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ORIGINAL RESEARCH

WILEY CLINICAL ORAL IMPLANTS RESEARCH

Losartan reverses impaired osseointegration in spontaneously hypertensive rats

Gabriel Mulinari-Santos[1,2,3,4] | Fábio Roberto de Souza Batista[1] | Franziska Kirchweger[2,3] | Stefan Tangl[2,3] | Reinhard Gruber[3,4,5] | Roberta Okamoto[6]

[1]Department of Oral Surgery and Integrated Clinic, Araçatuba Dental School, Universidade Estadual Paulista "Júlio de Mesquita Filho", Araçatuba, Brazil

[2]Karl Donath Laboratory for Hard Tissue and Biomaterial Research, Department of Oral Surgery, School of Dentistry, Medical University of Vienna, Vienna, Austria

[3]Austrian Cluster for Tissue Regeneration, Vienna, Austria

[4]Department of Oral Biology, Dental School, Medical University of Vienna, Vienna, Austria

[5]Department of Periodontology, School of Dental Medicine, University of Bern, Bern, Switzerland

[6]Department of Basic Science, Araçatuba Dental School, Universidade Estadual Paulista "Júlio de Mesquita Filho", Araçatuba, Brazil

Correspondence
Reinhard Gruber, Department of Oral Biology, Dental School, Medical University of Vienna, Vienna, Austria.
Email: reinhard.gruber@meduniwien.ac.at

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Abstract

Objective: Hypertension is not only associated with cardiovascular diseases but also with alterations in bone quality. Hypertension therefore might be a risk factor for osseointegration. Preclinical studies suggest that losartan, an angiotensin II receptor blocker widely used to treat hypertension, has a beneficial effect in graft consolidation. Here, we determine the effect of hypertension and losartan on osseointegration.

Methods: We used spontaneously hypertensive rats (SHR) and normotensive Wistar albinus rats receiving losartan (30 mg/kg, p.o.) or left untreated. After 1 week, titanium miniscrews were inserted into the tibia. Sixty days after implantation, implant stability was evaluated by removal torque measurement considered the primary endpoint. Microcomputed tomography and histomorphometric analysis were secondary endpoints.

Results: Losartan increased the removal torque in the hypertensive SHR group to levels of the Wistar controls. While the cortical parameters of osseointegration remained unchanged, losartan increased medullary bone formation. Microcomputed tomography revealed a higher bone volume per tissue volume and trabecular thickness in the SHR rats treated with losartan. Histomorphometric analysis further showed that losartan significantly increased the thickness of newly formed bone in medullary area in hypertensive SHR rats. Losartan did not significantly alter the parameters of osseointegration in normotensive rats.

Conclusions: The data presented suggest that the angiotensin II receptor antagonist losartan increases the medullary parameters of osseointegration in a tibia model of spontaneously hypertensive rats. Considering the study limitations, understanding the impact of hypertension and the respective drugs on osseointegration requires further research.

KEYWORDS

bone, losartan, osseointegration, renin-angiotensin system, spontaneously hypertensive rats

1 | INTRODUCTION

Hypertension is a major risk factor for premature death worldwide, mainly for causing cardiovascular complications (Tibazarwa & Damasceno, 2014). Every fifth person worldwide today suffers from hypertension and these numbers increase further (Kearney et al., 2005). In addition to cardiovascular complications, hypertension is associated with impaired calcium metabolism (McCarron, Yung, Ugoretz, & Krutzik, 1981; Wright & Rankin, 1982), decreased bone mineral density (Javed et al., 2012; Manrique et al., 2012), osteoporosis (Cappuccio, Kalaitzidis, Duneclift, & Eastwood, 2000), and consequently bone fractures (Vestergaard, Rejnmark, & Mosekilde, 2009; Yamamoto et al., 2015). Hypertension also negatively affects bone regeneration (Gealh et al., 2014; Manrique et al., 2015) and alveolar bone quality (Bastos et al., 2010), both crucial elements for the osseointegration of dental implants (Isidor, 2006, Schenk and Buser, 1998). Even though there is a lack of evidence from epidemiological studies identifying uncontrolled hypertension as a risk factor in implant dentistry, treatment with antihypertensive drugs was associated with an increased survival rate of osseointegrated implants (Wu et al., 2016).

Losartan, an angiotensin II receptor blocker (Al-Majed, Assiri, Khalil, & Abdel-Aziz, 2015), is prescribed particularly in the population over 60 years with more than half having hypertension (Ong, Cheung, Man, Lau, & Lam, 2007). The possible benefit exceeds the reduction in cardiovascular complications because antihypertensive drugs were associated with increased bone mass (Chen et al., 2015; Izu et al., 2009; Ma et al., 2010; Shimizu et al., 2008; Zhou et al., 2017). Losartan consequently improves the physicochemical properties of bone (Donmez et al., 2016) and reduces the fracture risk (Yamamoto et al., 2015). Losartan further supports fracture healing (Rajkumar et al., 2013) and graft consolidation (Gealh et al., 2014). These findings are consistent with the effects of antihypertensive drugs to increase survival rates of dental implants (Wu et al., 2016), also after sinus augmentation (Garcia-Denche et al., 2013). However, the possible impact of hypertension and the treatment of losartan on the early stages of osseointegration remain unclear.

Spontaneously hypertensive rats (SHR; Okamoto & Aoki, 1963; Pinto, Paul, & Ganten, 1998) are widely used to study the role of losartan and other angiotensin II receptor antagonists in vivo (Gealh et al., 2014; Soltis, 1993; You et al., 2008; Zhang et al., 2013). SHR rats develop hypertension around 5–6 weeks of age (Okamoto & Aoki, 1963). Losartan at least partially reduced periodontitis (Santos et al., 2015) and orthodontic tooth movement in SHR rats (Moura et al., 2016). When untreated, SHR present delayed alveolar bone healing suggesting that also osseointegration could be negatively affected (Manrique et al., 2015). To test this hypothesis, osseointegration was evaluated in SHR rats treated with losartan. The clinical relevance of this approach with respect to bone biology is to define if treatment with losartan under hypertension condition support or even increase the parameters of osseointegration.

2 | MATERIAL AND METHODS

2.1 | Study design and ethics

The Ethics Committee in the Use of Animals of Araçatuba Dental School (CEUA-2016–404) approved this study. The study was performed in 2016 at the Department of Oral Surgery and Integrated Clinic of the Araçatuba Dental School in accordance with the ARRIVE guidelines. A total of 32 male rats was used, 16 adult male Wistar rats (*Rattus norvegicus*, albinus) and 16 SHRs (body weight, 275–350 g). The animals were kept in cages in an environment with stable temperature (22°C ± 2°C), controlled light cycle (12 hr light, 12 hr dark), balanced feed (Ração Mogiana Alimentos SA, Campinas, Brazil), and controlled amounts of water. The animals were divided into four groups: Wistar, Wistar losartan, SHR, and SHR losartan. Randomization was performed by a computer-generated list. The sample was kept as small as possible, taking into account the statistical planning. All evaluations were performed under calibration and blinding examination.

2.2 | Losartan treatment

Losartan (Biosintetica, São Paulo, Brazil) was applied daily at 30 mg in drinking water per kg body weight, 7 days prior to implant placement until euthanasia (Gealh et al., 2014). Systolic blood pressure was checked preoperatively and every day until euthanasia by tail-cuff indirect plethysmography using a Physiograph®, MK-III-S (Narco Bio-systems, Houston, TX), adapted for measurements in rats, according to previous studies (Gealh et al., 2014; Manrique et al., 2015). Losartan controlled the blood pressure of all animals.

2.3 | Implant placement

GMS and FRSB performed the surgeries. As recently reported (Faverani et al., 2017; Ramalho-Ferreira, Faverani, Prado, Garcia, & Okamoto, 2015), animals received 50 mg/kg of ketamine intramuscularly and 5 mg/kg xylazine (mepivacaine; 0.3 ml/kg 2%, adrenaline 1:100,000, Septodont, Saint-Maur-des Fossés, France). Bone was exposed by an incision of the proximal metaphysis. Ten millimeter below the knee joint, a hole was drilled with a 1.4 mm diameter spiral bur mounted on an electric motor (BLM 600®; Driller, São Paulo, SP, Brazil) at a rotational speed of 1,000 rpm under irrigation with 0.9% sodium chloride (Fisiológico®, Laboratórios Biosintética Ltda®, Ribeirão Preto, SP, Brazil). Grade 4 titanium screws with 1.5 mm diameter and 3.5 mm length with an acid-etched surface (Emfills, Itu, São Paulo, Brazil) were implanted bilaterally in each tibia, with bicortical stabilization. Wounds were closed with resorbable sutures (Poliglactina 910, Vicryl, Ethicon, Johnson & Johnson Prod, São José dos Campos, Brazil). Animals received a single injection of intramuscular pentabiotic (0.1 ml/kg; Fort Dodge Saúde Animal Ltda, Campinas, São Paulo, Brazil) and sodic dipyrone (1 mg/kg; Ariston, Indústrias Químicas e Farmacêuticas Ltda, São Paulo, Brazil). Sixty days after implant placement animals received a lethal dose of

thiopental (150 mg/kg body weight; Cristália, Ltda., Itapira, SP, Brazil).

2.4 | Biomechanical test

Removal torque was determined on the left tibias of eight rats per group (Ramalho-Ferreira et al., 2015). Exposed implants were connected in an adapted implant hexagon and a digital torque (Conexão, São Paulo, Brazil), and the removal torque was measured. An anticlockwise movement was applied by increasing the removal torque until the implant rotated inside the bone tissue at the maximum torque peak in Newton centimeter (Ncm).

2.5 | Microcomputerized tomography (μCT)

The right tibias of eight rats per group were fixed in 10% buffered formalin (Reagentes Analíticos®, Dinâmica Odonto-Hospitalar Ltda, Catanduva, SP, Brazil) for 48 hr, washed in running water for 24 hr, and stored in 70% alcohol. Tibiae were scanned in the longitudinal plane with a SkyScan 1,172 (Bruker microCT, Aartselaar, Belgium) at 70 kV/114 mA with an integration time of 1 x 380 ms in a standard configuration (tube current: 165 μA, image pixel size: 9.92 μm, filter for beam hardening aluminum–copper: 0.5 mm, frame averaging: 4, rotation step: 0.6°). Region of interest (ROI) was a 0.5 mm high and 0.8 mm wide rectangular area in the most central slice of the first two medullary implant threats with 50 slices in the proximal and distal direction (Figure 1). Thus, the analysis was based on 100 slices as previously described (Faverani et al., 2017). Images were converted to gray scale values between 70 and 100 representing trabecular bone but not titanium. Morphological parameters were calculated with a software (SkyScan, Leuven, Belgium) in a standard configuration (smoothing of 1, correction of rings artifacts of 8, cone beam hardening of 24%) according to the American Society of Bone and Mineral Research (Dempster et al., 2013): bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.S), and trabecular number (Tb.N).

2.6 | Sample processing and histomorphometric analysis

Subsequent to μCT, these samples were dehydrated in ascending grades of alcohol and embedded in light-curing resin (Technovit 7200, Kulzer & Co., Hanau, Germany). Undecalcified thin ground sections were prepared along the longitudinal axis of the implant and the shaft of the tibia according to Donath (Donath, 1988). Following a Levai–Laczko stain, the specimens were digitized with the Olympus dotSlide 2.4 (Olympus, Tokyo, Japan) at a resolution of 0.312 μm/pixel. A 200 μm ROI parallel to the contour of the implant was defined as described elsewhere (Kuchler et al., 2011). Using the Definiens Developer XD2® software (Version 2.0.0; Munich, Germany), bone and soft tissue were classified from digital images. The classified areas were manually corrected using Adobe Photoshop® software (Adobe, San Jose, CA). For histomorphometric analysis, the cortical thickness from the periosteal to the endosteal margin (ct.Th), the percentage of newly formed bone per tissue area (nB.Ar/T.Ar), the percentage of new bone to implant-contact (nBIC), and the percentage of old bone-to-implant contact (oBIC) were evaluated in the cortical compartment. In the medullary compartment, the thickness of the newly formed layers of bone on the implant surface (nB.Th), nBIC, and nB.Ar/T.Ar were calculated.

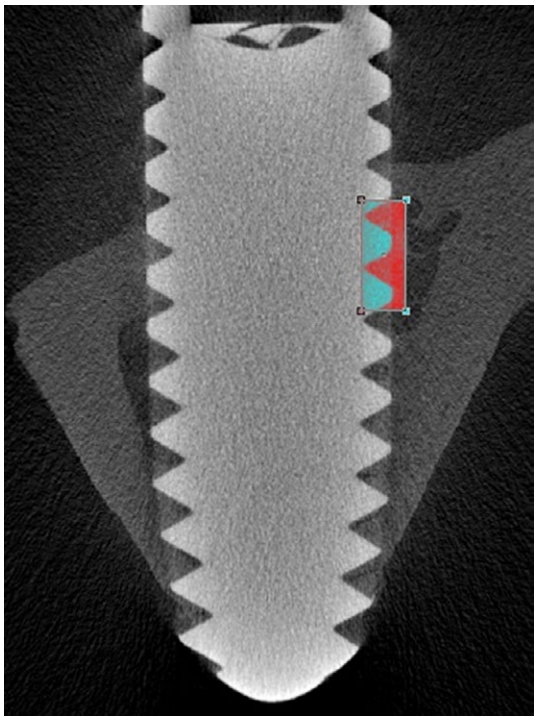

FIGURE 1 ROI demarcation. The region of interest was used to calculate the morphometric parameters with a red rectangular area of 0.5 mm high and 0.8 mm in the most central slice of the first two medullary implant threats with 50 slices in the proximal and distal direction

2.7 | Statistics

The statistical tests were performed in the GraphPad Prism 7 program (GraphPad Software; La Jolla; USA). For the quantitative parameters obtained from biomechanics (removal torque) and μCT (BV/TV; Tb.Th; Tb.N; Tb.S), normality and homoscedasticity tests were applied to verify the distribution of the data in the normality curve. The Shapiro-Wilk test was computed in order to check for normality. Based on this, two-way ANOVA followed by post hoc Tukey tests were chosen to analyze biomechanical and μCT data. Histomorphometric parameters were compared in the medullary and cortical compartments by nonparametric Kruskal–Wallis test followed by post hoc Dunn test. In case of a significant interaction effect, post hoc pairwise tests were conducted to test the losartan effect both in the control and the hypertensive group. p-values <0.05 were considered statistically significant for all analyses. The Benjamin–Hochberg procedure was applied to correct for multiple testing, and significance was assigned at the 5% level. The analysis

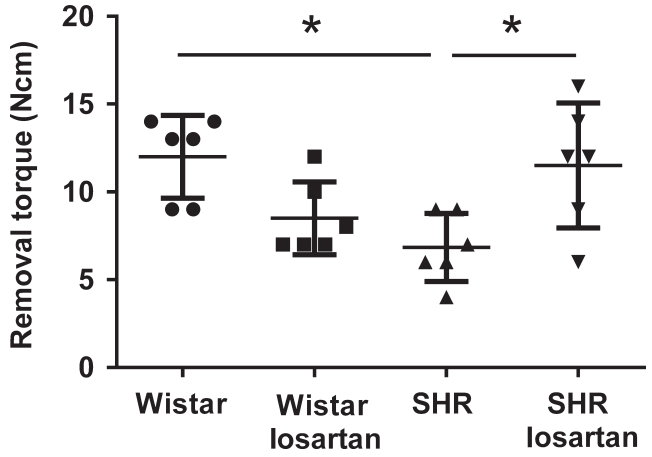

FIGURE 2 Biomechanical evaluation of removal torque. Sixty days after implant placement, animals' removal torque was determined on the left tibias of eight rats for each of the four experimental groups: Wistar, Wistar Losartan, spontaneously hypertensive rats (SHR), and SHR Losartan. Removal torque was increased until the implant rotated inside the bone and the maximum torque in Newton centimeter (Ncm) is reported. The * indicates significant statistical difference ($p < 0.05$)

of data did not demonstrate an interaction effect of the variables influenced by losartan and the hypertension ($p > 0.05$).

3 | RESULTS

3.1 | Biomechanics

We first determined whether hypertension in the SHR negatively affects the resistance against removal by the torque wrench. In support of this hypothesis, the removal torque was significantly lower in the SHR group compared to the Wistar group (6.0 ± 2.1 Ncm vs. 13.0 ± 2.5 Ncm; Tukey test; $p = 0.006$; $df = 3$), respectively (Figure 2). Importantly, losartan reversed the negative impact of hypertension on the removal torque to levels of Wistar animals (12.0 ± 2.3 Ncm vs. 13.0 ± 2.1 Ncm; Tukey test; $p = 0.48$; $df = 3$). Surprisingly, in the Wistar group, there was a trend toward a reduction in removal torque by losartan that, however, not reached the level of significance (13.0 ± 2.1 Ncm vs. 8.0 ± 1.1 Ncm; Tukey test; $p = 0.51$; $df = 2$). Thus, biomechanical testing exposed the beneficial effects of losartan on implant stability in hypertensive rats (Figure 2).

3.2 | Microcomputerized tomography

Consistent with the biomechanical findings, losartan increased the mean BV/TV in the hypertensive SHR group compared to the untreated SHR rats (52.5% ± 0.6% vs. 45.6% ± 0.6%; Tukey test; $p = 0.02$; $df = 2$) but not in the normotensive Wistar control group (52.5% ± 0.6% vs. 51.6% ± 0.4%; Tukey test; $p = 0.98$; $df = 3$; Figure 3a). The anabolic effect of losartan on BV/TV was caused by increasing the thickness of the bone trabeculae in the losartan-treated SHR animals compared to the respective SHR controls (0.13 ± 0.129 mm vs. 0.096 ± 0.003 mm; Tukey test; $p = 0.01$; $df = 2$; Figure 3b). The number of bone trabeculae remained unchanged in this setting (Figure 3c). The anabolic changes by losartan only caused a moderate decrease in trabecular separation (Figure 3d). Taken together, μCT structural analysis suggests that losartan exerts anabolic effects on peri implant bone in a hypertensive rat model.

3.3 | Histology

Figure 4 provides an overview of the bicortical implant integration, with a small seam of bone formation occurring in the medullary compartment. Considerable bone formation was observed on the periosteal and the endosteal surface of the cortical bone. No obvious differences were visible when comparing the four groups. At a higher magnification, plexiform bone characterized the periosteal

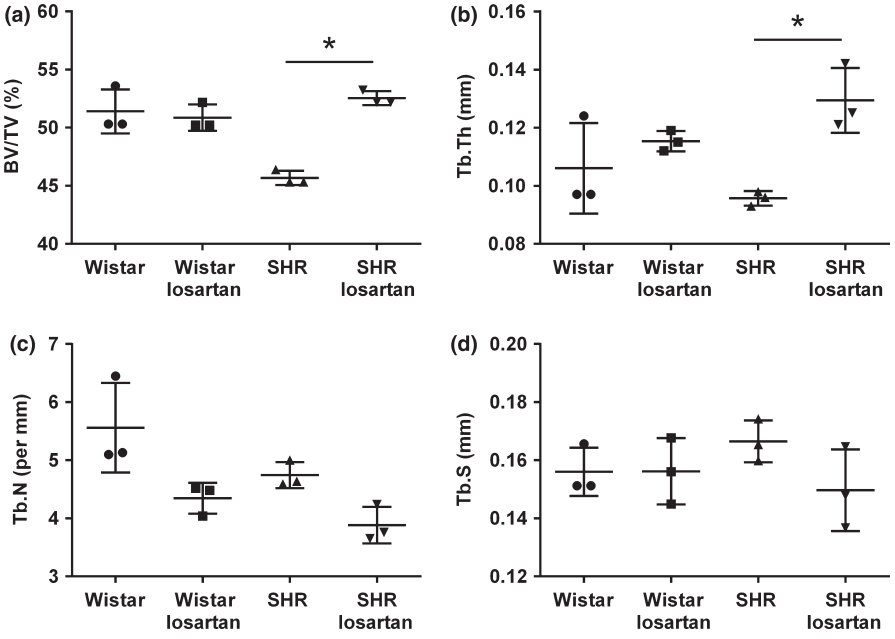

FIGURE 3 Microcomputerized tomography of the medullary peri implant bone. The formalin fixed right tibias of three rats per group were scanned in the longitudinal plane with a region of interest 0.5 mm high and 0.8 mm wide in the most central slice of the first two medullary implant threads with 50 slices in the proximal and distal direction. Morphological parameters were calculated and reported as (a) bone volume per tissue volume (BV/TV); (b) trabecular thickness (Tb.Th); (c) trabecular number (Tb.N.); and (d) trabecular separation (Tb.S). The * indicates significant statistical difference ($p < 0.05$)

FIGURE 4 Histology of the implants. Histological images at 60 days of the bicortical implant integration for Wistar, Wistar Losartan, SHR, and SHR Losartan. Formation of a thin layer of bone occurred in the medullary compartment. Considerable bone formation was observed on the periosteal and the endosteal surface of the cortical bone. All groups showed similar osseointegration. Undecalcified thin-ground sections were prepared along the implant axis and Levai–Laczko stained

region of the cortical compartment (Figure 5), while a thin layer of woven bone was found in the medullary compartment (Figure 6). Peri implant bone had undergone bone remodeling. Erythrocytes indicate the presence of the blood vessels. No signs of inflammations were observed. Again, these histological findings applied to all four groups.

3.4 | Histomorphometry of the medullary compartment

Losartan significantly increased the average thickness of the newly formed layers of bone on the implant surface in the medullary compartment (nB.Th) of the hypertensive SHR group and, surprisingly, also compared to the two groups of normotensive animals (0.061 ± 0.005 vs. 0.041 ± 0.004; Kruskal–Wallis test; $p = 0.0008$; $df = 2$; Kruskal–Wallis; Figure 7a). There was also a trend of a lower medullary nBIC in hypertensive SHR compared to normotensive Wistar rats, with no considerable changes caused by losartan (0.027 ± 0.006 vs. 0.03 ± 0.004; Kruskal–Wallis test; $p = 0.599$; $df = 3$; Figure 7b). The medullary nB.Ar/T.Ar was similar among all four groups (51.4; 50.8; 47.9; 52.3, respectively; Kruskal–Wallis test; $p = 0.592$, $df = 4$; Figure 7c). Thus, losartan increased the thickness of the peri implant bone, but has no impact on the coverage of the implant surface.

3.5 | Histomorphometry of the cortical compartment

As expected, the thickness of the cortical bone was lower in the hypertensive SHR compared to normotensive Wistar rats (0.39 ± 0.004 vs. 0.55 ± 0.006; Kruskal–Wallis test; $p = 0.0152$, $df = 5$; Figure 8a). However, losartan failed to return the cortical thickness to levels of normotensive rats (Figure 8a). Hypertension had no significant effects on the other parameters of osseointegration, for example, nB.Ar/T.Ar (0.30 ± 0.003; Kruskal–Wallis test; $p = 0.8112$, $df = 3$; Figure 8b) and BIC (0.54 ± 0.004; Kruskal–Wallis test; $p = 0.7892$, Figure 8c,d); thus, no effects of losartan were noticed.

4 | DISCUSSION

Hypertension delays bone regeneration in extraction sockets (Manrique et al., 2015) and the angiotensin II receptor blocker losartan improves microcirculation in fracture healing (Rajkumar et al., 2013) and graft consolidation (Gealh et al., 2014) in preclinical models. Losartan causes an anabolic shift of bone remodeling in OVX animals via the angiotensin-converting enzyme2/angiotensin 1–7/Mas pathway (Abuohashish, Ahmed, Sabry, Khattab, & Al-Rejaie, 2017). Thus, losartan supports bone regeneration and bone remodeling in rodent models. Considering that osseointegration follows the principles of bone regeneration and bone remodeling we have raised the hypothesis that losartan reverse impaired osseointegration under hypertensive conditions in spontaneously hypertensive rats. In support of this hypothesis, we report here that losartan increased implant stability at day 60 in hypertensive rats as determined by biomechanical testing. Histomorphometric and μCT analysis at least help to explain the biomechanical data on the structural level.

The cortical compartment mainly accounts responsible for the biomechanical stability of implants (Miyamoto, Tsuboi, Wada, Suwa, & Iizuka, 2005). In support of the biomechanical observations, there is a trend that Losartan increased the absolute cortical thickness in hypertensive rats, which is a consequence of a positive bone remodeling balance. The lack of significance of cortical thickness can be caused by the underpowered study design. Based on the existing data, at least 60 animals are necessary to reach power of 0.8% and 5% type 1 error. Interestingly, neither hypertension nor the therapy with losartan reduced the relative parameters of osseointegration, indicating that at day 60, which is the selected duration of our study, bone defects in a rat model are already undergoing remodeling

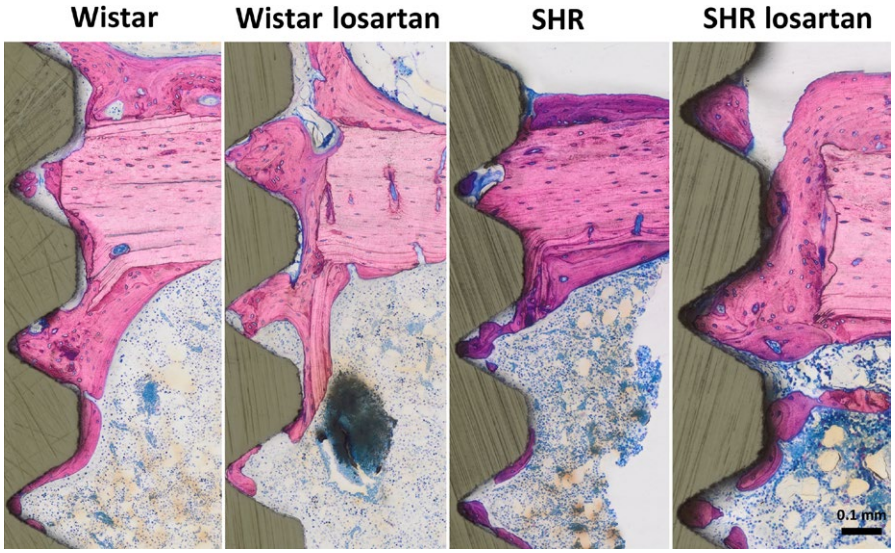

FIGURE 5 Histology of the cortical compartment. Histological pictures of the implant in the tibia, showing the cortical compartment of each group at a higher magnification. Plexiform bone, is observed in the periosteal region and the cortical compartment. Undecalcified thin-ground sections, Levai–Laczko stained

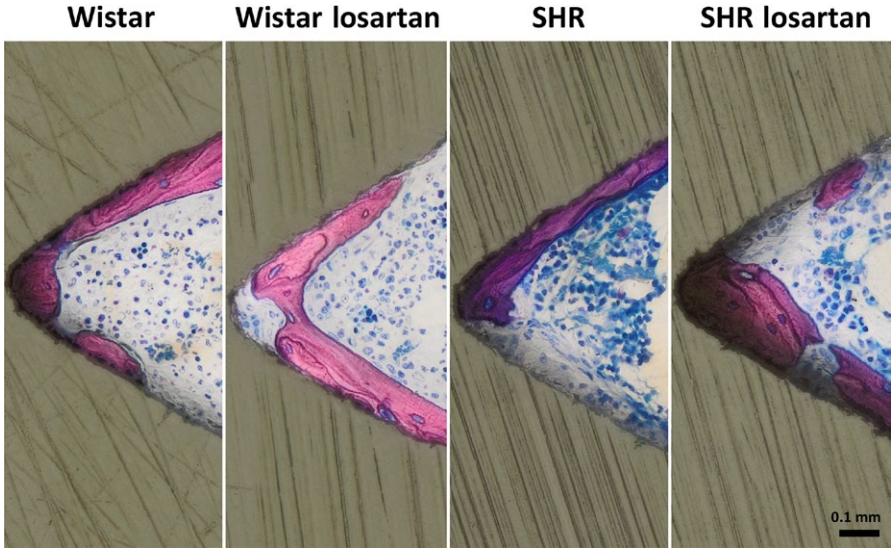

FIGURE 6 Histology of the medullary compartment. Histological pictures of the implant in the tibia, depicting the medullary compartment of each group at a higher magnification. A thin layer mainly consisting of woven bone that had been partly remodeled characterized the bone formation in the medullary compartment. Signs of remodeling can be observed. Undecalcified thin-ground sections, Levai–Laczko stained

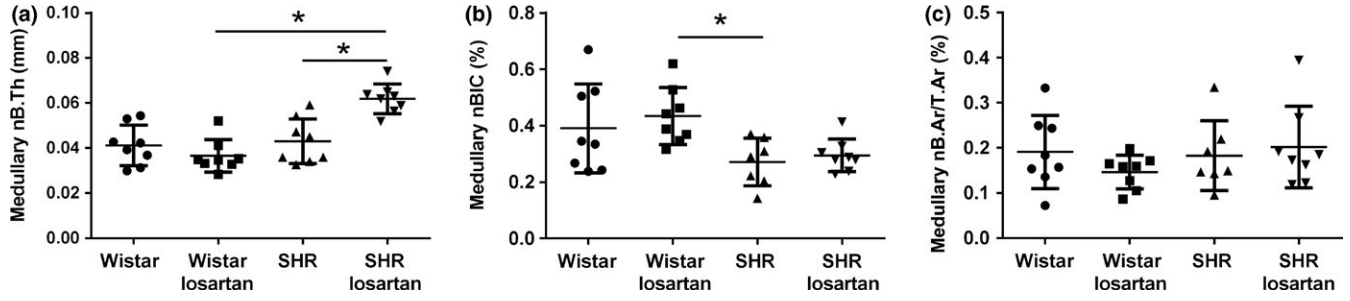

FIGURE 7 Histomorphometric results of the medullary compartment. Scatter plots summarizing the histomorphometric parameters in the medullary compartment. The data represent 200 μm wide areas immediately adjacent to the implant surfaces. (a) The thickness of newly formed bone on the implant surface (nB.Th); (b) new bone to implant contact (nBIC), and (c) the newly formed bone per tissue area (nB.Ar/T.Ar) were calculated. The * indicates significant statistical difference ($p < 0.05$)

(Puricelli et al., 2010) and also in the cortical peri implant area. This claim is supported by histology showing clear signs of lamellar bone in the area of newly formed bone. It can be speculated that new bone in the losartan group is more mature than in the untreated hypertensive rats and possibly contribute to the increased implant stability losartan.

The medullary compartment also contributes to the biomechanical stability of the implants as is reflected by μCT and histomorphometric analysis. Even though the μCT analysis is underpowered, there is a clear trend toward a beneficial effect of losartan to increase medullary bone thickness being associated with the BV/TV in hypertensive SHR rats. Considering also the lower trabecular number, the data suggest that losartan causes a positive shift in bone remodeling under hypertensive conditions. Losartan not necessarily increases the formation of new bone on the implant surface. In support of this concept are our findings from histomorphometry where losartan increased medullary bone thickness in hypertensive SHR rats, even above the levels of normotensive controls. Again, losartan had no impact on the parameters that reflect the formation of new bone on the implant surface. Taken together, these data led us to suggest that losartan increases the amount of existing bone in the medullary compartment while not being responsible for the initiation of bone formation.

The clinical relevance of our findings that losartan supports osseointegration in a hypertensive rat model, is strongly corroborated by other clinical observations, where antihypertensive drugs improved the rates of implant survival (Garcia-Denche et al., 2013; Wu et al., 2016). Clinical observations were from implants loaded at least 1 year, not including implants that are lost during the early period prior to functional loading (Garcia-Denche et al., 2013; Wu et al., 2016). Our model at least partially reflects this clinical scenario because the 60-day observation period integrates bone regeneration but also the continuous process of bone remodeling (Puricelli et al., 2010). Support for this hypothesis comes from studies showing that hypertension is linked to osteoporosis, and antihypertensive drugs reduce the fracture risk, respectively (Cappuccio et al., 2000; Vestergaard et al., 2009). The clinical relevance of the present study is maybe twofold: first, losartan prevents systemic bone loss in hypertensive patients, thereby also supporting the quality of the alveolar bone before implants are placed (Bastos et al., 2010; Fabris et al., 2017). Secondly, losartan supports bone remodeling after implants were placed causing a better biomechanical stability in the long term.

New insight on the role of losartan in implant dentistry also leads to new questions. As already stated, what remains to be determined is if the positive effect of losartan on biomechanical implant stability is a consequence of a positive balance of bone remodeling. Moreover, we cannot explain if the beneficial effects of losartan on osseointegration are indirect via the control of blood pressure or if losartan also exerts direct effects on cells involved in bone regeneration and remodeling. For example, losartan decreases the suppressive effects of angiotensin II on osteogenic differentiation markers in vitro (Nakai et al., 2015). Our data are in favor of the indirect effect as losartan fails to push osseointegration in normotensive rats. One limitation is that we have used outbred Wistar albinus and not inbred Wistar–Kyoto rats, the latter showing genetic heterogeneity but remain the closest genetic control for the SHR (Kurtz, Montano, Chan, & Kabra, 1989). Moreover, it remains unclear if antihypertensive drugs other than losartan cause similar changes in a SHR model and if the effects observed can be reproduced in other models of hypertension (Zhou et al., 2017). SHR rats are a model with a high concentration of plasmatic renin (Bagby, McDonald, & Mass, 1979). Therefore, it can justify the effect of losartan only in SHR model. Losartan controlled the renin angiotensin system of the SHRs, and it does not happen with the normotensive model. Also, the long bones of rats may not respond similarly as the alveolar bone to losartan and that one time-point of observation provides limited insight into the sequential process of osseointegration. Clearly further research is necessary to further reveal this positive effect of losartan on osseointegration.

In conclusion, evidence presented herein suggests that losartan can reverse impaired osseointegration under hypertensive

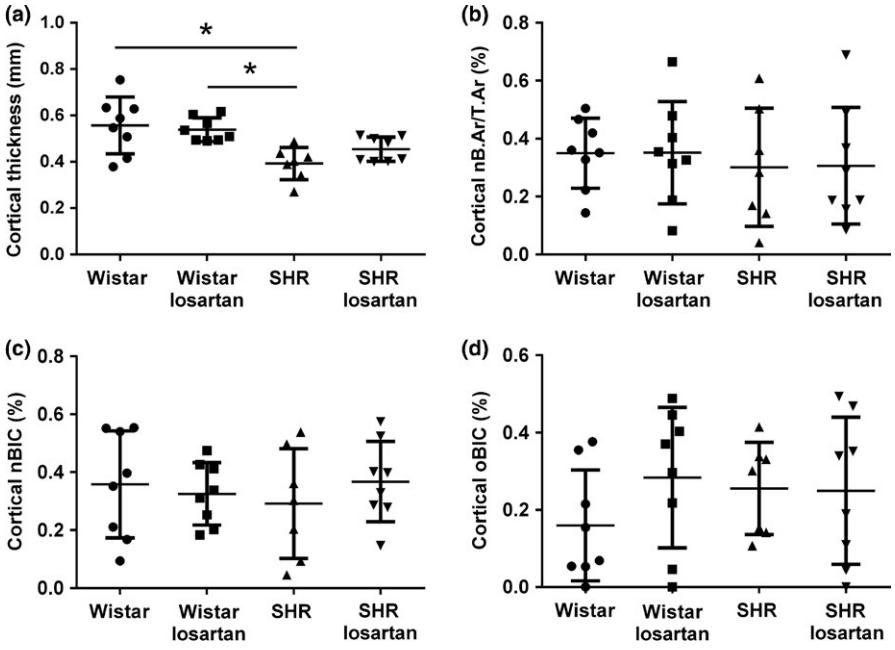

FIGURE 8 Histomorphometric results of the cortical compartment. Scatter plots summarizing the histomorphometric parameters in the cortical compartment. The data represent 200 μm wide areas immediately adjacent to the implant surfaces. (a) The cortical thickness (Ct. Th); (b) the percentage of newly formed bone per tissue area (nB.Ar/T.Ar); (c) the percentage of new bone to implant contact (nBIC), and (d) the percentage of old bone-to-implant contact (oBIC) were evaluated. The * indicates significant statistical difference ($p < 0.05$)

condition. The present preclinical data add to the accumulating knowledge that hypertension is a risk factor in dental implantology and that losartan, being an antihypertensive drug, can help to overcome these limitations.

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ORCID

Franziska Kirchweger http://orcid.org/0000-0003-2381-3664
Stefan Tangl http://orcid.org/0000-0002-9023-4745
Reinhard Gruber http://orcid.org/0000-0001-5400-9009

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SUPPORTING INFORMATION

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