Influence of chronic hyperhomocysteinemia on the features of bone metabolism in the case of lipopolysaccharide-induced periodontitis

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Abstract

Background. Periodontal disease is the second most common oral health problem after dental caries. This increasing prevalence makes it not only a health problem, but also a social issue. The pathogenesis of periodontal disease is associated with a number of adverse exogenous and endogenous factors, including hyperhomocysteinemia (HHcy).

Objectives. This study aimed to determine the features of bone metabolism in rats with lipopolysaccharide (LPS)-induced periodontitis combined with chronic thiolactone HHcy.

Material and methods. Forty-eight white, non-linear, mature rats were divided into 4 groups: control ($n = 12$); LPS-induced periodontitis ($n = 12$); chronic thiolactone HHcy ($n = 12$); and periodontitis combined with HHcy ($n = 12$). The rats were sacrificed the day after the last LPS injection or the day after the last homocysteine (Hcy) thiolactone administration. Bone metabolism was determined based on the activity of alkaline phosphatase (ALP) and acid phosphatase (AP) in blood serum and periodontal homogenate.

Results. A decrease in ALP activity (by 40.1%; $p = 0.001$) and the mineralization index (MI) (3.5 times; $p < 0.001$) with an increase in AP activity (2.0 times; $p < 0.001$) was observed in the periodontal homogenate of rats with LPS-induced periodontitis. In the case of LPS-induced periodontitis combined with chronic thiolactone HHcy, more pronounced changes in the activity of phosphatases and in MI were established as compared to rats with LPS-induced periodontitis only.

Conclusions. Chronic thiolactone HHcy enhances disturbances in bone metabolism in LPS-induced periodontitis. The osteotoxic effect of HHcy is associated with the activation of osteoclastogenesis and enhanced bone resorption. However, further research is required on the subject.

Keywords: periodontitis, bone metabolism, hyperhomocysteinemia

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Introduction

Inflammatory disorders that involve both soft and hard periodontal structures, such as gingivitis and periodontitis, are described as periodontal diseases. Nowadays, periodontal disease is the second most common oral health problem after dental caries,[1] and its increasing prevalence makes it not only a health problem, but also a social issue.[2] The prevalence of periodontal disease in the European population is above 50%, with 10% suffering from a severe form of the disease. The prevalence of the disease increases with age, reaching 70–85% in patients aged 60–65 years.[3] In Ukraine, the prevalence of periodontal disease among the general population is ranging from 92% to 98%.[4]

The early identification of periodontitis can help prevent the early loss of teeth. The treatment of periodontitis revolves around the debridement of the plaque biofilm and calculus which accumulate around the dentition. It is also pivotal to perform debridement and employ plaque control measures around dental restorations and at their interface with periodontal hard and soft tissues in order to prevent the initiation and progression of periodontitis. Additionally, the therapeutic strategies used in the treatment of periodontitis include the administration of antimicrobials, anti-inflammatory agents and antioxidants, systemically and topically.[5]

Several factors, of both exogenous and endogenous origin, are associated with the pathogenesis of periodontal disease. One of such factors is a high level of homocysteine (Hcy) – hyperhomocysteinemia (HHcy).[6] The first study on the association of Hcy with periodontitis appeared in 2004.[7] In 2015, Bhardwaj et al. suggested using Hcy as a marker of inflammation in patients with periodontitis.[8] Similar findings, including an increased level of Hcy in both the blood plasma and saliva of patients with inflammatory and destructive periodontal disease were obtained in other studies.[9–11]

The possible mechanisms resulting in increased Hcy levels in periodontitis involve the hyperproduction of pro-inflammatory cytokines, including interleukins (IL) (IL-1β, IL-6 and IL-8) as well as tumor necrosis factor alpha (TNF-α), in periodontal tissues. These mediators initiate an inflammatory cascade that can potentially disrupt methionine and Hcy homeostasis, leading to HHcy.[8,10]

On the other hand, metabolic dysregulation during periodontitis increases systemic inflammation, which leads to a decrease in the levels of vitamins, in particular B6, B12 and folic acid, which play important roles as cofactors in Hcy metabolism.[12]

There are only a few publications that focus on the course of experimental periodontitis in the case of HHcy. Krivosheeva et al. found that experimental HHcy complicated the course of periodontitis in rats.[13] Probable mechanisms responsible for the negative effect of HHcy on the course of periodontitis may be the activation of oxidative stress, which primarily causes endothelial damage and the development of endothelial dysfunction,[14,15] and the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is known to participate in the regulation of the adaptive immune response and stimulate the synthesis of pro-inflammatory cytokines (TNF-α, IL-1β), inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and leukocyte adhesion molecules, which promote the migration of leukocytes into the vessel wall, thereby increasing the cytotoxicity of leukocytes.[16]

It is worth noticing that recent evidence points to the same mechanism with regard to peri-implant tissues. Thus, Guarnieri et al. compared gingival tissue healing at the surgically manipulated periodontal sites and at the sites receiving implants and healing abutments with machined (MS) vs. laser-microtextured (LMS) surfaces, placed with one-stage protocol.[17] The researchers found that both MS and LMS implant sites presented a higher pro-inflammatory state in the early phase after surgery (1–4 weeks).[17]

This study aimed to determine the features of bone metabolism in rats with lipopolysaccharide (LPS)-induced periodontitis combined with chronic thiolactone HHcy.

Material and methods

Study design

Inbred white male rats (n = 48) were housed in a room with controlled temperature (22 ±2°C) and a 12/12 h light/dark cycle at the Laboratory Animal Facility of I. Horbachevsky Ternopil National Medical University, Ukraine, for the duration of the experiment. The animals had unrestricted access to food and water. The experimental procedures including animal treatment fully complied with relevant regulations.[18] The experimental design and protocol were approved by the Bioethics Committee of I. Horbachevsky Ternopil National Medical University (protocol No. 64 of May 17, 2021).

The study animals were randomly placed into one of 4 groups as follows: control (group 1; n = 12); animals with model periodontitis (group 2; n = 12); animals with chronic thiolactone HHcy (group 3; n = 12); and animals with periodontitis combined with HHcy (group 4; n = 12). The rats in group 2 received injections of 40 µL (1 mg/mL) of *Escherichia coli* (*E. coli*) LPS (Sigma-Aldrich, St. Louis, USA) into gingival tissues every other day for 2 weeks.[4] The rats in group 3 were administered Hcy thiolactone intragastrically (100 mg/kg of body weight in 1% solution of starch) once a day for 42 days.[19] In group 4, chronic thiolactone HHcy was induced as described above. From the 29th day after the start of HHcy

induction, the animals were injected with LPS into the gum tissue for 14 days concurrently with the ongoing Hcy thiolactone treatment.

The animals were euthanized with cardiac puncture under sodium thiopental anesthesia on the day following the last LPS injection (groups 2 and 4) or the day after the last Hcy thiolactone administration (group 3). The collected blood and periodontal samples were used for further investigations.

To confirm the development of HHcy in the blood serum of the animals, the total Hcy level was determined by means of a solid-phase enzyme-linked immunosorbent assay (ELISA), using an Axis Shield reagent kit (Axis Shield Diagnostics, Dundee, UK) according to the manufacturer's protocol and a Multiskan™ FC analyzer (Thermo Fisher Scientific, Vantaa, Finland). The Hcy level was expressed in μmol/L.

Bone metabolism was determined based on the activity of alkaline phosphatase (ALP) as a marker of osteoblast functioning and acid phosphatase (AP) as a marker of the intensification of osteoclast activity. The activity of phosphatases was expressed in μkat/L (in blood serum) or μkat/kg (in periodontal homogenate). The mineralization index (MI) was determined based on the ratio of ALP to AP.

Statistical analysis

The experimental data was compiled and analyzed using Microsoft Office Excel (v. 2016; Microsoft Corp., Redmond, USA) and Statistica, v. 7 (StatSoft Inc., Tulsa, USA). The Kolmogorov–Smirnov test was used to determine the normality of data distribution. If the values did not conform to normal distribution, the Kruskal–Wallis test was performed to compare 3 or more groups, followed by the Mann–Whitney test with the Bonferroni correction for the pairwise comparisons of the groups. The results were presented as median (*Me*) and interquartile (*IQR*) range. The results were considered statistically significant at the probability level (p-value) <0.05.

The association between the studied indices was established based on the results of the correlation analysis using Spearman's rank correlation coefficient. The linear correlation coefficient (r) and its probability (p-value) were calculated. The association was considered very weak at r = 0.10–0.30, weak at r = 0.31–0.50, moderate at r = 0.51–0.70, strong at r = 0.71–0.90, and very strong at r = 0.91–0.99. The direction of the association, positive or negative (inverse), was also assessed. The correlation coefficient was regarded as statistically significant at $p < 0.05$.

Results

The results showed that the blood serum Hcy level in rats with only LPS-induced periodontitis increased by 47.4% as compared to control, but this change was not statistically significant ($p = 0.215$). In animals with LPS-induced periodontitis combined with chronic thiolactone HHcy, this index increased 3.8 times as compared to control ($p < 0.001$) and was 2.6 times higher than in the case of LPS-induced periodontitis alone ($p = 0.002$). It should be noted that in animals with isolated chronic thiolactone HHcy, the serum level of Hcy increased 3.4 times as compared to control ($p < 0.001$), but it did not differ significantly from the serum Hcy level observed for the group with LPS-induced periodontitis combined with chronic thiolactone HHcy ($p = 0.999$) (Table 1).

We found that the activity of serum ALP in the case of LPS-induced periodontitis decreased by 22.1% as compared to control, but this change was not statistically significant ($p = 0.091$). In animals with isolated chronic thiolactone HHcy, an increase in this index by 94.6% as compared to control was found ($p = 0.001$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, the activity of serum ALP increased by 22.1% ($p = 0.893$) in relation to controls and was by 56.7% higher than in the case of LPS-induced periodontitis alone ($p = 0.005$) (Table 2).

While analyzing ALP activity in periodontal homogenate, it was found that in rats with LPS-induced periodontitis, this index significantly decreased by 40.1% as compared to control ($p = 0.001$). In animals with isolated chronic thiolactone HHcy, the periodontal ALP activity

Table 1. Level of homocysteine (Hcy) in the blood serum of rats with lipopolysaccharide (LPS)-induced periodontitis without comorbid pathology and combined with chronic thiolactone hyperhomocysteinemia (HHcy)

Parameter	Experimental group				Kruskal–Wallis criterion (H), p-value	p-values
	group 1 (control)	group 2 (periodontitis)	group 3 (HHcy)	group 4 (periodontitis + HHcy)		
Hcy [μmol/L]	7.70 (7.40–8.15)	11.35 (10.15–11.65)	26.39 (23.99–29.63)	29.04 (26.45–33.12)	26.41 $p < 0.001$*	$p_{1-2} = 0.215$ $p_{1-3} < 0.001$* $p_{1-4} < 0.001$* $p_{2-3} = 0.035$* $p_{2-4} = 0.002$* $p_{3-4} = 0.999$

Data presented as median (interquartile range) (*Me* (*IQR*)). p_{1-2}, p_{1-3}, p_{1-4}, p_{2-3}, p_{2-4}, p_{3-4} – probability of differences between the particular groups (numbers in lowercase correspond to group numbers); * statistically significant.

Table 2. Indices of bone metabolism in the blood serum and periodontal homogenate of rats with lipopolysaccharide (LPS)-induced periodontitis without comorbid pathology and combined with chronic thiolactone hyperhomocysteinemia (HHcy)

Parameter	Experimental group				Kruskal–Wallis criterion (H), *p*-value	*p*-values
	group 1 (control)	group 2 (periodontitis)	group 3 (HHcy)	group 4 (periodontitis + HHcy)		
Blood serum ALP [μkat/L]	12.90 (12.15–13.55)	10.05 (9.50–10.85)	25.10 (24.15–28.40)	15.75 (13.35–16.65)	41.79 $p < 0.001$*	$p_{1-2} = 0.091$ $p_{1-3} = 0.001$* $p_{1-4} = 0.893$ $p_{2-3} < 0.001$* $p_{2-4} = 0.005$* $p_{3-4} = 0.091$
Blood serum AP [μkat/L]	5.77 (5.42–6.24)	9.40 (8.00–9.75)	15.00 (12.55–15.60)	24.33 (20.86–25.54)	44.09 $p < 0.001$*	$p_{1-2} = 0.215$ $p_{1-3} < 0.001$* $p_{1-4} < 0.001$* $p_{2-3} = 0.215$ $p_{2-4} < 0.001$* $p_{3-4} = 0.215$
Blood serum MI (ALP/AP)	2.24 (2.10–2.33)	1.13 (1.04–1.26)	1.65 (1.61–2.07)	0.65 (0.59–2.07)	41.51 $p < 0.001$*	$p_{1-2} = 0.001$* $p_{1-3} = 0.970$ $p_{1-4} < 0.001$* $p_{2-3} = 0.082$ $p_{2-4} = 0.230$ $p_{3-4} < 0.001$*
Periodontal homogenate ALP [μkat/kg]	7.10 (6.60–7.90)	4.25 (3.70–4.95)	4.95 (4.75–5.30)	2.01 (1.82–2.17)	40.75 $p < 0.001$*	$p_{1-2} = 0.001$* $p_{1-3} = 0.070$ $p_{1-4} < 0.001$* $p_{2-3} = 0.999$ $p_{2-4} = 0.006$* $p_{3-4} = 0.001$*
Periodontal homogenate AP [μkat/kg]	4.18 (3.95–4.23)	8.55 (8.00–9.20)	7.10 (6.35–8.15)	19.19 (15.88–20.11)	43.12 $p < 0.001$*	$p_{1-2} < 0.001$* $p_{1-3} = 0.157$ $p_{1-4} < 0.001$* $p_{2-3} = 0.384$ $p_{2-4} = 0.007$* $p_{3-4} < 0.001$*
Periodontal homogenate MI (ALP/AP)	1.74 (1.67–1.85)	0.50 (0.47–0.56)	0.71 (0.67–0.80)	0.11 (0.10–0.14)	43.27 $p < 0.001$*	$p_{1-2} < 0.001$* $p_{1-3} = 0.166$ $p_{1-4} < 0.001$* $p_{2-3} = 0.348$ $p_{2-4} < 0.001$* $p_{3-4} < 0.001$*

Data presented as *Me* (*IQR*). ALP – alkaline phosphatase; AP – acid phosphatase; MI – mineralizaton index; p_{1-2}, p_{1-3}, p_{1-4}, p_{2-3}, p_{2-4}, p_{3-4} – probability of differences between the particular groups (numbers in lowercase correspond to group numbers); * statistically significant.

did not change significantly as compared to the control group ($p = 0.070$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, this index decreased 3.5 times as compared to control ($p < 0.001$) and was by 52.7% lower than in the case of LPS-induced periodontitis alone ($p = 0.006$) (Table 2).

Regarding the activity of serum AP in the case of LPS-induced periodontitis, this index increased by 62.9% as compared to the control group, but this change was not statistically significant ($p = 0.215$). In animals with isolated chronic thiolactone HHcy, a significant 2.6-fold increase of AP activity was found as compared to controls ($p < 0.001$). For the combination of LPS-induced periodontitis and chronic thiolactone HHcy, this index significantly increased 4.2 times as compared to control ($p < 0.001$) and was 2.6 times higher than in the case of LPS-induced periodontitis alone ($p < 0.001$) (Table 2).

In periodontal homogenate, AP activity was found to be significantly higher (2.0 times) in rats with LPS-induced periodontitis as compared to controls ($p < 0.001$). In animals with isolated chronic thiolactone HHcy, the periodontal AP activity did not change significantly as compared to the control group ($p = 0.157$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, this index significantly increased 4.6 times as compared to control ($p < 0.001$) and was 2.2 times higher than in the case of LPS-induced periodontitis alone ($p = 0.007$) (Table 2).

An important index that characterizes the condition of bone tissue is the ratio of ALP activity to AP activity, which is defined as MI. The results of our study showed that in the case of LPS-induced periodontitis, the serum MI significantly decreased by 49.6% as compared to the control group ($p = 0.001$). In animals with

isolated chronic thiolactone HHcy, a decrease in MI by 26.3% as compared to control was found, but this change was not statistically significant ($p = 0.970$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, the index decreased 3.4 times as compared to control ($p < 0.001$) and was 42.5% lower than in the case of LPS-induced periodontitis only; however, the latter change was not statistically significant ($p = 0.230$) (Table 2).

Regarding the changes of MI in periodontal homogenate, in rats with LPS-induced periodontitis, this index decreased 3.5 times as compared to the control group ($p < 0.001$). In animals with isolated chronic thiolactone HHcy, a 2.5-fold decrease in the periodontal MI as compared to control was found, but this change was not statistically significant ($p = 0.166$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, the index decreased 15.8 times as compared to control ($p < 0.001$) and was 4.5 times lower than in the case of LPS-induced periodontitis only ($p < 0.001$) (Table 2).

Analyzing the correlation linkages between the serum level of Hcy and the bone metabolism indices, we did not found any significant correlations in group 2 (LPS-induced periodontitis only). At the same time, in rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, a strong direct correlation between the serum Hcy level and the serum AP activity ($r = 0.89$; $p < 0.001$), as well as a moderate direct correlation between the serum Hcy level and the periodontal AP activity ($r = 0.66$; $p = 0.019$) were found. Of particular interest are significant correlations between the serum Hcy level and AP activity in blood serum ($r = 0.89$; $p < 0.001$), AP activity in periodontal homogenate ($r = 0.75$; $p = 0.005$) and MI in blood serum ($r = -0.71$; $p = 0.010$) in animals with isolated chronic thiolactone HHcy (Table 3).

Discussion

The leading role in the pathogenesis of periodontitis is played by the disorders of bone metabolism, which consists in maintaining balance between resorption processes (mediated by osteoclasts) and the formation of bone tissue (mediated by osteoblasts). A number of factors affect and coordinate bone remodeling on both systemic and local levels, ensuring the elimination of micro-damage in the bone matrix, the preservation of bone micro-architectonics and the maintenance of bone strength. The effects of the regulators of bone metabolism are realized through the main signaling pathways of osteoblastogenesis (canonical wingless-beta-catenin (Wnt/β-catenin)) and osteoclastogenesis (the system of receptor activator for NF-κB ligand (RANKL)/receptor activator for NK-κB (RANK)/osteoclastogenesis inhibitory factor (OCIF)).[20–22]

Among the markers of bone metabolism, the determination of phosphatases is relatively widely studied and used. In bone tissue, ALP is synthesized by osteoblasts and their precursors, and is involved in the mineralization of the bone matrix. The activity of ALP is interpreted as an index of bone tissue formation. Acid phosphatase is a lysosomal enzyme that characterizes the activity of osteoclasts and reflects the processes of bone matrix degradation.

The results of our study showed that LPS-induced periodontitis without comorbid pathology in rats is accompanied by decreases in ALP activity and MI, with a simultaneous increase of AP activity in periodontal homogenate, which indicates diminishing the function of osteoblasts in periodontal bone tissue and the activation of osteoclasts, and hence osteoresorption. Chronic thiolactone HHcy exacerbates the disorders of bone metabolism in the case of periodontitis. It is confirmed by more pronounced changes in all studied indices as compared to animals with LPS-induced periodontitis only. A moderate direct

Table 3. Correlation linkages between the level of homocysteine (Hcy) in blood serum and the indices of bone metabolism in the case of lipopolysaccharide (LPS)-induced periodontitis without comorbid pathology and combined with chronic thiolactone hyperhomocysteinemia (HHcy) (r_{xy})

Parameter		Experimental groups		
		group 2 (periodontitis)	group 3 (HHcy)	group 4 (periodontitis + HHcy)
Hcy [μmol/L]	blood serum ALP [μkat/L]	$r = -0.49$ $p = 0.105$	$r = 0.01$ $p = 0.974$	$r = 0.20$ $p = 0.533$
	periodontal homogenate ALP [μkat/kg]	$r = -0.19$ $p = 0.564$	$r = -0.08$ $p = 0.814$	$r = -0.10$ $p = 0.755$
	blood serum AP [μkat/L]	$r = 0.10$ $p = 0.765$	$r = 0.89$ $p < 0.001$*	$r = 0.89$ $p < 0.001$*
	periodontal homogenate AP [μkat/kg]	$r = 0.25$ $p = 0.428$	$r = 0.75$ $p = 0.005$*	$r = 0.66$ $p = 0.019$*
	blood serum MI (ALP/AP)	$r = -0.32$ $p = 0.305$	$r = -0.71$ $p = 0.010$*	$r = -0.51$ $p = 0.092$
	periodontal homogenate MI (ALP/AP)	$r = -0.31$ $p = 0.327$	$r = -0.47$ $p = 0.123$	$r = -0.32$ $p = 0.313$

* statistically significant.

correlation between the serum Hcy level and AP activity in periodontal homogenate (r = 0.66; p = 0.019) indicates the association between Hcy and osteoclast activity in the case of experimental periodontitis.

Vacek et al. suggest that in addition to the direct Hcy action on the bone matrix, Hcy can modulate bone remodeling via increased osteoclast activity and decreased osteoblast activity.[23]

Homocysteine can also directly affect osteoclast performance. Homocysteine has been demonstrated to exert a potent stimulatory inluence on oxidant signaling, while osteoclasts are known to be sensitive to elevated reactive oxygen species (ROS) levels.[24] In vitro experiments enriching growth media with Hcy for the bone marrow cell culture demonstrated that in these cells, the upregulation of the formation of osteoclasts and the downregulation of apoptosis were caused by an increased production of ROS.[25] Elevated Hcy also disrupts balance between the phosphorylation and dephosphorylation of protein kinases (PKs) modulating bone cell remodeling; this can cause the cell-wide disruption of molecular mechanisms in bone marrow-derived osteoclasts. For instance, Hcy has been shown to increase the phosphorylation of P38 mitogen-activated protein kinases (MAPKs) mediated by RANKL.[25]

Patients with HHcy show a higher risk of fractures due to decreased bone mineral density, as a result of bone resorption caused by elevated osteoclast activity.[26] Additionally, in human bone marrow stromal cells, elevated Hcy levels activate the caspase-dependent apoptosis pathway, which also results in compromised bone repair.[27] Bone resorption is slowed down in unfavorable oxidizing environments, which are activated by Hcy binding as a ligand to peroxisome proliferator-activated receptor gamma (PPAR-γ) expressed in bone cells.[28] A study done on a HHcy mouse model showed the attenuation of PPAR-γ, tissue inhibitor of matrix metalloproteinase 4 (TIMP-4) and thioredoxin (an antioxidant), while inducing matrix metallopeptidase 9 (MMP-9), TIMP-3, and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase 4 (Nox4).[29]

A correlation between Hcy and the bone turnover markers detected in serum, such as deoxypyridinoline[30] and C-terminal telopeptide of type I collagen,[31] was found in some studies.

However, the effect of Hcy on osteoblast activity is comparatively little understood. Osteoblasts are involved in bone formation, and in vitro studies show that elevated concentrations of Hcy result in a moderate increase in primary human osteoblast activity.[32] However, the extent of this effect is less prominent than the effects on primary human osteoclasts described above. This indicates the lack of balance between osteoblast and osteoclast activity. In the HS-5 cell line, Hcy has been shown to induce apoptosis in NF-κB-activated primary human bone marrow stromal cells through the ROS-mediated mitochondrial pathway.[26] The resulting escape of cytochrome c from the mitochondria activates caspases 3 and 9, and is likely to produce an apoptotic effect on osteoblasts. In a study by Herrmann et al., the inhibition of osteoblast activity was supported by the reduced circulating osteocalcin level (by 40%) observed in rats with HHcy compared to controls.[33]

In another research by Herrmann et al., the bone tissue of rats with HHcy showed an increased accumulation of Hcy, with 65% of it bound to collagen of the extracellular matrix.[34] The accumulation of Hcy produced a "spongy" bone appearance and resulted in decreased bone strength.[34] This bone-specific accumulation of Hcy is a mechanism likely to underlie the detrimental effects of HHcy on bone tissue. Furthermore, Hcy has been found to downregulate the mRNA expression of protein-lysine 6-oxidase (LOX), an enzyme essential for cross-linking in collagen.[35]

Limitations

There are some limitations to this study. Firstly, the study sample size was small; therefore, the results are presented as preliminary. Bone metabolism was only determined with the use of biochemical markers, without imaging methods, such as computed tomography (CT) scanning. Further investigations are needed to explore the clinical implications of these findings.

Conclusions

The disruption of bone remodeling by LPS-induced periodontitis results in imbalance between bone matrix synthesis by osteoblasts and bone turnover by osteoclasts. Moreover, chronic thiolactone HHcy enhances the violations of bone metabolism in LPS-induced periodontitis. The osteotoxic effect of HHcy is associated with the activation of osteoclastogenesis and enhanced bone resorption. However, it is likely that there are other mechanisms of negative effects of HHcy on bone metabolism, which require further research.

Ethics approval and consent to participate

The experimental procedures including animal treatment fully complied with relevant regulations. The experimental design and protocol were approved by the Bioethics Committee of I. Horbachevsky Ternopil National Medical University (protocol No. 64 of May 17, 2021).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. GBD 2017 Oral Disorders Collaborators; Bernabe E, Marcenes W, Hernandez CR, et al. Global, regional, and national levels and trends in burden of oral conditions from 1990 to 2017: A systematic analysis for the Global Burden of Disease 2017 study. *J Dent Res*. 2020;99(4):362–373. doi:10.1177/0022034520908533
2. Deszczyńska K, Górska R, Haładyj A. Clinical condition of the oral cavity in overweight and obese patients. *Dent Med Probl*. 2021;58(2):147–154. doi:10.17219/dmp/127873
3. Peres MA, Macpherson LM, Weyant RJ, et al. Oral diseases: A global public health challenge. *Lancet*. 2019;394(10194):249–260. doi:10.1016/S0140-6736(19)31146-8
4. Shcherba V, Kyryliv M, Bekus I, Krynytska I, Marushchak M, Korda MA. A comparative study of connective tissue metabolism indices in experimental comorbidity-free periodontitis and periodontitis combined with thyroid dysfunction. *J Med Life*. 2020;13(2):219–224. doi:10.25122/jml-2019-0113
5. Balaji TM, Varadarajan S, Jagannathan R, et al. Melatonin as a topical/systemic formulation for the management of periodontitis: A systematic review. *Materials (Basel)*. 2021;14(9):2417. doi:10.3390/ma14092417
6. Škovierová H, Vidomanová E, Mahmood S, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. *Int J Mol Sci*. 2016;17(10):1733. doi:10.3390/ijms17101733
7. Montebugnoli L, Servidio D, Miaton RA, Prati C, Tricoci P, Melloni C. Poor oral health is associated with coronary heart disease and elevated systemic inflammatory and haemostatic factors. *J Clin Periodontol*. 2004;31(1):25–29. doi:10.1111/j.0303-6979.2004.00432.x
8. Bhardwaj S, Venkatesh Prabhuji ML, Karthikeyan BV. Effect of non-surgical periodontal therapy on plasma homocysteine levels in Indian population with chronic periodontitis: A pilot study. *J Clin Periodontol*. 2015;42(3):221–227. doi:10.1111/jcpe.12374
9. Ślebioda Z, Szponar E, Dorocka-Bobkowska B. Vitamin D and its relevance in the etiopathogenesis of oral cavity diseases. *Arch Immunol Ther Exp (Warsz)*. 2016;64(5):385–397. doi:10.1007/s00005-016-0384-z
10. Mallapragada S, Kasana J, Agrawal P. Effect of nonsurgical periodontal therapy on serum highly sensitive capsule reactive protein and homocysteine levels in chronic periodontitis: A pilot study. *Contemp Clin Dent*. 2017;8(2):279–285. doi:10.4103/ccd.ccd_140_17
11. Penmetsa GS, Bhaskar RU, Mopidevi A. Analysis of plasma homocysteine levels in patients with chronic periodontitis before and after nonsurgical periodontal therapy using high-performance liquid chromatography. *Contemp Clin Dent*. 2020;11(3):266–273. doi:10.4103/ccd.ccd_650_18
12. Stanisic D, Jovanovic M, George AK, et al. Gut microbiota and the periodontal disease: Role of hyperhomocysteinemia. *Can J Physiol Pharmacol*. 2021;99(1):9–17. doi:10.1139/cjpp-2020-0215
13. Krivosheeva EM, Fefelova EV, Sepp AV, Borodulina II, Borodulina NV. The effectiveness of adaptogens in experimental periodontitis against the background of hyperhomocysteinemia [in Russian]. *Bulletin ESSC SB RAMS*. 2010;3(73):221–225. https://cyberleninka.ru/article/n/effektivnost-adaptogenov-pri-eksperimentalnom-parodontite-na-fone-gipergomotsisteinemii. Accessed September 1, 2021.
14. Tyagi N, Sedoris KC, Steed M, Ovechkin AV, Moshal KS, Tyagi SC. Mechanisms of homocysteine-induced oxidative stress. *Am J Physiol Heart Circ Physiol*. 2005;289(6):H2649–H2656. doi:10.1152/ajpheart.00548.2005
15. Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L. Reactive oxygen species: Key regulators in vascular health and diseases. *Br J Pharmacol*. 2018;175(8):1279–1292. doi:10.1111/bph.13828
16. Wang XJ, Tian DC, Wang FW, et al. Astaxanthin inhibits homocysteine-induced endothelial cell dysfunction via the regulation of the reactive oxygen species-dependent VEGF-VEGFR2-FAK signaling pathway. *Mol Med Rep*. 2019;19(6):4753–4760. doi:10.3892/mmr.2019.10162
17. Guarnieri R, Miccoli G, Reda R, Mazzoni A, Di Nardo D, Testarelli L. Sulcus fluid volume, IL-6, and Il-1b concentrations in periodontal and peri-implant tissues comparing machined and laser-microtextured collar/abutment surfaces during 12 weeks of healing: A split-mouth RCT. *Clin Oral Implants Res*. 2022;33(1):94–104. doi:10.1111/clr.13868
18. Council of Europe. European Treaty Series – No.123: European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg, France: Council of Europe; 1986. https://rm.coe.int/168007a67b. Accessed September 1, 2021.
19. Stangl GI, Weisse K, Dinger C, Hirche F, Brandsch C, Eder K. Homocysteine thiolactone-induced hyperhomocysteinemia does not alter concentrations of cholesterol and SREBP-2 target gene mRNAS in rats. *Exp Biol Med (Maywood)*. 2007;232(1):81–87. PMID:17202588.
20. Czupkallo L, Rahnama M, Kielbowicz D, Lobacz M, Kozicka-Czupkallo M. Bone metabolism and RANKL/RANK/OPG trail in periodontal disease. *Curr Issues Pharm Med Sci*. 2017;29(4):171–175. doi:10.1515/cipms-2016-0036
21. Infante M, Fabi A, Cognetti F, Gorini S, Caprio M, Fabbri A. RANKL/RANK/OPG system beyond bone remodeling: Involvement in breast cancer and clinical perspectives. *J Exp Clin Cancer Res*. 2019;38(1):12. doi:10.1186/s13046-018-1001-2
22. Kobayashi Y, Uehara S, Udagawa N, Takahashi N. Regulation of bone metabolism by Wnt signals. *J Biochem*. 2016;159(4):387–392. doi:10.1093/jb/mvv124
23. Vacek TP, Kalani A, Voor MJ, Tyagi SC, Tyagi N. The role of homocysteine in bone remodeling. *Clin Chem Lab Med*. 2013;51(3):579–590. doi:10.1515/cclm-2012-0605
24. Behera J, Bala J, Nuru M, Tyagi SC, Tyagi N. Homocysteine as a pathological biomarker for bone disease. *J Cell Physiol*. 2017;232(10):2704–2709. doi:10.1002/jcp.25693
25. Koh JM, Lee YS, Kim YS, et al. Homocysteine enhances bone resorption by stimulation of osteoclast formation and activity through increased intracellular ROS generation. *J Bone Miner Res*. 2006;21(7):1003–1011. doi:10.1359/jbmr.060406
26. Schalinske KL, Smazal AL. Homocysteine imbalance: A pathological metabolic marker. *Adv Nutr*. 2012;3(6):755–762. doi:10.3945/an.112.002758
27. Kim DJ, Koh JM, Lee O, et al. Homocysteine enhances apoptosis in human bone marrow stromal cells. *Bone*. 2006;39(3):582–590. doi:10.1016/j.bone.2006.03.004
28. Stunes AK, Westbroek I, Gustafsson BI, et al. The peroxisome proliferator-activated receptor (PPAR) alpha agonist fenofibrate maintains bone mass, while the PPAR gamma agonist pioglitazone exaggerates bone loss, in ovariectomized rats. *BMC Endocr Disord*. 2011;11:11. doi:10.1186/1472-6823-11-11
29. Mishra PK, Tyagi N, Sen U, Joshua IG, Tyagi SC. Synergism in hyperhomocysteinemia and diabetes: Role of PPAR gamma and tempol. *Cardiovasc Diabetol*. 2010;9:49. doi:10.1186/1475-2840-9-49
30. Dhonukshe-Rutten RA, Pluijm SM, de Groot LC, Lips P, Smit JH, van Staveren WA. Homocysteine and vitamin B12 status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. *J Bone Miner Res*. 2005;20(6):921–929. doi:10.1359/JBMR.050202
31. Bode MK, Laitinen P, Risteli J, Uusimaa P, Juvonen T. Atherosclerosis, type 1 collagen cross-linking and homocysteine. *Atherosclerosis*. 2000;152(2):531–532. doi:10.1016/s0021-9150(00)00548-7
32. Herrmann M, Umanskaya N, Wildemann B, et al. Stimulation of osteoblast activity by homocysteine. *J Cell Mol Med*. 2008;12(4):1205–1210. doi:10.1111/j.1582-4934.2008.00104.x
33. Herrmann M, Wildemann B, Claes L, et al. Experimental hyperhomocysteinemia reduces bone quality in rats. *Clin Chem*. 2007;53(8):1455–1461. doi:10.1373/clinchem.2007.086272
34. Herrmann M, Tami A, Wildemann B, et al. Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone. *Bone*. 2009;44(3):467–475. doi:10.1016/j.bone.2008.10.051
35. Thaler R, Spitzer S, Rumpler M, et al. Differential effects of homocysteine and beta aminopropionitrile on preosteoblastic MC3T3-E1 cells. *Bone*. 2010;46(3):703–709. doi:10.1016/j.bone.2009.10.038