Facilitation of Reparative Dentin Using a Drug Repositioning Approach With 4-Phenylbutric Acid

Eui-Seon Lee

Kyungpook National University

Yam Aryal

Kyungpook National University

Tae-Young Kim

Kyungpook National University

Ji-Youn Kim

Gachon University

Hitoshi Yamamoto

Tokyo Dental College

Chang-Hyeon An

Kyungpook National University

Seo-Young An

Kyungpook National University

Youngkyun Lee

Kyungpook National University

Wern-Joo Sohn

Daegu Haany University

Jae-Kwang Jung

Kyungpook National University

Jung-Hong Ha

Kyungpook National University

Jae-Young Kim (✉ jykim91@knu.ac.kr)

Kyungpook National University

Research Article

Keywords: drug repositioning, cavity preparation, chemical chaperone, dentin-pulp complex, reparative dentin formation

Posted Date: December 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-119295/v1>

Abstract

For hard tissue formation, cellular mechanisms, involved in protein folding, processing, and secretion play important roles in the endoplasmic reticulum (ER). In pathological and regeneration conditions, ER stress hinders proper formation and secretion of proteins, and tissue regeneration by unfolded protein synthesis. 4-Phenylbutyric acid (4PBA) is a chemical chaperone that alleviates ER stress through modulation in proteins folding and protein trafficking. However, previous studies about 4PBA only focused on the metabolic diseases rather than on hard tissue formation and regeneration. Herein, we evaluated the function of 4PBA in dentin regeneration using an exposed pulp animal model system via a local delivery method as a drug repositioning strategy. Altered morphological changes and cellular physiology were examined with histology and immunohistochemistry. The 4PBA treatment modulated the inflammation reaction and resolved ER stress in the early stage of pulp exposure. In addition, 4PBA treatment activated blood vessel formation and TGF- β 1 expression in the dentin-pulp complex. Micro-computed tomography and histological examinations confirmed the facilitated formation of the dentin bridge in the 4PBA-treated specimens. These results suggest that proper modulation of ER stress would be an important factor for secretion and patterned formation in dentin regeneration.

Introduction

The endoplasmic reticulum (ER) functions as a site for protein folding, lipid biosynthesis, and calcium homeostasis ¹. Numerous proteins, either destined for cell-surface or for secretion, undergo process of synthesis and modification such as folding and assembly ². Trafficking and translocation of proteins in the ER would cause loads of stress to the ER regulation due to the large number of proteins that need to be executed ³. However, several molecular chaperones, such as multi-domain families of proteins, are located at the lumen of the ER, which help in achieving correct protein folding and assembly ⁴. The process of balancing between the load of protein synthesis and its protein folding ability is referred to as ER homeostasis. When there is a disruption in ER homeostasis ⁵, ER stress can be triggered which is caused by the presence of mis-folded and unfolded proteins ⁶. For those mis-folded proteins remain in the ER whether to be completed or to be degraded ⁷. When there is a disruption of these processes, ER stress can be triggered, the overloaded status of ER which is provoked by presence of mis-folded and unfolded proteins and in response to ER stress, the unfolded protein response (UPR) activates ^{8,9}. The unfolded protein response (UPR) activates in response to ER stress when ER capacity for protein folding is deficient. UPR activation alleviates ER stress by increasing ER abundance, decreasing synthesis of proteins, and augmenting the ER chaperone protein synthesis to sustain homeostatic balance; however, when ER stress could not be restored by UPR, apoptosis takes place ^{10,11}.

Dentin is typically classified into formation stages, namely, primary, secondary, and tertiary. During odontogenesis, primary dentin is formed by odontoblasts until the tooth becomes functional. Secondary dentin is formed after root completion and continues lifelong. Particularly, in the pathological condition, odontoblasts form a specific dentin—the tertiary dentin. Tertiary dentin, whether reactionary or reparative,

is formed in response to an external stimulus, caries, or abrasion. Reactionary dentin is formed from pre-existing odontoblast, and reparative dentin, which is elaborated by odontoblast-like cells that form after the death of the original odontoblasts^{12,13}. Proper regeneration of dentin still remains as an unsolved problem in dental fields. Tissue regeneration requires high fidelity of restoration of structure including vesicle secretion for tissue function^{14,15}. Odontoblasts and pulp cells react against the damage through reinitiating differentiation of odontoblasts from stem cells and progenitor cells in dental pulp resulting in secretion of the reparative dentin matrix for normal physiologic function of tooth^{14,15}. During dentin formation, ER stress regulation plays a significant role especially in the secretory stage¹⁶. Several previous reports suggested that ER stress would affect formation of dentin. When ER stress regulator gene, *Tmbim6* was knocked out, the dentin and enamel structures were disturbed by altering odontoblast and enamel differentiation¹⁷. Mutated dentin forming genes of which could cause Dentinogenesis Imperfecta and dentin dysplasia may cause a high level of ER stress^{18–20}. Odontoblasts and pulp cells react against the damage by reinitiating differentiation of the odontoblasts from stem cells and progenitor cells in the dental pulp resulting in the secretion of the reparative dentin matrix for normal physiologic function of the tooth¹⁹.

4-Phenylbutyric acid (4PBA) is an FDA-approved drug intended to treat congenital diseases in the urea cycle²¹. However, 4PBA is a known chemical chaperone that functions as an ER stress antagonist²²; a low-molecular weight compound which stabilizes protein conformations, enhancing the protein folding ability of ER and protein trafficking to alleviate ER stress²³. A number of previous studies have also proved the therapeutic effects of 4PBA in proteostasis. Mainly, 4PBA facilitates metabolic syndrome and metabolism such as obesity and diabetes^{24–26}. The chemical chaperone 4PBA is also known to alleviate ER stress-mediated cell death and genetic disorders caused by protein mis-folding^{27,28} and protein mis-folding genetic disorders^{27,29}. Furthermore, various effects of 4PBA in inflammatory disorders^{26,30}, neurological diseases, and cancers are well known.

Pulp cavity preparation is the most well-known method of dentin-pulp restoration. In this procedure, the tooth itself receives stress even in protein modulation and secretion of ER. This study hypothesizes that 4PBA, not applied in dental treatment yet, is involved in the modification and secretion of protein matrix for dentin formation by attenuating ER stress when added to the process of pulp cavity preparation.

Results

Altered histological structures including odontoblast localization

Histological changes in the pulp and dentin of the upper molar were evaluated using H&E and Masson's trichrome staining (Fig. 1A1–B4). After 3 and 5 days of operation, 4PBA-treated specimens showed more obvious blood vessel formation in the pulp (Fig. 1A2, A4, B2, and B4 arrow heads), and adjacent to the dentin wall, more number of nucleus in the cells developed heterochromatin staining patterns (Fig. 1A2, A4, B2, and B4). In addition, beneath the exposed pulp area, increased cell number

which showed plasma-like structure was detected (Fig. 1 *B2'* and *B4'*). According to the histological changes between the 4PBA and DMSO groups, an immunolocalization pattern for NESTIN was performed at 3 and 5 days after cavity preparation (Fig. 1 *C1–C4*). After 3 days, NESTIN, an indicator of active odontoblasts, is more strongly localized in cells along the dentin wall in the 4PBA-treated group (Fig. 1 *C2* and *C2'*) than it did in the DMSO group after 3 days (Fig. 1 *C1* and *C1'*). Similarly, after 5 days from 4PBA treatment, the localization of NESTIN showed stronger positive reactions in active odontoblasts than those of control (Fig. 1 *C3* and *C4*).

Altered localization patterns of ER stress, inflammation, and TGF- β 1 proteins

To examine the direct effect of 4PBA, we employed immunostaining against GRP78 and HRD1 (Fig. 2 *A1–A4* and *B1–B4*). The localization pattern of GRP78, a key indicator for induction of ER stress, was examined (Fig. 2 *A1–A4*). In the 4PBA-treated group, there was a decrease in localization level in both groups after 3- and 5-day compared to controls (Fig. 2 *A1, A2, A3*, and *A4*). In addition, we have examined the localization pattern of HRD1, a marker of ER protein quality control by ER-associated degradation of mis-folded proteins (Fig. 2 *B1–B4*). DMSO group, after 3 days, showed broad cytoplasmic localization pattern of HRD1 at the entire pulp area (Fig. 2 *B1–B1"*). Whereas nucleic localization pattern of HRD1 was examined in the damaged upper pulp cavity of the 4PBA-treated group after 3 days (Fig. 2 *B2–B2"*). After 5 days, the entire region of the pulp cavity in both the DMSO- and 4PBA-treated groups showed the nucleic localization of HRD1 (Fig. 2 *B3–B3"* and *B4–B4"*). Furthermore, we also examined MPO localization to evaluate the modulation pattern of inflammation in the exposed pulp (Fig. 2 *C1–C4*). MPO-positive cells were mainly apparent in the 4PBA-treated group at 3-day specimens (Fig. 2 *C2'*). The 4PBA-treated group after 3 days showed mild positive reaction against MPO and control group showed slight stronger positive reactions of MPO (Fig. 2 *C1, C2*). Similarly, at 5 days after drug treatment, the DMSO group (Fig. 2 *C3'*) showed stronger localization of MPO than that of the 4PBA group (Fig. 2 *C4'*). In addition, localization of TGF- β 1, known as an important regulator of various cellular events including proliferation, differentiation, and reparative dentinogenesis, was also carefully examined (Fig. 2 *D1–D4*). After 3 days, the 4PBA-treated group showed less positive reaction against TGF- β 1 at the exposed pulp area and dentin wall (Fig. 2 *D2*) when compared to controls (Fig. 2 *D1*). After 5 days from the treatment, the DMSO- and 4PBA-treated groups showed weak localization patterns of TGF- β 1 similarly in the dental pulp (Fig. 2 *D3* and *D4*). These localization patterns of TGF- β 1 would suggest that 4PBA treatment activates the earlier TGF- β 1 expression in the exposed pulp cavity. This histogenesis and the altered localization patterns of proteins from the 4PBA treatment would suggest that 4PBA treatment alters the cellular physiology such as odontoblast activation, blood vessel formation, and inflammatory cell migration in the exposed pulp. Furthermore, the intensities of immunostaining were quantified as none (-), exist (+), strong (++) and strongest (+++) and prepared as supplementary table 1.

Micro-CT and dentin bridge evaluations

Micro-CT evaluation was performed at 42 days after pulp cavity access preparation with treatment of 100 μ M 4PBA (Fig. 3). The coronal and horizontal views of 42 days after cavity preparation showed

significant differences in the quality of the regenerated hard tissue (Fig. 3A1 – A3) between the DMSO- and 4PBA-treated groups; the hard tissue density of the 4PBA group was much higher than that of the control group (Fig. 3B1 – B3). The percentage of hard tissue in the region of interest where marked with asterisks in the control group was 66.6%, whereas in the 4PBA treated group it was 77.4% (Fig. 3). These micro-CT image analyses were confirmed by histological examination (Fig. 4). In the DMSO-treated group, the hard tissue deposition appeared to be incomplete, and some inflammatory cells were still observed below the exposed areas (Fig. 4A1 and A2). In contrast, the 4PBA-treated group showed complete dentin bridge formation of which dentinal tubules are absent and irregularly arranged, similar with a bone-like structure; regenerated hard tissue continues with reparative dentin (Fig. 4B1 and B2).

Discussion

Formation of dentin is mainly contributed by cellular physiology of odontoblasts. These specific secreting cells are precisely regulated and modulated by ER regulation and vesicle trafficking to form the specifically featured dentin matrices through protein synthesis, folding, trafficking, secretion, and patterned apposition^{17,19,20}. Previous studies reported that modulation of ER stress would be an important cellular pathway for differentiation and maintenance of dentin^{18,31}. When dentin and pulp are exposed, several cellular reactions would be initiated to regenerate dentin and to protect the pulp cells^{32,33}. For example, when carious lesion or influence of the adverse effects of filling materials are affecting exposed pulp, the inflammatory processes are gradually increasing from mild to severe³². Until now, most of studies on pulp therapy did only focus on the inflammation controls and some growth factor treatments^{34–36}. Because, in clinic, these two tactical approaches showed the somehow qualified results with newly mineralized hard tissue and conserve the vitality of pulp^{32,34}. However, these approaches are required to be more developed for overcoming the limitations such as better quality of newly synthesized hard tissue and more biocompatible and prolonged guaranteed pulp condition.

In this study, we hypothesized that in the exposed dentin and pulp complex, ER stress would be occurred unavoidably to repair the dentin structure and maintain the vitality of the pulp as examined in other wound sites^{17,19,20}. In inflammation condition, a number of studies revealed the vicious circle of escalation of pathological conditions between ER stress and inflammation responses^{26,37}. Inflammation disturbs protein folding, leading to the accumulation of unfolded or mis-folded proteins inside the ER which in turn results in UPR³⁰. Furthermore, sustained ER stress induces chronic activation of UPR, which would eventually lead to severe inflammation and even cell death³⁸. Recently, studies on ER regulation and vesicle trafficking made use of chemical chaperone treatments to subdue a range of incurable diseases^{24,26,27}. Similarly, in this study, in order to prevent pathological conditions and promote dentin regeneration after pulp exposure, we examined the function of the chemical chaperone 4PBA, one of the candidate drugs for relieving ER stress, as a drug repositioning approach, in exposed pulp cavity, using a well-established animal model system³³. Drug repositioning is intended to apply alternative uses for a drug that is developed by another investigator and besides its original medical purpose. It has

advantages in financial and time savings for drug development with the lower failure risk and reduced drug investigating time and costs^{39–41}.

Recent reports elaborated 4PBA has chaperone properties and stabilizes protein conformation in ER which facilitates ER stress and UPR activation^{25,42}. We performed local delivery of 4PBA with concentration of 100 μ M which was determined in *in vitro* cell culture condition⁴³ and it is comparably lower than oral medication for systematic applications^{19,26,27,44}. This low concentration of drug local delivery was available in pulp cavity preparation model system and would confirm the merits including harmless, economic and efficient method for treatment. Interestingly, our results showed that local delivery of 4PBA presented the excellent morphological changes with dentin bridge and altered cellular changes in late and early time period of exposed pulp and dentin. Based on results, we proposed that two key factors for successive pulp therapy would be management of ER stress and control of initial inflammatory status of the pulp which influences the quality of newly mineralized hard tissue after treatment

As we expected, 4PBA showed the excellent function for resolving ER stress from the exposed pulp (Fig. 2). After precise examinations of localization patterns of ER stress related molecules, GRP78 and HRD1, we suggest that the regulation of inflammation of dental pulp and formation of reparative dentin would be resulted from the proper modulation of ER stress by treatment of 4PBA^{45,46}. HRD1 plays a critical role in ER-associated degradation (ERAD) of mis-folded/unfolded proteins as it protects cells from ER stress-induced cell death^{24,47,48}. We have observed interesting result after precise examination of localization patterns of HRD1. The differential localization patterns of HRD1 between controls and the 4PBA group is worth noting. The localization pattern of HRD1 in control group showed cytoplasmic positive localization generally at the entire pulp area. In the 4PBA-treated group after 3 days, there was a decrease in the localization compared to controls, and localization pattern was observed only at a limited area of the dentin wall, which was a nucleic localization (Fig. 2B1-B1" and B2-B2"). These results coincide with a previous report that found that cytoplasmic localization of HRD1 would take place when secretory cells are impaired²⁴. The results support that 4PBA treatment in the exposed dentin alleviates ER stress and ERAD by providing the proper condition of tissue regeneration.

4PBA treatment activated the MPO expression in the dentin-pulp complex after 3 days of cavity preparation (Fig. 2). Furthermore, histological examinations after 4PBA treatment showed less enucleated cells and more blood vessel formation than those of the control group (Fig. 1). Then, after 5 days, 4PBA-treated specimens showed less positive reaction against MPO than the control. This histological observation and the localization pattern of MPO would suggest that 4PBA initiates blood vessel formation for rapid control of inflammation through neutrophil-mediated inflammation modulation after the dentin injuries. Localization patterns of NESTIN and TGF- β 1 were carefully examined with histological alterations (Fig. 1C and Fig. 2D). At 3 days, both NESTIN and TGF- β 1 increased their positive localizations compared to controls. These results confirmed the previous report that elevated levels of NESTIN would stimulate cell proliferation and invasion by stimulating the TGF- β 1 signaling pathway

^{35,49,50}. To examine the morphological changes of reparative dentin formation, we examined the micro-CT 6 weeks after cavity preparation and its histologic images to understand the structural alteration of 4PBA molecular reactions (Fig. 3 and 4). As we observed at early-onset cellular changes, long-term regeneration of reparative dentin in area of exposed pulp with dentin bridge formation (Fig. 4). Our results show that 4PBA treatment in exposed pulp and dentin complex would modulate inflammation controls through blood vessel formation which improves healing capacity and also it promotes the active odontoblasts to produce reparative dentin through TGF beta 1 signaling pathways. We suggest that 4PBA would be a feasible treatment after pulpal cavity preparation to facilitate dentin regeneration, modulating pulp inflammation and alleviating ER stress. However, to reveal the detailed cellular physiology and signaling pathway for controlling inflammation, it is necessary to employ the immunostainings against various proteins including CD31, F4/80, and IL1 β in the near future. We recommend 4PBA as a feasible treatment after pulp capping to facilitate dentin regeneration, modulating pulp inflammation and alleviating ER stress.

Overall, we examined the function of the FDA-approved drug, 4PBA in dentin regeneration as a drug repositioning approach. This attempt would be a plausible answer to extend and develop new drugs and techniques in the dental field. For example, as a drug repositioning strategy, many ER stress-related drugs such as melatonin, simvastatin, and TUDCA should be tested in the dental field ^{25,43,51}. In addition, we suggest examining the synergistic effects of ER stress-relieving molecules in combination with previously established treatment including triple antibiotic paste and Wnt signaling-related small molecules ^{33,52}.

Methods

Animals

All experiments involving animals were performed in accordance with the guidelines of the Kyungpook National University, School of Dentistry, Intramural Animal Use and Care Committee (KNU-2015-136) as previous study mentioned ³³. For the pulp access cavity preparation, at least 15 adult 8-week-old male ICR mice in each group were euthanized 3, 5, and 42 days after pulp exposure.

Histology and immunohistochemistry

Histology and immunostaining were performed as described previously ³³. Primary antibodies were directed against NESTIN (1:400; cat.no. ab11306; Abcam), glucose regulatory protein 78 (GRP78; 1:400; cat.no. ab21685; Abcam), HRD1 (1:400; cat.no. NB100-2526; Novus Biologicals), MPO (1:200; cat.no. bs-4943R; Bioss), and TGF- β 1 (1:100; cat.no. ab92486; Abcam), and the secondary antibodies used in the present study were biotinylated goat anti-rabbit or anti-mouse immunoglobulin G. Immunocomplexes were visualized using a diaminobenzidine tetrahydrochloride reagent kit (cat.no. C09-12; GBI Labs).

Pulp cavity preparation

The animal experiment was performed as previously described³³. The animals were anesthetized by intraperitoneal injections of Avertin (Sigma-Aldrich, USA). Pulpal cavity was mechanically prepared in pulp chamber of first right molar of 8 wks male mice using a 0.6-mm round burr in a high-speed handpiece with water spray under a dissecting microscopy (S6, Leica). Pluronic F-127 medium containing 100 µM 4PBA or 0.07% DMSO was treated in the pulpal cavity. The left upper molars were used as the untreated controls. After treatment, the exposed teeth were double-sealed with Dy-cal (Dentsply Caulk, Milford, DE) and light-cured composite resin with a bonding system.

Micro-CT imaging

The samples after 6 weeks were analyzed using micro-CT imaging (Skyscan1272; Bruker, Kontich, Belgium). The specimens were scanned through 360° at a spatial resolution of 4904×3280 pixels with a pixel size of 2 µm. The image data were reconstructed and analyzed using Dataviewer and CTAn (Bruker) to quantify the volume of hard tissue formation³³.

Declarations

Acknowledgments

Supported by the National Research Foundation of Korea (NRF) NRF-2018R1A2A3075600, NRF-2017R1A5A2015391.

Author contributions

Eui-Seon Lee contributed to conceptualization, data acquisition, data analysis, interpretation, preparation of the main manuscript test. Yam Prasad Aryal and Tae-Young Kim contributed to analysis and interpretation of data. Jae-Kwang Jung and Jung-Hong Ha contributed to data acquisition and analysis, experimental designing, interpretation of data. Jae-Young Kim contributed to conceptualization and designing of manuscript, data analysis, interpretation and critically revised the manuscript. All authors reviewed the manuscript.

Competing interests

Authors deny any conflicts of interest related to this study.

Ethical approval

All experiments involving animals were performed according to the guidelines of the Kyungpook National University, School of Dentistry, Intramural Animal Use and Care Committee (KNU-2015-136).

References

1. Sarvani, C., Sireesh, D. & Ramkumar, K. M. Unraveling the role of ER stress inhibitors in the context of metabolic diseases. *Pharmacol. Res.***119**, 412–421 (2017).
2. Schröder, M. & Kaufman, R. J. ER stress and the unfolded protein response. *Mutat. Res.***569**, 29–63 (2005).
3. Hartl, F. U. & Hayer-Hartl, M. Converging concepts of protein folding in vitro and in vivo. *Nat. Struct. Mol. Biol.***16**, 574–581 (2009).
4. Saibil, H. Chaperone machines for protein folding, unfolding and disaggregation. *Nat. Rev. Mol. Cell Biol.***14**, 630–642 (2013).
5. Ron, D. & Harding, H. P. Protein-folding homeostasis in the endoplasmic reticulum and nutritional regulation. *Cold Spring Harb. Perspect. Biol.***4**, (2012).
6. Hubbard, S. C. & Ivatt, R. J. Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.***50**, 555–583 (1981).
7. Ellgaard, L., Molinari, M. & Helenius, A. Setting the standards: quality control in the secretory pathway. *Science***286**, 1882–1888 (1999).
8. Kozutsumi, Y., Segal, M., Normington, K., Gething, M.-J. & Sambrook, J. The presence of malformed proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature***332**, 462–464 (1988).
9. Zimmer, T., Ogura, A., Ohta, A. & Takagi, M. Misfolded membrane-bound cytochrome P450 activates KAR2 induction through two distinct mechanisms. *J. Biochem.***126**, 1080–1089 (1999).
10. Merksamer, P. I. & Papa, F. R. The UPR and cell fate at a glance. *J. Cell Sci.***123**, 1003–1006 (2010).
11. Hetz, C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.***13**, 89–102 (2012).
12. Goldberg, M., Kulkarni, A. B., Young, M. & Boskey, A. Dentin: structure, composition and mineralization. *Front. Biosci. (Elite Ed)***3**, 711–735 (2011).
13. Cajazeira Aguiar, M. & Arana-Chavez, V. E. Ultrastructural and immunocytochemical analyses of osteopontin in reactionary and reparative dentine formed after extrusion of upper rat incisors. *J. Anat.***210**, 418–427 (2007).
14. Simon, S. & Smith, A. J. Regenerative endodontics. *Br. Dent. J.***216**, 1–4 (2014).
15. Angelova Volponi, A., Zaugg, L. K., Neves, V., Liu, Y. & Sharpe, P. T. Tooth Repair and Regeneration. *Curr. Oral Heal. Reports***5**, 295–303 (2018).
16. Cameron, T. L. *et al.* Cartilage-specific ablation of XBP1 signaling in mouse results in a chondrodysplasia characterized by reduced chondrocyte proliferation and delayed cartilage maturation and mineralization. *Osteoarthr. Cartil.***23**, 661–670 (2015).
17. Aryal, Y. P. *et al.* An endoplasmic reticulum stress regulator, Tmbim6, modulates secretory stage of mice molar. *J. Cell. Physiol.***234**, 20354–20365 (2019).
18. Brookes, S. J., Barron, M. J., Dixon, M. J. & Kirkham, J. The unfolded protein response in amelogenesis and enamel pathologies. *Front. Physiol.***8**, (2017).

19. Brookes, S. J., Barron, M. J., Boot-Handford, R., Kirkham, J. & Dixon, M. J. Endoplasmic reticulum stress in amelogenesis imperfecta and phenotypic rescue using 4-phenylbutyrate. *Hum. Mol. Genet.***23**, 2468–2480 (2014).
20. Liang, T. *et al.* Mutant Dentin Sialophosphoprotein Causes Dentinogenesis Imperfecta. *J. Dent. Res.***98**, 912–919 (2019).
21. Iannitti, T. & Palmieri, B. Clinical and experimental applications of sodium phenylbutyrate. *Drugs R. D.***11**, 227–249 (2011).
22. Zeng, M. *et al.* 4-PBA inhibits LPS-induced inflammation through regulating ER stress and autophagy in acute lung injury models. *Toxicol. Lett.***271**, 26–37 (2017).
23. Yam, G. H. F., Gaplovska-Kysela, K., Zuber, C. & Roth, J. Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis. *Investig. Ophthalmol. Vis. Sci.***48**, 1683–1690 (2007).
24. Wu, T. *et al.* HRD1, an Important Player in Pancreatic β -Cell Failure and Therapeutic Target for Type 2 Diabetic Mice. *Diabetes***69**, 940 LP – 953 (2020).
25. Ozcan, L. *et al.* Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metab.***9**, 35–51 (2009).
26. Luo, Z.-F. *et al.* Effects of 4-phenylbutyric acid on the process and development of diabetic nephropathy induced in rats by streptozotocin: regulation of endoplasmic reticulum stress-oxidative activation. *Toxicol. Appl. Pharmacol.***246**, 49–57 (2010).
27. Singh, O. V, Pollard, H. B. & Zeitlin, P. L. Chemical rescue of deltaF508-CFTR mimics genetic repair in cystic fibrosis bronchial epithelial cells. *Mol. Cell. Proteomics***7**, 1099–1110 (2008).
28. Sawada, N. *et al.* Involvement of hypoxia-triggered endoplasmic reticulum stress in outlet obstruction-induced apoptosis in the urinary bladder. *Lab. Invest.***88**, 553–563 (2008).
29. Vij, N., Fang, S. & Zeitlin, P. L. Selective inhibition of endoplasmic reticulum-associated degradation rescues DeltaF508-cystic fibrosis transmembrane regulator and suppresses interleukin-8 levels: therapeutic implications. *J. Biol. Chem.***281**, 17369–17378 (2006).
30. Kim, H. J. *et al.* Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF- κ B/HIF-1 α signaling pathway. *Sci. Rep.***3**, 1142 (2013).
31. Kim, J. W. *et al.* Transcriptional factor ATF6 is involved in odontoblastic differentiation. *J. Dent. Res.***93**, 483–489 (2014).
32. Goldberg, M., Njeh, A. & Uzunoglu, E. Is Pulp Inflammation a Prerequisite for Pulp Healing and Regeneration? *Mediators Inflamm.***2015**, 347649 (2015).
33. Jung, J. K. *et al.* Bortezomib Facilitates Reparative Dentin Formation after Pulp Access Cavity Preparation in Mouse Molar. *J. Endod.***43**, 2041–2047 (2017).
34. Tran, X. V. *et al.* Reparative Mineralized Tissue Characterization after Direct Pulp Capping with Calcium-Silicate-Based Cements. *Mater. (Basel, Switzerland)***12**, (2019).

35. Niwa, T. *et al.* The dynamics of TGF- β in dental pulp, odontoblasts and dentin. *Sci. Rep.***8**, 4450 (2018).
36. Bae, C. H. *et al.* Wntless regulates dentin apposition and root elongation in the mandibular molar. *J. Dent. Res.***94**, 439–445 (2015).
37. Qi, W. *et al.* Attenuation of diabetic nephropathy in diabetes rats induced by streptozotocin by regulating the endoplasmic reticulum stress inflammatory response. *Metabolism***60**, 594–603 (2011).
38. Rao, R. V *et al.* Coupling endoplasmic reticulum stress to the cell death program. Mechanism of caspase activation. *J. Biol. Chem.***276**, 33869–33874 (2001).
39. Birjandi, A. A., Suzano, F. R. & Sharpe, P. T. Drug Repurposing in Dentistry; towards Application of Small Molecules in Dentin Repair. *Int. J. Mol. Sci.***21**, (2020).
40. Pushpakom, S. *et al.* Drug repurposing: progress, challenges and recommendations. *Nat. Rev. Drug Discov.***18**, 41–58 (2019).
41. Nosengo, N. Can you teach old drugs new tricks? *Nature* vol. 534 314–316 (2016).
42. Ozcan, U. *et al.* Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science***313**, 1137–1140 (2006).
43. Ranga Rao, S., Subbarayan, R., Ajitkumar, S. & Murugan Girija, D. 4PBA strongly attenuates endoplasmic reticulum stress, fibrosis, and mitochondrial apoptosis markers in cyclosporine treated human gingival fibroblasts. *J. Cell. Physiol.***233**, 60–66 (2018).
44. Basseri, S., Lhoták, S., Sharma, A. M. & Austin, R. C. The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response. *J. Lipid Res.***50**, 2486–2501 (2009).
45. Kaufman, R. J. Orchestrating the unfolded protein response in health and disease. *J. Clin. Invest.***110**, 1389–1398 (2002).
46. Lee, A. S. The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. *Methods***35**, 373–381 (2005).
47. Sun, S. *et al.* IRE1 α is an endogenous substrate of endoplasmic-reticulum-associated degradation. *Nat. Cell Biol.***17**, 1546–1555 (2015).
48. Wei, J. *et al.* ER-associated ubiquitin ligase HRD1 programs liver metabolism by targeting multiple metabolic enzymes. *Nat. Commun.***9**, 3659 (2018).
49. Toyono, T., Nakashima, M., Kuhara, S. & Akamine, A. Expression of TGF-beta superfamily receptors in dental pulp. *J. Dent. Res.***76**, 1555–1560 (1997).
50. Bokhari, A. A. *et al.* Nestin suppression attenuates invasive potential of endometrial cancer cells by downregulating TGF- β signaling pathway. *Oncotarget***7**, 69733–69748 (2016).
51. Karakida, T. *et al.* Potential for Drug Repositioning of Midazolam for Dentin Regeneration. *Int. J. Mol. Sci.***20**, (2019).

Figures

Figure 1

Histological examination using H&E and MTC and immunostaining of NESTIN after pulp access preparation. Arrow heads indicate blood vessels. Scale bars: A1 – B4, C1 – C4 = 200 μm; B1’ – B4’, C1’ – C4’ = 50 μm.

Figure 2

Immunohistochemistry staining with GRP78, HRD1, MPO, and TGF- β 1 after 3 days and 5 days pulp access preparation. Arrow heads indicates nucleic localization patterns of HRD1 immunostaining. Scale bars: A1–A4, B1–B4, C1–C4 = 200 μ m; A1'–A4', B1'–B4', C1'–C4' = 50 μ m; B1''–B4'' = 20 μ m.

Figure 3

Micro-CT and histology of 0.07% DMSO (A1–A5) and 100 μ M 4PBA (B1–B5) at 42 days after pulp cavity access preparation. The 4PBA applied cavity preparation tooth shows more deposition of dentin compared to DMSO control tooth. Arrow heads indicate the region of interest.

Figure 4

Histological structure using H&E staining and Masson's trichrome staining of reparative dentin formation 42 days after 4PBA treatment. The exposed dentin was not completely restored with hard tissues in the DMSO-treated groups (Fig. 4 A1 and A2). The reparative dentin formation of the 4PBA-treated tooth was completely restored with bone-like structures, dentin bridge (Fig. 4 B1 and B2). Arrow heads indicate regions of interest and dotted lines refer formation of dentin bridge. Scale bars = 50 μ m.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementary.docx](#)