The Influence of Cryopreservation on the Oligosaccharide Expression of Tooth Germ - Sugar Chain Expression of Frozen Tooth Germs -

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Abstract: To explore the influence of preservation by freezing on tissues of tooth germ, we examined changes in histological architectures and glycoconjugate expression detected by lectins, in tooth germs, with or without freezing, of postnatal 5 day rats. The results acquired were as follows: tissue architectures and cellular morphologies were similar between frozen and control groups. Stellate cells of enamel medulla and pulpal cells were, however, slightly atrophic at the control group. With lectin staining, there are no remarkable changes between frozen and control groups. From these results, we conclude that the influence of preservation by freezing was relatively small.

Key words: tooth germ, cryopreservation, histochemistry

Introduction

In clinical dentistry, transplantation at the stage of tooth germ has hardly been challenged. Furthermore, if frozen tooth germs could be transplantable, the chance of clinical application would spread, but the experimental researches regarding the transplantation of frozen tooth germs are quite limited to be available. Therefore, we examined changes in histological architecture and sugar chain expression detected by lectin histochemistry, Alcian blue (AB) and Periodic acid Schiff (PAS) staining, in frozen and non-frozen tooth germs, since sugar chains are known to participate in cell growth, tissue development and differentiation, and hard tissue formation.

Materials and Methods

The first molar teeth from the maxilla of 5 days postnatal Sprague-Dawely (SD) type rats were extracted deliberately under a stereoscopic microscope. These tooth germs were soaked in the HANK'S balanced salt solution immediately, washed by the Dulbecco's Modified Eagle's Medium (DMEM) solution inside the clean bench, and soaked in a cell banker. Next, the tooth germs were placed quiet in a deep freezer at -85℃ overnight, kept frozen in liquid nitrogen for 1 week, constituting the frozen tooth germs group. Tooth germs at the same postnatal 5 days were extracted and used immediately for the unfrozen control group.

Sample processing for light microscopy:

Frozen tooth germs and non-frozen tooth germs were fixed with 4% paraformaldehyde at 4 ℃ for 3 days, decalcified with 10% ethylenediaminetetra-acetic acid solution at pH7.4 for 5 days, and embedded in paraffin.

As for the tissue construction of tooth germ, the stratum intermedium cells, ameloblasts, odontoblasts, the cells of enamel free area, pulpal cells and the stellate reticulum cells were recognized with H E staining. Among those cell groups, we examined those ameloblasts, odontoblasts and pulpal cells which make influence on tooth germ development.

As lectin histochemistry, the biotinized lectin of 18 types (25 υg/mL, summarized in Table. 1) was challenged, which are widely used for the histochemical analysis of sugar chain structure. Regarding the estimation of lectin binding, negative and postive stains were expressed as – and +, and exceedingly strong positivity was designated as + +.

In addition, in order to explore the carbohydrate complex of the intercellular matrix components which form the tooth germ, PAS reaction and Alcian blue stains(AB,pH1.0 and pH2.5), both of which detect the acidic mucopolysaccharide staining, were carried out.

Results

Light microscopic findings (based upon H-E staining)

A)Control group

Tooth germs, having three dental cusps, were in the enamel matrix formation stage. The dentin was formed a little thicker at the dental cusps, and thinner at the cervical area. The Hertwig's epithelial root sheath was observed at the apical tip, though the root formation did not initiate. At the pulpal side of the dentin, predentin was clearly recognized as homogeneously eosinophilic and non-calcifying substance.

B) Frozen group

Tissue construction of frozen tooth germs was well maintained similar to that of the control group. However, the cells of stellate reticulum and pulpal cells showed obscure and atrophic cell processes, subsequently widening intercellular space.

Lectin histochemistry

A) The control group

The 18 lectins were dichotomized into non-specific binding and specific binding members. The whole cells and matrix of the tooth germ were positive for the former lectins, while limited cells or matrices were specifically stained with the latter lectins.

(1) Non-specific binding member contained RCA-I, WGA, PSA, PHA-E, ConA and LCA.

(2) Specific binding member consisted of HPA, VVA, SBA, UEA-I, PNA, sWGA, DBA, PHA-L, GS-I and GS-IB4. Among these lectins, HPA, VVA, SBA, PNA, sWGA, DBA, PHA-L and GS-I were positive for the cells raised as the examination target. UEA-I and GS-IB4 were positive for the cells other than the examination target, and consequently these data were excluded from the data shown in the table 2. As for SBA, VVA and DBA, the binding was localized at the perinuclear and Golgi area of the ameloblasts

as granular particles, and distributed diffusely throughout the cytoplasm of odontoblasts.

None of the tooth germ component was positive for MPA and SJA.

B) Frozen group

The pattern of lectin binding in the frozen group was basically the same as that of the control group, including the species of lectins belonging to either non-specific or specific binding member. As for the binding of DBA, however, the strength of staining weakened at ameloblasts in comparison with those of the control. The exploration by pigmentation technique for the compound polysaccharides

AB stain (pH 1.0) showed the positivity at stellate reticulum cells and extracellular matrices of both the control and frozen group. The staining was enhanced at the cervical region of the pulp.

AB stain (pH 2.5) gave overall the same positivity as AB stain (pH 1.0), although the strength of staining weakened slightly.

As for PAS stain, red colored positive signal was detected at the matrix element of the dentin and was unclear at the stellate reticulum cells and extracellular matrices.

Discussion

Freeze preservation of tooth germ

An established freeze preservation for culture cells was applied in the present study. Morphologically, frozen tooth germ contained atrophic pulpal cells compared with the non-frozen control. The future application of the technique to human tooth germs should receive various verifications with multiple approaches.

Lectin histochemistry

Frozen tooth germs showed almost same staining pattern with the control tooth germs, except for the staining strength of DBA at ameloblasts. These cell and tissue-specific expression of sugar chains were preserved under the frozen condition, suggesting minimal influence by freezing on the component (cellular and tissue) of tooth germs.

Staining for the compound polysaccharides

It is notable that sulfate-containing GAGs in the tooth germ are associated with the dentin formation. The strong positivity for AB (pH 1.0) at the stromal components indicate that many amount of sulfate-containing GAGs exist in the non-frozen tooth germ. This positivity was not impaired with freezing, and consequently frozen tooth germs were supposed to retain the activity of dentin formation.

Conclusions

To explore the influence of preservation by freezing on tissues of tooth germ, we examined changes in histological architectures and glycoconjugate expression detected by lectins, in tooth germs, with or without freezing, at postnatal 5 day in rats. The results acquired were as follows:

1. Although tissue organization of the frozen tooth germ group and the control group was almost identical, the stellate reticulum cells and pulpal cells of the enamel pulp were slightly atrophi in the frozen group.
2. The 18 types of lectin binding characteristics concerning the sugar chain expression were almost identical between the frozen tooth germ group and the control group.
3. Concerning the compound carbohydrates in the intercellular matrix, the results given by both AB and PAS stainings showed no substantial difference between two groups.

Consequently, our data suggested that the technique of organ preservation by freezing could make very little influences on the tissue organization, and the morphological and sugar chain characteristics for cellular components of tooth germ .

Table 1: Lectins used in this studies and their binding specificities

Lectins		Binding specificities
RCA-I	*Ricinus communis*(castor bean)	β Gal1,4GlcNAc
WGA	*Triticum vulgare*(wheat germ)	GlcNAc(β 1,4GlcNAc)$_{1\text{-}2}$
PSA	*Pisum sativum*(pea)	Fucosylated core region of bi- and triantennary N-glycosidically linked oligosaccharides
PHA-E	*Phaseolus vulgaris* erythroagglutinin(kidney bean)	Bisected complex oligosaccharides
Con-A	*Canavalia ensiformis*	α Man
LCA	*Lens culinaris*(lentil)	Fucosylated core region of bi- and triantennary N-glycosidically linked oligosaccharides
PHA-L	*Phaseolus vulgaris* leukoagglutinin(kidney bean)	GlcNAcβ 1,2Man
HPA	*Helix pomatia*(snail)	Terminal GalNAcα1,3GalNAc
sWGA	succinyl *Triticum vulgare*(wheat germ)	GlcNAc(β 1,4GlcNAc)$_{1\text{-}2}$
SBA	*Glycine max*(soybean)	Terminal α and β GalNAc
VVA	*Ulex europeus I*(gorse seed)	α Fuc
DBA	*Dolichos biflorus*(horse gram)	Terminal α GalNAc
PNA	*Arachis hypogaea*(peanut)	Terminal Galβ 1,3GalNAc
GS-I	*Griffonia simplicifolia*	α Gal
GSI-B_4	*Griffonia simplicifolia* I_4	Terminal α Gal
UEA-I	*Ulex europeus I*(gorse seed)	α Fuc
MPA	*Maclura pomifera*(hedge apple tree)	Terminal Gaβ 1,3GalNAc
SJA	*Saphora Japonica*(pagoda tree)	TerminalGalβ 1,3GalNAc

GalNAc, N-acetyl galactosamine; GlcNAc, N-acetyl glucosamine; Fuc, fucose; Man, mannose; Gal, galactose;

Table 2: Summary of lectin bindings in control and frozen tooth germs

Lectin	ameloblasts		odontoblasts		dental pulp		blood vessels	
	Ctr	F	Ctr	F	Ctr	F	Ctr	F
RCA-I	++	++	++	++	++	++	++	++
WGA	++	++	++	++	++	++	++	++
PSA	++	++	++	++	++	++	++	++
PHA-E	++	++	++	++	++	++	++	++
ConA	++	++	++	++	++	++	++	++
LCA	++	++	++	++	++	++	++	++
PHA-L	++	++	−	−	−	−	−	−
HPA	++	++	−	−	−	−	−	−
sWGA	−	−	+	+	−	−	−	−
SBA	++	++	+	+	−	−	−	−
VVA	++	++	+	+	−	−	−	−
DBA	+	−	+	+	−	−	−	−
PNA	++	++	−	−	++	++	−	−
GS-I	++	++	−	−	−	−	++	++
GSI-B_4	−	−	−	−	−	−	−	−
UEA-I	−	−	−	−	−	−	−	−
MPA	−	−	−	−	−	−	−	−
SJA	−	−	−	−	−	−	−	−

Ctr, control(non-frozen) ; F, frozen group