Abstract of Thesis

Name (Huang Hailing)	
Title	Evaluation of inflammatory response during the wound healing process in caries-induced pulpitis model (う蝕により誘発された歯髄炎モデルの創傷治癒過程における炎症反応の評価)

[Background]

Rodent animal models for pulp capping have been widely used in dental research because of the similarity of their tooth structure and cellular processes with those of humans. However, most pulp capping studies using rodents have been conducted on non-infected sound teeth, which are not true mimics of the clinical situation. Our previous study has shown that some intrinsic proteinases released from the organic dentin matrix during caries progression could affect the pulpal wound healing process. Therefore, existing animal models may not be able to accurately evaluate the inflammatory transition after vital pulp therapy.

The objective of this study was to establish a rat caries-induced pulpitis model and to evaluate inflammatory changes during the wound healing process after pulp capping in a reversible pulpitis model induced by caries.

[Materials and Methods]

All animal studies were conducted under the approval of the Institutional Animal Care and Use Committee of Osaka University Graduate School of Dentistry (No. R-01-017-0).

Experiment 1: Establishment of caries-induced pulpitis model for direct pulp capping

Dental caries was induced by 5-day continuous inoculation of *Streptococcus mutans* MT8148 into the oral cavity of 18-day-old Sprague Dawley rats (n=30) fed with a cariogenic diet including 56% sucrose and 7% wheat flour. Stages of caries progression were classified as shallow (less than outer 1/3 of dentin thickness), moderate (between middle1/3 and inner 1/3 of dentin), or severe (inner 1/3 of dentin) by micro-CT analysis of the demineralized layer of dentin near the mesial horn of the pulp.

To evaluate the inflammatory status of the pulp in different stages of caries, the carious teeth were collected and stained with hematoxylin and eosin (H&E). Brown & Brenn stain was performed to detect bacterial invasion. To confirm that immune reaction of dental pulp was stimulated by bacterial invasion, immunohistochemical staining against Toll-like receptor 2 (TLR2) and proliferating cell nuclear antigen (PCNA) were investigated. Furthermore, to identify the boundary line between reversible and irreversible pulpitis, double immunofluorescent staining of CD68/CD206 was investigated.

Experiment 2: Tertiary dentin formation in caries-induced pulpitis model

To evaluate the wound healing process of the pulp under different inflammatory conditions, direct pulp capping using ProRoot MTA (Dentsply-Sirona, York, PA, USA) was performed in sound teeth and in moderately and severely carious teeth (n=5). The specimens were collected 28 days after pulp capping. The volume of newly formed tertiary dentin (n=5) was assessed by micro-CT. The data were reconstructed and quantitatively analyzed using three-dimensional reconstruction imaging software (TRI/3D-BON; Ratoc System Engineering, Japan). And H&E staining was performed to evaluate the histological status of the tertiary dentin.

Experiment 3: Assessment of inflammatory changes during the wound healing process of reversible pulpitis after after pulp capping with ProRoot MTA

To investigate inflammatory changes during the wound healing process, double immunofluorescent staining of CD68 and CD206 was performed to monitor spatiotemporal

localization of macrophages on day 1, 3, 7, and 14 post capping in moderate caries teeth. Cell proliferative capacity was also investigated by immunohistochemical staining against PCNA. CD68(+)/CD206(-) cells were considered to be M1 macrophages, whereas CD68(+)/CD206(+) cells were considered to be M2 macrophages. Sound teeth were used as a control group.

[Results and Discussion]

Experiment 1

H&E staining showed that thick reactionary dentin was formed beneath the carious lesion in the moderate and severe groups, and only the severe group showed apparent inflammatory cell infiltration. Brown & Brenn staining revealed bacterial invasion through the dentinal tubules in the moderate and severe groups, but no positive staining was detected in the pulp space from any of the specimens. Immunohistochemical staining against TLR2 and PCNA also showed that positive cells were distributed near the odontoblast layer beneath the carious lesion in the moderate and severe groups, and no positive expression was found in the sound and shallow groups. Double immunofluorescent staining against CD68 and CD206 showed M2 macrophages were predominant in the moderate group, whereas M1 macrophages were predominant in the severe group. These results indicate that the immune reaction have already occurred under moderate and severe carious conditions and moderate caries was considered to be reversible pulpitis, while severe caries was considered to be irreversible pulpitis.

Experiment 2

When investigating the wound healing process 28 days after direct pulp capping, the sound group showed a well-arranged odontoblast-like layer beneath a newly formed dentin bridge. The moderate caries group showed irregular structured complete tertiary dentin formation, which supported that moderate caries-induced inflammation of the pulp indicates reversible pulpitis. While the severe caries group showed incomplete hard tissue formation with defects away from the capping area, and the inflammation was still remained. This result indicated the pulp was under irreversible inflammation before treatment in the severe carious teeth. Quantification of newly formed tertiary dentin exhibited less volume in the moderate caries group than in the sound group ($P < 0.05$, Student's t-test, n = 5). These results indicate that the immune reaction in the inflamed pulp could affect tertiary dentin formation.

Experiment 3

During the wound healing process after pulp capping in the moderate caries group, M2 macrophages were predominantly distributed in the injured pulp. The maximum population was observed on day 3 ($P < 0.05$, ANOVA and Tukey's HSD test), and started to decrease after day 7, whereas no significant changes in the M2 macrophage population were observed in the sound group throughout the experiment ($P > 0.05$, one-way ANOVA). These results suggest that the healing process under inflammatory conditions due to carious stimulation might be different from that of healthy pulp with mechanical exposure. M2 macrophages might play an important role in reversible inflammation of the dental pulp in wound healing. The proliferative capacity was higher in the moderate caries group ($P < 0.05$, Student's t-test) on day 1 and day 3 compared with the sound group. The population of PCNA (+) cells also reached a maximum on day 3, and started to decrease after day 7 in the caries group, indicating that M2 macrophages might be involved in promoting cell proliferation in the inflamed pulp in the early stages of the wound healing process.

[Conclusion]

We successfully established a caries-induced reversible pulpitis model for direct pulp capping. M2 macrophages may play a pivotal role in the wound healing process of the inflamed pulp.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、う蝕により惹起された歯髄炎の創傷治癒メカニズムについて、動物実験モデルを用いて解明することを目指したものである。

その結果、本研究にて確立したう蝕由来ラット歯髄炎モデルを用いて、可逆性歯髄炎および不可逆性歯髄炎におけるマクロファージの挙動の詳細を明らかにした。

以上の研究成果は、う蝕由来歯髄炎の創傷治癒メカニズムの一端を解明し、新規の歯髄保存療法を開発する上で重要な知見を提供するものであり、本研究は博士（歯学）の学位授与に値するものと認める。