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学位論文内容の要旨

博士の専攻分野の名称　　博士（歯学）　　氏　名　YANG SHAN

学　位　論　文　題　名

Effects of different calcium hydroxide pastes on dentine resorption of osteoclasts in vitro

（異なる水酸化カルシウム製剤が in vitro における破骨細胞による象牙質吸収に与える影響）

キーワード（５つ）　Calcium hydroxide, Osteoclast, External infection-related resorption (EIR), Hydrophilic paste, Hydrophobic paste

External infection-related resorption (EIR), a common complication following dental trauma such as avulsion or luxation, is exacerbated by microbial activity from the root canal system. Calcium hydroxide has been widely used in clinical treatments due to its antimicrobial properties and its ability to create an alkaline environment conducive to bone and root repair. By neutralizing acids and inhibiting collagenase activity, calcium hydroxide promotes tissue regeneration and reduces resorption at the site of injury. However, the effectiveness of this treatment relies heavily on the ability of calcium hydroxide medicaments to penetrate dentinal tubules, reaching the resorptive areas and exerting their therapeutic effects.

The study focused on two commercially available calcium hydroxide-based medicaments: the hydrophilic Calcipex® II and the hydrophobic Vitapex®. Both medicaments are frequently used in root canal treatments but differ significantly in their vehicle composition, which influences their dissolution, ion release, and penetration ability. Hydrophilic vehicles, such as those in Calcipex® II, enable faster ion release and diffusion, creating a more immediate alkaline environment. Conversely, hydrophobic vehicles like those in Vitapex® offer slower, sustained release, potentially making them more suitable for long-term applications. Despite these differences, comparative data on their penetrability and effects on osteoclastic activity have been limited.

To address this gap, an in vitro model was developed to evaluate the penetration of these medicaments into dentinal tubules and their subsequent impact on osteoclast activity. Single-rooted human teeth were selected, with criteria ensuring complete root formation and the absence of prior treatment or significant structural damage. The teeth were instrumented, disinfected, and prepared to a standardized thickness of 1 mm from the canal to the external root surface. The roots were divided into three groups: one

filled with Calcipex® II, one with Vitapex®, and a control group left unfilled. The prepared roots were immersed in saline and sealed to simulate clinical conditions.
The diffusion of hydroxide ions through dentinal tubules was assessed via pH measurements over a seven-day period, using a two-layer 12-well plate setup to monitor changes in the surrounding solution. Osteoclast precursor cells were cultured on the root surfaces, and their differentiation and resorptive activity were analyzed using tartrate-resistant acid phosphatase (TRAP) staining and scanning electron microscopy (SEM). TRAP-positive cells, indicative of osteoclast activity, were quantified, and the density of resorption pits on the root surfaces was measured to evaluate the medicaments' effects on osteoclastic function.

The results revealed that both Calcipex® II and Vitapex® effectively increased the pH of the surrounding medium within the first three days, demonstrating their ability to diffuse hydroxide ions through dentinal tubules. The rapid pH increase suggests that both medicaments establish an alkaline environment early in the treatment process, which is critical for inhibiting osteoclastic activity. However, no significant differences in pH levels were observed between the two medicaments, indicating similar short-term penetrability in terms of hydroxide ion diffusion.

When osteoclast activity was evaluated, significant differences emerged between the groups. In the Calcipex® II group, fewer TRAP-positive cells were observed, and these cells were smaller in size compared to those in the Vitapex® group and the control group. This suggests that Calcipex® II was more effective in inhibiting osteoclast differentiation. Furthermore, the Calcipex® II group exhibited the lowest density of resorption pits, indicating reduced osteoclastic resorptive activity. In contrast, the Vitapex® group showed moderate inhibition of osteoclast activity, with higher numbers of TRAP-positive cells and resorption pits compared to Calcipex® II, but still fewer than the control group. These findings highlight the superior inhibitory effects of the hydrophilic Calcipex® II paste on osteoclast differentiation and activity.

The differences in effectiveness can be attributed to the vehicle composition of the two medicaments. The hydrophilic vehicle in Calcipex® II facilitates rapid ion release upon contact with tissue fluids, establishing a strong alkaline environment that inhibits osteoclast differentiation and resorptive activity. Conversely, the oily vehicle in Vitapex® limits ion solubility and diffusion, resulting in slower ion release and reduced immediate efficacy. While the hydrophobic nature of Vitapex® may offer advantages for prolonged treatment, its short-term effectiveness in creating an inhibitory environment for osteoclasts is comparatively lower.

The study also highlighted discrepancies between the findings of pH measurements and osteoclast activity assays. While both medicaments exhibited similar penetrability in terms of hydroxide ion diffusion, the inhibitory effects on osteoclast activity were more pronounced with Calcipex® II. This divergence could be due to experimental differences, such as the replacement of culture medium during osteoclast assays, which

may have influenced ion concentration gradients and localized effects.

The in vitro model developed in this study provides a valuable platform for evaluating the penetration and efficacy of intracanal medicaments under simulated clinical conditions. By allowing the simultaneous observation of pH changes and osteoclast activity, the model offers insights into the mechanisms through which calcium hydroxide medicaments exert their therapeutic effects. However, the study also identified limitations in the model, including potential inaccuracies in pH measurement due to solution saturation and the need for more refined techniques to evaluate penetrability and biological effects.

In conclusion, the hydrophilic Calcipex® II paste demonstrated superior short-term penetrability and inhibitory effects on osteoclast activity compared to the hydrophobic Vitapex® paste. These findings suggest that Calcipex® II is more suitable for clinical scenarios requiring rapid ion diffusion and immediate therapeutic effects, such as the treatment of EIR. Conversely, Vitapex® may be preferred for cases where sustained ion release and long-term effects are desired. The study underscores the importance of vehicle composition in the design and selection of intracanal medicaments and provides a foundation for future research.