjugates during molar tooth germ development in mice

Materials and Methods: Sixteen tooth germs were obtained from BALB/c mice embryos 15 to 18 days post-gestation and fixed in 10% formalin. After routine tissue processing, 5 µm sections were cut and stained with BSA1-B4 and PNA using the lectin histochemical method. All slides were evaluated by light microscopy.

Results: Both lectins showed positive reaction in the tooth germ but with spatiotemporal differences. During bell stage, the reaction was strong with BSA1-B4 but moderate with PNA. Strong PNA uptake was observed in the odontoblastic and ameloblastic nuclei along with the apical cytoplasm of the ameloblasts.

Conclusion: Although the lectins that were used in the present study recognize the same terminal sugar residue, they reacted with different disaccharide sequences with various penaltomer sugars. Therefore it may be assumed that the pattern of affinity for different parts of the developing tooth germ such as ameloblasts and odontoblasts is different in various lectins.

Key Words: Development; Glycoconjugates; Lectin; Tooth

INTRODUCTION

The developing tooth is an excellent model to study the molecular mechanisms of morphogenesis. It is well known that tooth morphogenesis and differentiation of the dental cells are guided by interactions between epithelial and mesenchymal tissues [1-7]. Dental epithelium in mice is capable of inducing tooth formation just before the bud stage, whereas its tooth-forming potential relocates from the epithelium to the dental mesenchyme. [8-11]. However, the molecular mechanisms involved in dental cell differentiation are not well understood.

One of the critical molecules involved in embryonic development is glycoconjugates that are detected by lectins. Glycoconjugates have important roles in cell-cell interaction, cell migration and proliferation [12-17]. Lectins are glycoproteins that can specifically react with terminal sugars. 'Lectin' comes from the Greek word 'legere', which means 'to select'. Most lectins act in a non-enzymatic manner and demonstrate a non-immune origin. Lectins are constantly encountered in nature [18]. These molecules may bind to a carbohydrate moiety occurring free in a solution or as a part of a protein/particulate body and can agglutinate cells and/or precipitate glycoconjugates. Lectins extracted from plants can conjugate
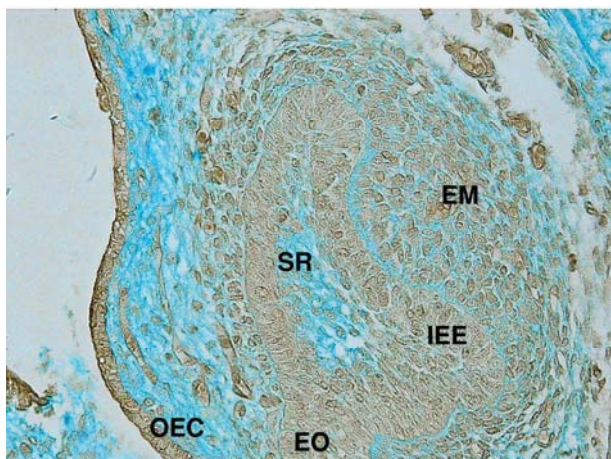
Fig 1. Cap stage; BSA-1 Reaction; EO; Enamel Organ, IEE; Inner Enamel Epithelium, OEC; Oral Epithelium Cells, SR; Stellate Reticulum × 200
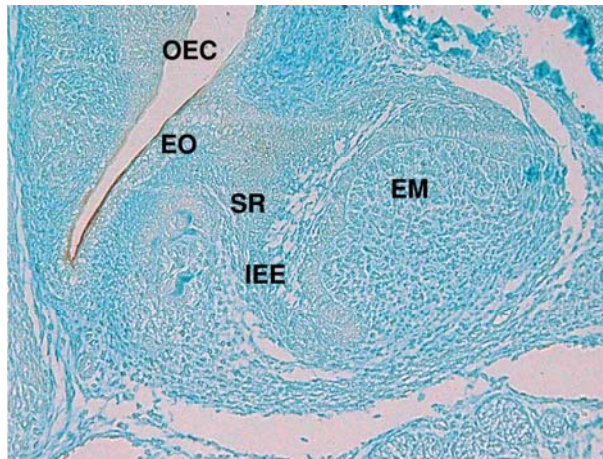

Fig 2. Cap stage; PNA Reaction: EO; Enamel Organ, IEE; Inner Enamel Epithelium, OEC; Oral Epithelium Cells, SR; Stellate Reticulum × 200

with labels and are good tools to detect terminal sugars in different tissues [12-13]. These molecules have the potential to participate in both differentiation and maturation [19]. Many studies have been performed to describe the distribution of oligosaccharide chains in developing teeth [12-16,19,20]; however, the distribution pattern of these molecules shows a considerable amount of diversity in developing and tumoral tissues [12-16,21,22]. Therefore, it seems that further investigation can be helpful to elucidate the roles of these sugar residues in mouse models. The aim of the present study was to evaluate the structure and distribution of Glycoconjugates during molar tooth germ development in mice.

MATERIALS AND METHODS

BALB/c mice with an age range of 2-4 months, weighing approximately 25-30 grams were used in the present study. Males and females were caged together overnight. Observa-

tion of the vaginal plug was considered as day zero of gestation. The pregnant mice were kept at standard conditions with free access to water and food. Sixteen mice embryos were collected from the 15th to 18th days of gestation. Their heads were fixed, processed and sectioned serially.

Lectin Histochemistry:

The Lectin histochemical method was used for detecting glycoconjugates. Deparaffinized and dehydrated 5 μm sections were immersed in 0.1 M PBS containing 0.1mM $CaCl_2$, $MgCl_2$ and $MnCl_2$ (PBSc), followed by 1% H_2O_2 in methanol for 10-15 minutes. After rinsing with PBSc, they were incubated with peroxidase conjugated lectins including PNA (L7381; Sigma, USA) and BSA1-B4 (L5391; Sigma, USA) that bind galactose (Gal) and N acetyl galactoseamine (Gal-Nac) for two hours at room temperature, respectively. The lectins were diluted to a concentration of 10μg/ml [23,24].

Table 1. Lectin reactivity of the cells and ECM in the tooth germ at cap stage

Lectins	Oral Epithelial Cells	Basement Membrane	Enamel Organ	Ecto-mesenchyme	Dental Sac	Extracellular Matrix	Capillary Endothelium
BSA-1	+++ *	–	+++	+++	+++	–	–
PNA	–	–	+	–	–	–	–

* Evaluation of binding indicates staining intensity on a subjectively estimated scale: no staining (–), little staining (+), moderate staining (++), intense staining (+++)
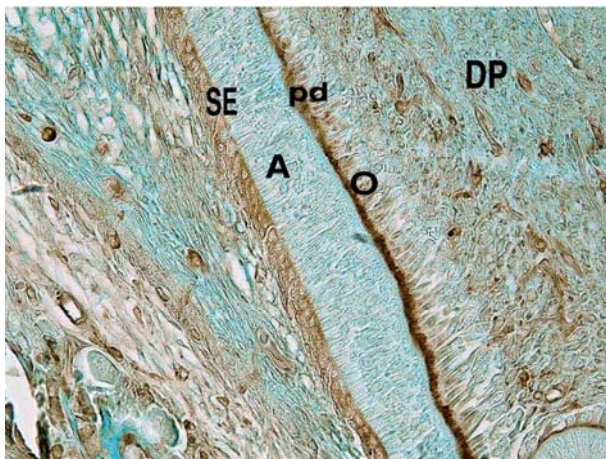
Fig 3. Bell stage; BSA-1 Reaction: A; Ameloblasts, O; Odontoblasts, pd; predentine, DP; Dental Papilla, SR; Stellate Reticulum × 400
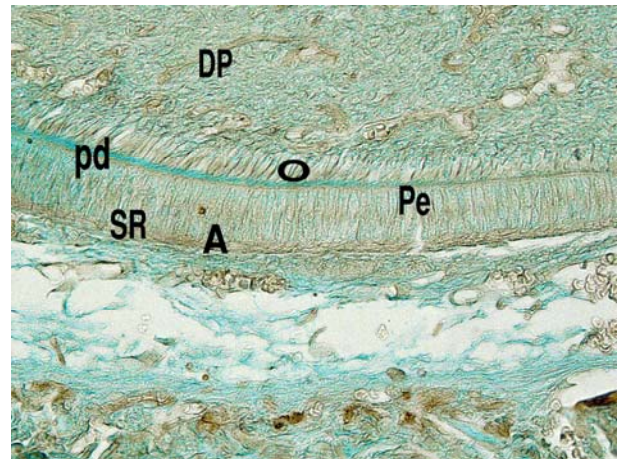

Fig 4. Bell stage; PNA Reaction: A; Ameloblasts, O, Odontoblasts, pd; predentine, DP; Dental Papilla, SR; Stellate Reticulum, Pe; Preenamel × 400

Following final washing with buffer, the binding sites were visualized by incubating sections in 0.03% diaminobenzidine containing 200μl H_2O_2 in PBS for 10 minutes. The sections were counterstained with alcian blue. To compare the intensity of positive staining, we used an arbitrary scale from 0 to 4, ranging from no- to strong reaction [25]. It has been shown that retinal epithelium and endothelial cells in different tissues can react with PNA [26] and BSA1-B4 [27], respectively; therefore a composite of several tissues including retinal pigmented epithelium, testis and … were used as positive control. Negative control was obtained by omitting lectins in a number of slides in order to establish negative reaction. The study protocol was approved by the Ethics committee of Birjand University of Medical Sciences.

RESULTS

A positive reaction for both Lectins was identified in the studied tooth germ, but with spatiotemporal differences. BSA1-B4 reaction occurred during the cap stage with "strong" intensity in the oral epithelium, enamel organ and ectomesenchyme which was limited to the cells (Fig 1). The extracellular matrix (ECM) began to take-up lectin at the bell stage. The predentin and odontoblasts of the apical region were more reactive to BSA1-B4 in this stage. In addition to the thick amorphous matrix under the ameloblasts, the dental papilla and the endothelial cells also demonstrated lectin uptake (Fig 2).

Oral epithelial cells showed positive reaction to PNA, but the basement membrane was negative. The enamel and ectomesenchyme were also positive but stained "weakly" for this lectin (Fig 3). At the bell stage a "strong" reaction to PNA was observed in the Golgi zone of the odontoblasts and ameloblasts in addition to the apical cytoplasm of the ameloblasts (Fig 4). Lectins have been suggested to label the Golgi compartment of cells in odontogenic tissues. The thick amorphous matrix under the ameloblasts was PNA positive but the preden tin was negative except for a thin layer of newly formed dentin adjacent to the ameloblasts. Dispersed reaction was seen in the dental papillae. Lectin reactivity in different stages of tooth development is shown in Tables 1 and 2.

DISCUSSION

Glycoconjugates have been suggested to participate in many critical events of embryogenesis by establishing the dental morphologic

Table 2. Lectin reactivity of the cells and ECM in the tooth germ at bell stage.

Lectins	Ameloblasts			Odontoblasts			Pre-dentine	Dental Papilla	Capillary Endothelium
	Apical area	Nucleus	Base	Apical area	Nucleus	Base			
BSA-1	–	+	+++	+++	+++	–	+++	++	+++
PNA	+++	++	+++	–	++	–	–	+	+

no staining (–), little staining (+), moderate staining (++), intense staining (+++)

pattern and ECM secretion [19]. They regulate cell-cell and cell-matrix interactions [8-17]. Our data indicated differential expression of lectin residues in various parts of the tooth germ that highlights the importance of these sugar residues in tooth development.

In the present study, PNA positive residues were found in ameloblasts which is in accordance with a study conducted by Lemous et al [14]. It has been shown that Peanut lectin influences epithelial proliferation in some cancers [21,22]. PNA can affect normal pancreatic growth [28] and PNA residues may also have the same function in regulation of cell proliferation during tooth development. In addition it has been suggested that PNA binding structures may be important for cellular interaction during morphogenetic processes. Ameloblasts have an inductive effect on the dental papilla resulting in the formation of odontoblasts. PNA reacted residues may be related to these types of interactions.

It has been demonstrated that an N-acetylgalactosamine rich matrix component is differentially expressed during odontogenesis [16-17]. However, according to the results obtained in the current investigation, predentin reaction to PNA was negative. Proteoglycans like D-galactose and N-acetylgalactosamine sugars bound to PNA are considered as important components of the dentin matrix. The production of these sugars in adult rat odontoblasts is related to PNA binding sites [29]. This was in contrast to the results obtained in the current investigation regarding PNA reaction in mouse tooth germs. A possible explanation could be that odontoblast secretion is dif-

ferent after tooth eruption; therefore considering that the present study was performed on mouse embryos, we did not find reactive residues of PNA in the predentin.

Growth hormone can regulate the glycoconjugate content of the extracellular matrix that is essential for normal tooth development [16], and becomes functional after birth. Various PNA expression patterns may be related to the effects of growth hormone.

According to our results, BSA1-B4 stained the cell surfaces in the cap and early bell stages. Moderate reaction to galactose has been reported in other studies [15]. The presence of glycoconjugates in calcified tissues like dentin may be related to mineral deposition. It has been suggested that highly charged plasma membranes produced by glycocalyces are responsible for the transportation of mineral and/or organic materials between ameloblasts and extracellular fluid that is organized by glycoconjugates. [12]. Amelogenins are major enamel proteins and their properties are similar to lectins, hence they can bind to glycoconjugates during enamel formation [30].

CONCLUSION

The presence or absence of the glycoconjugates may have a critical role in tooth development because of their spatiotemporal distribution. However, more investigation is required in order to gain a better understanding of their molecular mechanisms.

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