Exploring the staining potential of GSK-3 inhibitors in bovine teeth: a one-year laboratory investigation

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Abstract

GSK-3 inhibitors, such as Tideglusib (TG) and CHIR-99021 (CHIR), show promise in stimulating reparative dentin formation. The aim of this study was to assess the discoloration potential of TG and CHIR in an established *in vitro* model.

Enamel-dentin specimens made from bovine incisors were randomly allocated to five groups (n=15 each): group bovine blood (BB), group dimethyl sulfoxide (DMSO), group TG, group CHIR, and group mineral trioxide aggregate (MTA). Each specimen had a central cavity in which the respective material was applied and sealed with resin-based luting material. Color determination was conducted using a dental spectrophotometer at t_0 (before filling), t_1 (immediately after filling), t_2 (after one week), t_3 (after one month), t_4 (after three months), t_5 (after six months), and t_6 (after one year). Statistical analysis involved descriptive statistics, Kruskal-Wallis tests, and analysis of variance (α=0.05). Group BB and group CHIR exhibited the most significant decrease in lightness (ΔL^*) after one year (ΔL^*-4.7 and ΔL^* -5.7, respectively), whereas groups DMSO, TG, and MTA showed minimal changes (DMSO ΔL^*: -0.3; TG ΔL^*: 1.4; MTA ΔL^*: -0.5). Group BB and CHIR exhibited the highest ΔE values (6.4±0.6 and 6.5±0.8, respectively).

Unlike CHIR, TG did not result in discoloration exceeding the threshold of visual perception, defined by a ΔE value of 5.5, during the one-year observation period. This laboratory study therefore suggests that TG could be utilized for indirect or direct pulp capping without major discoloration concerns. However, additional research is required to corroborate these findings.

Introduction

Dental injuries and deep carious lesions can lead to pulp exposure. When signs and symptoms are absent, the primary objective of treating such lesions is to preserve the vitality and health of the pulp tissue (Ricucci et al. 2014; Bjørndal et al. 2019). Various operative treatment approaches are available for maintaining pulp vitality, including indirect and direct pulp capping, partial pulpotomy, and complete pulpotomy. Preserving pulp vitality offers numerous benefits. The dentin-pulp complex can continue fulfilling its developmental, defensive, and proprioceptive functions. In addition, teeth with a vital pulp have a more favorable long-term prognosis regarding tooth survival compared with those that have undergone root canal treatment (Caplan et al. 2005; Leong & Yap 2021).

The gold standard materials for indirect and direct pulp capping comprise calcium hydroxide pastes and hydraulic silicate cements, including but not limited to mineral trioxide aggregate (MTA), Biodentine, Endocem MTA, EndoSequence, and others (Duncan et al. 2019; Dammaschke et al. 2019; Parirokh et al. 2018). Hydraulic silicate cements exhibit high mechanical resistance and can set in both dry and moist environments (Camilleri & Pitt Ford 2006; Parirokh & Torabinejad 2010; Torabinejad & Parirokh 2010; Dammaschke et al. 2019). On the other hand, calcium hydroxide pastes are less mechanically stable and susceptible to resorption and porosities over time at the site of pulp capping (Dammaschke et al. 2019). Both hydraulic silicate cements and calcium hydroxide pastes release calcium hydroxide ions during the setting process, which contribute to their high antibacterial properties but may also cause local tissue damage owing to chemical irritation (Spångberg 1969; Meadow et al. 1984; Briseño & Willershausen 1992).

It has been observed that localized cell death resulting from this mechanism leads to the release of inflammatory mediators, which subsequently activate tissue

regeneration pathways and promote the formation of reparative dentin (BABB ET AL. 2017). This process involves the mobilization and proliferation of mesenchymal stem cells from the pulp tissue, which can differentiate into new odontoblast-like cells and initiate the secretion of reparative dentin (BABB ET AL. 2017).

Based on a molecular understanding of the cellular signaling pathways involved in regenerative/reparative dentin formation, researchers have investigated pharmacological treatments that directly activate these pathways using specific molecules, inducing regulated reparative processes and promoting cellular differentiation (NAKASHIMA & REDDI 2003; THESLEFF & TUMMERS 2008; GALLER ET AL. 2014). Collagen sponges loaded with these molecules have been utilized to administer the treatment by placing them on the exposed pulp. Following application, controlled degradation of these collagen sponges allows for their integration within the structure of reparative dentin (NEVES ET AL. 2017; ZAUGG ET AL. 2020).

The novel treatment approach under investigation involves the use of glycogen synthase kinase 3 (GSK-3) inhibitors as pharmacological agents. GSK-3, a protein kinase, modulates the Wnt signaling cellular pathway (VISHWAKARMA ET AL. 2015). Laboratory studies have demonstrated that GSK-3 inhibitors stimulate the proliferation and viability of human dental pulp stem cells (HANNA ET AL. 2023; KORNSUTHISOPON ET AL. 2023) and elicit the formation of reparative dentin in animal models following pulp exposure (NEVES ET AL. 2017; ZAUGG ET AL. 2020). The Wnt signaling cellular pathway assumes a crucial role in reparative dentin development in cases of pulp exposure. Remarkably, the activity of the Wnt signaling pathway can also be activated in dentin damage without pulp exposure, as observed in indirect pulp capping, resulting in the formation of reactionary dentin and thickening of the dentin wall beneath the "near exposure" (NEVES & SHARPE 2018). These findings suggest that novel medicaments

97 utilizing GSK-3 inhibitors hold promise for both direct and indirect pulp capping in the
98 future.
99 Tideglusib (TG), a non-competitive GSK-3 inhibitor, has been the subject of clinical
100 trials for Alzheimer therapy (ELDAR-FINKELMAN & MARTINEZ 2011; WANG ET AL. 2021).
101 Notably, no adverse reaction profile was described for TG (ELDAR-FINKELMAN &
102 MARTINEZ 2011). Another potent ATP-competitive GSK-3 inhibitor, CHIR-99021
103 (CHIR), an aminopyrimidine, has demonstrated its ability to activate the Wnt signaling
104 pathway, leading to enhanced self-renewal and pluripotency in mouse stem cells
105 (ELDAR-FINKELMAN & MARTINEZ 2011; WU ET AL. 2013). Both CHIR and TG have
106 exhibited promising outcomes in preclinical research regarding reparative dentin
107 formation, prompting considerations for their potential clinical application in dentistry
108 (NEVES ET AL. 2017; NEVES & SHARPE 2018; ZAUGG ET AL. 2020; ALAOHALI ET AL. 2022).
109 One drawback associated with early market-stage MTA products was their propensity
110 for significant tooth discoloration (NAIK & HEGDE 2005; LENHERR ET AL. 2012; KRASTL ET
111 AL. 2013; DETTWILER ET AL. 2016; ABUELNIEL ET AL. 2020). Recent investigations
112 attribute this discoloration to the presence of the radio-opaque marker bismuth oxide,
113 prompting the modification of formulations using alternative, non-discoloring radio-
114 opaque markers (KANG ET AL. 2015; KESKIN ET AL. 2015; XUEREB ET AL. 2016; MARCIANO
115 ET AL. 2019). These findings underscore the importance of laboratory research in
116 evaluating dental materials to identify potential adverse effects, including their
117 propensity for discoloration.
118 In recent years, there has been a surge in studies exploring the molecular mechanisms
119 and potential therapeutic applications of GSK-3 inhibitors. However, as of today, there
120 is a lack of published evidence regarding the discoloration potential of these inhibitors.
121 Thus, the objective of this study was to evaluate the discoloration potential of GSK-3
122 inhibitors in a well-established *in vitro* setup.

Material and methods

Sample size calculation

Based on data reported in a previous study, an a priori sample size calculation was performed (LENHERR ET AL. 2012). A change of luminosity mean values (Lmean) of 92 to 90 with a standard deviation of 2.2 was assumed as the effect size between the control group and the test groups. By setting the type I (two-sided) and type II error rates at 5% and 20% respectively, establishing a significance level of 5%, and aiming for a test power of 80%, a minimum of 10 samples per group was determined as necessary. To ensure a prudent safety margin, accounting for the possibility of subtle variations in discoloration outcomes, a total of 15 specimens per group were included.

Specimen preparation

A total of 75 specimens were produced using a methodology previously described in detail by LENHERR ET AL. (2012). The source material consisted of bovine incisors obtained from calves slaughtered in a commercial slaughterhouse and stored in tap water at room temperature. The crown and root of each tooth were separated at the cemento-enamel junction by using a diamond-coated cutting disk, which was continuously cooled with water. From the middle third of each crown, rectangular enamel-dentin slabs measuring 10 mm in length, 10 mm in width, and 3 mm in thickness were excised. Subsequently, an oral surface cavity with dimensions of 2.5 mm in diameter and 2.0 mm in depth was created in each enamel-dentin slab using a water-cooled cylindrical diamond bur. To ensure uniformity, the length, width, and thickness dimensions of all specimens were verified using a digital caliper.

Disinfection procedure

The specimens were treated by immersing them in 1% sodium hypochlorite (NaOCl; Toppharm Apotheke Hersberger, Basel, Switzerland) for a period of 30 minutes. Subsequently, they were thoroughly rinsed with tap water, dried using compressed air, and submerged in 20% ethylenediaminetetraacetic acid (EDTA, Toppharm Apotheke Hersberger, Basel, Switzerland) for 2 minutes to eliminate the smear layer. After rinsing with copious amounts of tap water and drying with air, the specimens were immersed in 1% NaOCl for an additional 3 minutes. Finally, the specimens were thoroughly washed with tap water and placed in 0.9% saline (Grosse Apotheke Dr. G. Bichsel, Interlaken, Switzerland) for storage in an area with indirect sunlight.

Experimental groups

The specimens were randomly allocated to five groups using computer-generated sequences created with free online software (www.randomizer.org). Each group consisted of 15 specimens (Tab. I), and the same amount of liquid (1µL) and collagen sponges (Kolspon; Eucare, Chennai, India) were used. The sponges were cut according to the dimensions 2 mm x 2 mm x 2 mm and placed in the cylindrical cavity prior to injecting the liquid. Group BB was designated as positive control group using bovine blood (Bell Schweiz, Oensingen, Switzerland). In group DMSO, dimethyl sulfoxide (DMSO, Sigma-Aldrich/ Merck, Darmstadt, Germany), a polar aprotic solvent and organosulfur compound, was dispensed onto the sponge previously placed in the cylindrical cavity of the specimens. In groups TG and CHIR, 50nM TG (Sigma-Aldrich/ Merck, Darmstadt, Germany) and 5µM CHIR (Sigma-Aldrich/ Merck, Darmstadt, Germany), respectively, were dissolved in DMSO and subsequently administered into each cavity. In group MTA, the non-discoloring formula of mineral trioxide aggregate (MTA) powder and liquid (PD-MTA, Produits Dentaires SA, Vevey, Switzerland) were thoroughly mixed at a ratio of 3:1 until a homogeneous mixture was achieved. The

resulting mixture had a creamy consistency and was promptly applied into the cylindrical cavities without any sponge.

After the application of the material in each group, the cylindrical cavities were sealed with self-adhesive resin-based luting material (RelyX Unicem2 Aplicap, 3M, St. Paul, MN, USA). The luting material was light cured for 20 s at an irradiance of 907 mW/cm^2 (SmartLite Focus, Dentsply Sirona, Charlotte, NC, USA). The tip of the curing light, whose emission spectrum ranged from 420 nm to 540 nm, was positioned as close as possible to the resin-based material without touching it during light curing. After light curing, the specimens were placed in individual test tubes (Standard Micro Test Tube 3810, Eppendorf, Hamburg, Germany) containing 0.9% saline (Grosse Apotheke Dr. G. Bichsel, Interlaken, Switzerland). The test tubes were then stored at room temperature, ensuring they were kept away from direct sunlight.

Spectrophotometric color determination

Color determination was performed using a dental spectrophotometer (Vita EasyShade Compact, Vita, Bad Säckingen, Germany) (LEHMANN ET AL. 2011). The measurements were performed exclusively under the illumination provided by the experimental setup's lamp (Lamp Trio 1 [max. 40W, 230V/50Hz] with a light-emitting diode bulb [OSRAM, 40W, Classic B, 230V, E14, SES], both purchased from Migros, Zurich, Switzerland) within a dark room. The spectrophotometric measurements were taken at multiple time points, including baseline (t0), immediately after filling (t_1), and subsequently after one week (t_2), one month (t_3), three months (t_4), six months (t_5), and one year (t_6). Before each group measurement, the spectrophotometer was calibrated following the manufacturer's instructions. The color parameters recorded and measured according to the Lab* color space were as follows: *L** (lightness), *a** (red-green coordinates), and *b** (blue-yellow coordinates).

Statistical analysis

To describe the appearance of color, descriptive statistics were used for *L*, a*, and b**values for each time interval and the color difference between baseline (t_0) and after one year (t_6) was calculated using the following formula:

$\Delta L^* = L^*_{t6} - L^*_{t0}$

$\Delta a^* = a^*_{t6} - a^*_{t0}$

$\Delta b^* = b^*_{t6} - b^*_{t0}$

To assess the relative color change (ΔE) after one year, the following formula was applied:

$\Delta E^*ab = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$

The normality of the data was assessed using both the Kolmogorov-Smirnov and Shapiro-Wilk tests. For between-group analysis, the Kruskal-Wallis test was employed. Univariate and multivariate analysis of variance (ANOVA) were conducted for ΔE and ΔL. Pairwise comparisons were performed using the Bonferroni and post-hoc Tukey tests. The significance level was set at $\alpha = 0.05$. All analyses were carried out by an unblinded investigator utilizing SPSS Statistics software (version 28, IBM, Armonk, NY, USA).

Results

All values showed a normal distribution except TG at t_0 and t_6 for value *a**. The *L*a*b** mean values at baseline (t_0) and after one year (t_6) and the differences over this period ($\Delta L^* t_6\text{-}t_0$, $\Delta a^* t_6\text{-}t_0$, $\Delta b^* t_6\text{-}t_0$) are reported in Tab. II. Group BB and CHIR presented the most pronounced changes in lightness (ΔL^*) during the observation period with a decrease of *L** after one year (BB ΔL^* -4.7 ±3.5 CHIR ΔL^* -5.7±3.3; Tab. II). A similar range of *L** values with only slight changes after one year were found for group DMSO

227 with L^* -0.3±1.7, group TG with ΔL^* 1.4±1.1 and group MTA with ΔL^* -0.5±1.5. A
228 decrease in L^* was observed in group DMSO and MTA, while L^* of group TG increased,
229 though not significantly. Group BB and CHIR showed significant changes in lightness
230 (ΔL^*) after one year of observation compared to the remaining groups DMSO, TG and
231 MTA (p<0.001), while no significant difference was found between BB and CHIR.
232 The relative color changes (ΔE) after one year were reported highest for group BB and
233 CHIR with 6.4±0.6 and 6.5±0.8, respectively. The remaining groups exhibited ΔE
234 values ≤ 4 (group DMSO: 2.7±0.4), TG: 4.0±0.2 and MTA: 2.8±0.2; Tab. II). Significant
235 changes were recorded between group BB to group DMSO (p<0.001), group TG
236 (p<0.01) and group MTA (p<0.001), but not to group CHIR (p<0.999). Group CHIR
237 also showed significant differences compared with these three groups (DMSO
238 p<0.001, TG p<0.005, MTA p<0.001).
239

Discussion

This study sought to explore the discoloration potential of two GSK-3 inhibitors, specifically TG and CHIR. These GSK-3 inhibitors have garnered attention as potential direct and indirect pulp capping materials for future use. Employing an established *in vitro* setup, first described by LENHERR ET AL. (2012), our findings did not substantiate the hypothesis that TG exhibited significant discoloration potential. However, significant changes in lightness and color were observed in the case of CHIR.

The observed alterations in color for CHIR hold clinical significance, as previous research has suggested that discolorations with a ΔE value exceeding 5.5 become perceptible to the average patient and could be deemed unacceptable (DAY ET AL. 2011). Additionally, WESTLAND ET AL. (2017) investigated the threshold values at which changes in color become noticeable to the average observer. Their study revealed that a change of 1.1 ΔL^*, 3.2 Δa^*, and 1.5 Δb^* could be discerned. CHIR therefore, requires additional assessment of its discoloration potential. Future research should focus on comprehensively investigating the characteristics of CHIR products to ascertain their suitability for clinical use in dentistry.

Compared with the findings of previous studies (LENHERR ET AL. 2012; DETTWILER ET AL. 2016), our present investigation revealed a noticeably reduced discoloration effect caused by bovine blood in the positive control group. This disparity can be attributed to a subtle modification in our experimental approach. Specifically, like groups DMSO, TG, and CHIR, the control group used only 1 µl of material, resulting in a minimal amount of blood being employed. Additionally, our study did not include any groups combining blood with drugs. These factors likely played a crucial role in the observed decrease in discoloration intensity, highlighting the importance of both the quantity of staining compounds and the interactions among different compounds in the process of tooth discoloration. This finding underscores the significance of carefully considering

the dosage and combinations of substances to gain a comprehensive understanding of discoloration mechanisms.

The L*a*b* color space is a three-dimensional color model that describes colors based on three components: L* represents lightness, a* represents the red-green axis, and b* represents the yellow-blue axis. When assessing color changes in the bovine specimens, the data of changes in L*a*b* values provided additional insight compared with the use of ΔE, which represents the overall color difference between two samples. While ΔE is a useful metric for comparing color changes, it does not provide detailed information about the specific aspects of color alteration, such as lightness or hue shifts. By analyzing the changes in L*a*b* values, specific aspects of color transformation could be evaluated. It is noteworthy to observe that, despite the rigorous process of sample randomization, the control group exhibited lower baseline L* values in comparison with the other groups. This disparity may introduce a subtle bias into the results, warranting consideration in the interpretation of the findings.

A potential hindrance to the clinical utilization of GSK-3 inhibitors lies in the limited availability of application forms. These inhibitors are solely accessible in a dissolved state within DMSO, commonly combined with collagen sponges. In contrast, calcium hydroxide exists in a paste form, enabling convenient application. However, ongoing investigations are exploring alternative application forms for GSK-3 inhibitors, aiming to enhance practicality and ease of use. For instance, recent studies conducted by ALAOHALI ET AL. (2022) and ATILA ET AL. (2022) have evaluated the feasibility of incorporating GSK-3 inhibitors into hydrogels. The utilization of hydrogels as a delivery system for GSK-3 inhibitors offers notable advantages. Hydrogels can encapsulate therapeutic agents, enabling controlled release and localized drug delivery. Additionally, hydrogels can be tailored to exhibit desired physical properties, including viscosity and gelation characteristics, allowing for precise application and retention at

292 the target site. Consequently, the utilization of hydrogels as an alternative application
293 form holds significant promise for facilitating the administration of GSK-3 inhibitors.
294 The findings of this laboratory study do not possess direct clinical relevance. Careful
295 consideration must be given to the inherent methodological limitations of this study.
296 First, the evaluation of possible discoloration resulting from the activation of the Wnt
297 pathway can only be effectively assessed in vital teeth. Therefore, it is crucial that
298 future studies investigating GSK-3 inhibitors in animal models monitor tooth color
299 changes over time and provide comprehensive data on discoloration or lack thereof.
300 Second, it should be noted that contact between GSK-3 inhibitors and saliva or dentinal
301 fluid could potentially lead to the formation of pigmented breakdown products, thus
302 increasing the likelihood of discoloration. In the present study, the materials were
303 applied under ideal laboratory conditions, without any fluid contamination.
304 Consequently, it remains unclear whether the application of GSK-3 inhibitors carries
305 inherent risks for tooth discoloration in clinical scenarios.
306 Third, the laboratory studies utilized bovine teeth specimens to assess the
307 discoloration potential of GSK-3 inhibitors. The utilization of bovine teeth, which exhibit
308 greater consistency in terms of shape and size compared to human teeth, served to
309 reduce variability across specimens. However, while the microstructure of bovine
310 dentin is similar to that of human dentin, notable differences exist in the shape and
311 diameter of dentinal tubules. Additional studies employing human teeth are therefore
312 necessary to enhance our understanding of the discoloration potential of GSK-3
313 inhibitors.
314 Owing to the absence of published studies investigating the discoloration potential of
315 GSK-3 inhibitors, direct comparisons with existing research cannot be drawn.
316 Nonetheless, this preliminary study offers valuable insights into the discoloration
317 potential of GSK-3 inhibitors. Considering the significance of dental esthetics in oral

health-related quality of life, it is important to take into account the discoloration potential of direct and indirect capping materials alongside their therapeutic effects. To comprehensively assess the true extent of the discoloration potential of TG, further *in vitro* and *in vivo* studies are imperative.

Conclusion

Within the limitations of this laboratory study, the following conclusions were drawn:

- CHIR and TG, two GSK-3 inhibitors capable of promoting reparative dentin formation, differ in their potential for inducing tooth discoloration *in vitro.*
- Whereas CHIR produced significant changes in lightness and color, the color changes caused by TG remained below the threshold of visual perception.
- Additional research is necessary to advance our understanding of the discoloration potential associated with GSK-3 inhibitors.

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Zusammenfassung

Einleitung

Massnahmen zur Vitalerhaltung bei der Behandlung von Zahnfrakturen und fortgeschrittenen Kariesläsionen haben das Ziel, die Vitalität der Pulpa zu erhalten. Gängige Methoden zur indirekten und direkten Pulpaüberkappung beinhalten die Verwendung von Kalziumhydroxidpasten und hydraulischen Silikatzementen. Entzündungsmediatoren fördern die reparative Bildung von Dentin durch die Aktivierung von Geweberegenerationswegen. Inhibitoren der Glykogensynthase-Kinase 3 (GSK-3), wie Tideglusib (TG) und CHIR-99021 (CHIR), haben sich als vielversprechend erwiesen, um die Proliferation von Pulpastammzellen und die Bildung von reparativem Dentin anzuregen. Obwohl das Verfärbungspotenzial herkömmlicher Überkappungsmaterialien bekannt ist, gibt es derzeit nur begrenzte wissenschaftliche Erkenntnisse über das Verfärbungspotenzial von GSK-3-Inhibitoren. Daher hatte diese Studie zum Ziel, das Verfärbungspotenzial von GSK-3-Inhibitoren zu bewerten.

Material und Methoden

In dieser Studie wurde ein etablierter *In-vitro*-Aufbau verwendet, um das Verfärbungspotenzial von GSK-3-Inhibitoren zu untersuchen. Insgesamt wurden 75 Proben aus Rinderschneidezähnen präpariert, wobei rechteckige Schmelz-Dentin-Proben mit standardisierten Kavitäten hergestellt wurden. Die Proben wurden einem Desinfektionsverfahren mit Natriumhypochlorit und Ethylendiamintetraessigsäure unterzogen. Anschliessend wurden sie zufällig fünf Versuchsgruppen zugewiesen: Positivkontrolle (Rinderblut RB), Dimethylsulfoxid (DMSO), TG, CHIR und Mineraltrioxidaggregat (MTA). Jede Gruppe bestand aus 15 Proben. Nach dem Einbringen der jeweiligen Materialien in die Kavitäten und dem Verschluss der Kavitäten mit Befestigungskomposit wurden die Proben in Reagenzgläsern mit

Kochsalzlösung bei Raumtemperatur gelagert. Die spektrophotometrische Farbbestimmung wurde zu den Zeitpunkten t_0 (Vor Einlage), t_1 (unmittelbar nach Einlage), t_2 (nach einer Woche), t_3 (nach einem Monat), t_4 (nach drei Monaten), t_5 (nach sechs Monaten) und t_6 (nach einem Jahr) durchgeführt. Statistische Analysen, einschließlich deskriptiver Statistik, Kruskal-Wallis-Test, ANOVA und paarweiser Vergleiche, wurden durchgeführt, wobei das Signifikanzniveau bei $\alpha=0.05$ festgelegt wurde.

Resultate

Die Ergebnisse der Studie zeigten signifikante Unterschiede in den Farbveränderungen zwischen den Versuchsgruppen während des einjährigen Beobachtungszeitraums. Die Gruppen RB und CHIR, die mit Rinderblut respektive mit CHIR-99021 behandelt wurden, wiesen die deutlichste Abnahme der Helligkeit (L*) vom Ausgangswert (Lt_0) nach einem Jahr (Lt_6) auf, was auf eine erhebliche Verfärbung hinweist (RB: ΔL* -4,7, CHIR: ΔL* -5,7). Die Gruppen DMSO, TG und MTA wiesen minimale Veränderungen von L* auf (ΔL* innerhalb ±1,5), die jedoch statistisch nicht signifikant waren. Die relativen Farbveränderungen (ΔE) nach einem Jahr waren bei den Gruppen RB und CHIR am höchsten, mit signifikanten Unterschieden im Vergleich zu den Gruppen DMSO, TG und MTA.

Diskussion

Die Ergebnisse zeigten, dass TG kein signifikantes Verfärbungspotenzial aufwies, während CHIR signifikante Helligkeits- und Farbveränderungen verursachte. Diese Farbveränderungen sind klinisch bedeutsam, da frühere Untersuchungen darauf hindeuten, dass Farbveränderungen, die einen ΔE-Wert von 5,5 überschreiten, von Patienten wahrgenommen werden. Die begrenzte Verfügbarkeit von GSK-3-Inhibitoren in geeigneten Applikationsformen bleibt eine Herausforderung. Jüngste Untersuchungen haben jedoch alternative Formen erforscht, wie z. B. die Einbindung

von GSK-3-Inhibitoren in Hydrogele, die Vorteile bei der kontrollierten Wirkstofffreisetzung und zielgenauen Applikation bieten. Diese Studie hat methodische Beschränkungen, daher ist es wichtig, weitere Untersuchungen an vitalen Zähnen durchzuführen und potenzielle Verfärbungsrisiken durch den Kontakt von GSK-3-Inhibitoren mit Speichel oder Dentinflüssigkeit zu bewerten. Trotz dieser Einschränkungen liefert die Studie wertvolle Erkenntnisse zum Verfärbungspotenzial von GSK-3-Inhibitoren. Erste Hinweise deuten darauf hin, dass TG möglicherweise günstigere Eigenschaften in Bezug auf das Verfärbungspotenzial aufweist als CHIR.

Résumé

Introduction

Les mesures de préservation de la vitalité lors du traitement de fractures dentaires et de lésions carieuses avancées ont pour objectif de préserver la vitalité de la pulpe. Les méthodes courantes de coiffage pulpaire indirect et direct comprennent l'utilisation de pâtes d'hydroxyde de calcium et de ciments silicatés hydrauliques. Les médiateurs de l'inflammation favorisent la formation réparatrice de la dentine en activant les voies de régénération tissulaire. Les inhibiteurs de la glycogène synthase kinase 3 (GSK-3), tels que le tidéglusib (TG) et le CHIR-99021 (CHIR), se sont révélés prometteurs pour stimuler la prolifération des cellules souches pulpaires et la formation de dentine réparatrice. Bien que le potentiel de coloration des matériaux de coiffage traditionnels soit connu, les connaissances scientifiques sur le potentiel de coloration des inhibiteurs de GSK-3 sont actuellement limitées. C'est pourquoi cette étude avait pour but d'évaluer le potentiel de coloration des inhibiteurs de GSK-3.

Matériels et methodes

Dans cette étude, un dispositif *in vitro* bien établi a été utilisé pour évaluer le potentiel de décoloration des inhibiteurs de GSK-3. Au total, 75 échantillons ont été préparés à

partir d'incisives bovines, en produisant des échantillons rectangulaires d'émail et de dentine avec des cavités standardisées. Les échantillons ont été soumis à un processus de désinfection avec de l'hypochlorite de sodium et de l'acide éthylènediaminetétraacétique. Ils ont ensuite été répartis au hasard dans cinq groupes expérimentaux : Contrôle positif (CP), diméthylsulfoxyde (DMSO), TG, CHIR et agrégat de trioxyde minéral (MTA). Chaque groupe était composé de 15 échantillons. Après l'introduction des matériaux respectifs dans les puits et la fermeture des puits avec du composite de fixation, les échantillons ont été stockés dans des tubes à essai contenant une solution saline à température ambiante. La détermination de la couleur par spectrophotométrie a été effectuée à plusieurs moments sur une période d'un an. Des analyses statistiques, y compris des statistiques descriptives, le test de Kruskal-Wallis, l'ANOVA et des comparaisons par paires, ont été effectuées, le niveau de signification étant fixé à $\alpha=0,05$.

Résultats

Les résultats de l'étude ont montré des différences significatives dans les changements de couleur entre les groupes expérimentaux au cours de la période d'observation d'un an. Le groupe CP, traité avec du sang bovin, et groupe CHIR, traité par CHIR-99021, a présenté la diminution la plus significative de la luminosité (L*) par rapport à la valeur initiale (Lt_0) après un an (Lt_6), ce qui indique une décoloration importante (CP : ΔL* -4,7. CHIR: ΔL* -5,7). Les groupes DMSO, TG et MTA ont présenté des variations minimes de L* (ΔL* à l'intérieur de ±1,5), mais qui n'étaient pas statistiquement significatives. Les changements de couleur relatifs (ΔE) au bout d'un an étaient les plus élevés pour les groupes CP et CHIR, avec des différences significatives par rapport aux groupes DMSO, TG et MTA.

Discussion

Les résultats ont montré que la TG ne présentait pas de potentiel de décoloration significatif, tandis que la CHIR provoquait des changements significatifs de luminosité et de couleur. Ces changements de couleur sont cliniquement significatifs, car des études antérieures ont suggéré que les changements de couleur dépassant une valeur ΔE de 5,5 sont perçus par les patients. La disponibilité limitée des inhibiteurs de GSK-3 dans des formes d'application appropriées reste un défi. Cependant, des études récentes ont exploré des formes alternatives, telles que l'incorporation d'inhibiteurs de GSK-3 dans des hydrogels, qui présentent des avantages en termes de libération contrôlée du principe actif et d'application ciblée. Cette étude présente des limites méthodologiques, il est donc important de réaliser d'autres études sur des dents vitales et d'évaluer les risques potentiels de décoloration liés au contact des inhibiteurs de GSK-3 avec la salive ou le liquide dentinaire. Malgré ces limites, l'étude fournit des informations précieuses sur le potentiel de coloration des inhibiteurs de GSK-3. Les premières indications suggèrent que la TG pourrait présenter des propriétés plus favorables que la CHIR en termes de potentiel de décoloration.

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Tables

Tab. I Composition of the materials used in the different experimental groups.

Group	Material	Composition/Details	Mixing information	Manufacturer
BB	Bovine blood	Positive control group	N/A	Bell Schweiz, Oensingen, Switzerland
DMSO	DMSO	Dimethyl sulfoxide	N/A	Sigma-Aldrich/ Merck, Darmstadt, Germany
TG	Tideglusib	Concentration: 50nM	Tideglusib dissolved in DMSO/PBS*	Sigma-Aldrich/ Merck, Darmstadt, Germany
CHIR	CHIR99021	Concentration: 5µM	CHIR 99021 in DMSO/PBS* dissolved	Sigma-Aldrich/ Merck, Darmstadt, Germany
MTA	PD-MTA	Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate, Calcium oxide, Calcium tungstate.	Full content of one sachet mixed with distilled water for 30 s to a creamy consistency	Produits Dentaires SA, Vevey, Switzerland

PBS: phosphate buffered saline (DMSO/PBS ratio = 0.2%v/v)

PD-MTA: Produits Dentaires Mineraltrioxidaggregat

Tab. II Mean values ± standard deviation (SD) and 95% confidence intervals (CI) of *L**, *a** and *b** values of the materials at baseline (t_0) and after one year (t_6) and the difference over this period ($\Delta L^*t_6\text{-}t_0$; $\Delta a^*t_6\text{-}t_0$; $\Delta b^*t_6\text{-}t_0$; $\Delta E t_6\text{-}t_0$)

Group	*L**					*a**					*b**					ΔE	
	t_0		t_6		$t_6\text{-}t_0$	t_0		t_6		$t_6\text{-}t_0$	t_0		t_6		$t_6\text{-}t_0$	$t_6\text{-}t_0$	
	Mean±SD	95% CI	Mean±SD	95% CI	ΔL^*	Mean±SD	95% CI	Mean±SD	95% CI	Δa^*	Mean±SD	95% CI	Mean±SD	95% CI	Δb^*	ΔE±SD	95% C
BB	86.1±0.8	84.4-87.8	81.4±0.7	80.0-82.8	-4.7	0.5±0.2	0.2-0.9	-0.3±0.2	-0.8-0.2	-0.8	20.7±0.4	19.8-21.5	20.1±0.6	18.7-21.4	-0.6	6.4±0.6	5.1-7.(
DMSO	90.0±0.7	88.6-91.5	89.7±0.5	88.6-90.8	-0.3**	-0.3±0.1	-0.7-(-0.02)	-0.5±0.1	-0.7-(-0.3)	-0.2*	19.9±0.5	18.8-20.9	17.8±0.6	16.6-19.1	-2.0	2.7**±0.4	1.7-3.(
TG	90.7±0.4	89.9-91.5	92.1±0.4	91.2-92.9	1.4**	-0.3±0.2	-0.6-0.03	-1.0±-0.1	-1.1-(-0.8)	-0.7	20.5±0.5	19.5-21.5	17.1±0.5	16.0-18.2	-3.5**	4.0*±0.2	3.7-4.3
CHIR	91.5±0.5	90.3-92.6	85.8±0.6	84.5-87.0	-5.7	-0.7±0.1	-1.0-(-0.5)	-1.0±0.1	-1.2-(-0.8)	-0.3	20.1±0.4	19.2-21.0	17.5±0.5	16.5-18.5	-2.6*	6.5±0.8	4.8-8.3
MTA	91.5±0.4	90.7-92.4	91.0±0.4	90.1-92.0	-0.5**	-0.6±0.1	-0.8-(-0.5)	-1.0±0.1	-1.2-(-0.9)	-0.4	19.8±0.4	19.0-20.7	17.6±0.3	16.9-18.4	-2.2	2.8**±0.2	2.4-3.(

BB: control group blood, DMSO: group DMSO, TG: group Tideglusib, CHIR: group CHIR99021, MTA: PD-MTA, L^*: lightness, a^*: red-green coordinates, b^*: blue-yellow coordinates, ΔE: relative color change. *$p < 0.05$ denotes a significant difference when compared with control group BB. **$p < 0.001$ indicates a highly significant difference when compared with control group BB.