Original Research
Endodontic Therapy

Relationship between hypertension and periapical lesion: an *in vitro* and *in vivo* study

Christine Men MARTINS[a]
Hajime SASAKI[b]
Kimito HIRAI[b]
Ana Cristina ANDRADA[c]
João Eduardo GOMES-FILHO[a]

[a]Universidade Estadual Paulista – Unesp, Araçatuba Dental School, Department of Restorative Dentistry, Aracatuba, SP, Brazil.

[b]The Forsyth Institute, Department of Immunology and Infectious Diseases, Cambridge, MA, USA.

[c]Harvard School of Dental Medicine, Division of Endodontics, Department of Restorative Dentistry and Biomaterials Science, Boston, MA, USA.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
João Eduardo Gomes Filho
E-mail: joao@foa.unesp.br

DOI: 10.1590/1807-3107BOR-2016.vol30.0078

Submitted: Feb 06, 2016
Accepted for publication: Mar 02, 2016
Last revision: Apr 08, 2016

Abstract: The aim of this study was to compare potential aspects of periapical lesion formation in hypertensive and normotensive conditions using hypertensive (BPH/2J) and wild-type control (BPN/3J) mice. The mandibular first molars of both strains had their dental pulp exposed. At day 21 the mice were euthanized and right mandibular molars were used to evaluate the size and phenotype of apical periodontitis by microCT. Proteins were extracted from periapical lesion on the left side and the expressions of IL1α, IL1β and TNFα were analyzed by ELISA. Bone marrow stem cells were isolated from adult mice femurs from 2 strains and osteoclast differentiation was evaluated by tartrate-resistant acid phosphatase (TRAP) *in vitro*. The amount of differentiated osteoclastic cells was nearly double in hypertensive mice when compared to the normotensive strain ($p < 0.03$). Periapical lesion size did not differ between hypertensive and normotensive strains ($p > 0.7$). IL1α, IL1β and TNFα cytokines expressions were similar for both systemic conditions ($p > 0.05$). Despite the fact that no differences could be observed in periapical lesion size and cytokines expressions on the systemic conditions tested, hypertension showed an elevated number of osteoclast differentiation.

Keywords: Hypertension; Periapical Diseases; Inflammation.

Introduction

The association between periapical inflammatory and infectious processes and systemic disease is raising researchers' interests lately. In the past, the focal infection theory was applied as a causal relationship between oral infections and heart disease, like infective endocarditis.[1] However, in 2012 the American Association of Endodontists stated that decades of research opposes the beliefs of focal infection theory proponents. To date, there is no valid, scientific evidence linking endodontically treated teeth and systemic diseases.[2]

Oral health will certainly improve overall good health,[3] and although there is no causal relationship,[2] an association between oral infections and certain systemic conditions, such as hypertension, can be inferred. This association may depend on common risk factors such as dysregulation of biological functions including immune response.[4,5,6,7]

The diagnosis of hypertension can be given by a systolic blood pressure higher than 140 mmHg, a diastolic blood pressure higher than 90 mmHg,

or both.[8] Essentially, hypertension is due to genetic factors associated with an unhealthy lifestyle, and renal disorder is the cause of secondary hypertension.[9]

It is known that hypertension can be considered an inflammatory disease.[10] The TLR4 signal links hypertension and periapical inflammation,[11] and lymphocytes T cells are responsible for hypertension development[12] mediated by angiotensin II.[13] Pro-inflammatory cytokines like TNFα and IL-6 are more often presented in a hypertensive condition[14] and the opposite is true for IL-10, an anti-inflammatory cytokine.[15]

Although the relationship between this systemic disorder and periapical lesions has been indicated, it is paramount that such connection be scientifically reinforced. Studies have shown correlation between hypertension and periodontal disease[16,17] as well as amount of salivary flow and its protein concentration.[18] Changes in hard-tissue structures like enamel, dentin and bone are also affected by hypertension.[18,19,20,21,22] Few studies can be found correlating endodontic disease and hypertension. Allareddy et al.,[23] conducted a retrospective study where they found that 24.6% of patients hospitalized for periapical abscesses were hypertensive.[23] Likewise, another study found that almost 8% of endodontically treated teeth in hypertensive patients were not considered satisfactory.[24]

Thus, the hypothesis tested in this study was that osteoclast differentiation from bone marrow cells (BMCs), periapical lesion sizes and inflammatory cytokine expression in hypertensive mice are higher than in normotensive ones. Therefore, this study aimed to compare potential aspects of periapical lesion formation in hypertensive and normotensive conditions.

Methodology

Animals

This experimental animal study was submitted to the approval of the Animal Experiment Committee of Forsyth Institute, no. 14/004. BPH/2j and its normal control, BPN/3J, mouse strains were purchased from Jackson Laboratory, Bar Harbor, Maine. The mice were maintained in accordance with the guidelines of the Animal Experiment Committee of Forsyth Institute.

Osteoclast differentiation from bone marrow cells

BMCs were isolated from femora of BPH/2J and BPN/3J strain mice. The sample size was 5 mice each divided in 40 wells. The initial density of cells was 1.5 × 106 cells/μL in a 96-well plate. The cells were incubated for 5 days in α-MEM containing 10% inactivated FBS with M-CSF (50 ng/mL). After the incubation, the adherent cells were collected as bone marrow-derived macrophages (BMMs). The BMMs were cultured in the presence of M-CSF (25 ng/mL) and RANKL (100 ng/mL) for 8 days. Osteoclast formation was evaluated by measuring the tartrate-resistant acid phosphatase (TRAP) activity as an early differentiation marker. After TRAP-staining, the cells with more than three nuclei were counted as TRAP-positive multinucleated cells.

Periapical lesion stimulation

Seven-week-old female and male mice were used. The sample size was 5 mice for the hypertensive group and 5 mice for the normotensive group. The mice were anesthetized via intra peritoneal (IP) injection with ketamine HCl (80 mg/kg) and xylazine (10 mg/kg) and were placed on a jaw-retraction board. The dental pulps of both mandibular first molars were exposed using an electric dental hand piece with a no. 1/4 round bur under a surgical microscope. The pulp chambers were open until the entrance of the canals could be visualized and probed with a size 6 endodontic file. On day 21 after pulp exposure, mice were sacrificed using a CO_2 gas chamber and mandibles were isolated and dissected free of soft tissue. Right hemimandibles were fixed in fresh 4% paraformaldehyde in PBS and scanned by Micro Tomography Computed to analyze the periapical lesion sizes. Left hemimandibles were immediately frozen for protein extraction and analysis of the pro-inflammatory cytokine expression.

General bone and periapical lesion phenotype

After fixation, the paraformaldehyde in the samples was reduced by distilled water and the samples were scanned in Micro Tomography Computed. The angles of the image were adjusted using the ImageJ program and the periapical lesion sizes were measured using

the Adobe Photoshop56 program. The periapical lesion sizes were recorded in a square micrometer.

Pro-inflammatory cytokine expression from periapical lesion proteins

Five samples of each group, hypertensive or normotensive, were used. For protein extraction, frozen periapical tissue samples were disrupted in a cell lysis buffer (Cell Signaling Technology, Danvers, USA) supplemented with 50 µg/mL gentamicin (Sigma-Aldrich, St. Louis, USA) using FastPrep-24 with matrix A (both MP Biomedicals, Solon, USA). The supernatant was collected after centrifugation and cytokine assays were performed using commercially available ELISA kits obtained from R&D Systems (DuoSets) and were used according to the manufacturer's instructions to evaluate periapical tissue levels of IL1α, IL1β and TNFα. The concentration of each cytokine was calculated with reference to a standard curve constructed using recombinant cytokines provided with each kit. Results were expressed as picograms of cytokines per milligram of periapical tissue.

Statistical analysis

Normality was defined by a Shapiro-Wilk test. Tukey's T-test was performed to assess the difference between the groups. Values of $p < 0.05$ were considered statistically significant.

Results

Osteoclast differentiation from bone marrow cells

The average number of differentiated osteoclast per well is described in Figure 1. The hypertensive group presented almost twice the number of differentiated osteoclast when compared with the normotensive group, 97 and 45, respectively. There is a statistically significant difference between the two groups ($p < 0.03$).

General bone and periapical lesion phenotype

The average periapical lesion sizes in hypertensive and normotensive conditions is shown in pixels in Figure 2. The average lesion size of the hypertensive group was 2.6 mm^3 compared to 2.7 mm^3 in the normotensive group. No statistically significant difference between the groups was observed ($p > 0.07$). There was no visible difference between the groups regarding the bone structure or phenotype pattern.

Pro-inflammatory cytokine expression from periapical lesion proteins

The averages of IL1α, IL1β and TNFα expressions are described in Figure 3. For all cytokines, the tendency is toward higher expression in the exposed group than in the control (unexposed) group. The hypertensive condition presented higher expression of cytokines IL1α and TNFα than the normotensive condition. This tendency was not viewed for IL1β. There was no significant difference between the systemic conditions or exposed and non-exposed groups ($p > 0.05$).

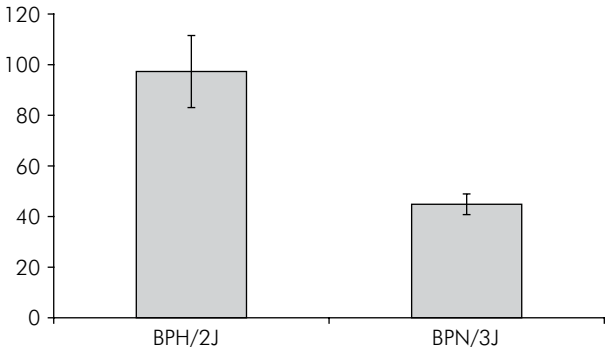

Figure 1. The value on x axis is the number of osteoclasts differentiated. n = 5 for each group. The difference is statistically significant between the hypertensive group (BPH/2J) and the normotensive group (BPN/3J).

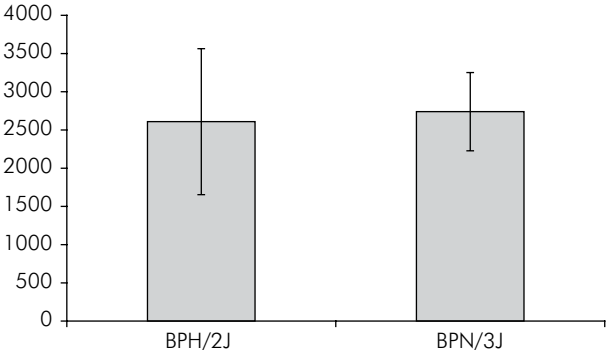

Figure 2. The average periapical lesion size in pixels in the hypertensive (BPH/2J) and normotensive (BPN/3J) conditions. The value is in mm^3. n = 5 for each group. No statistically significant difference was found.

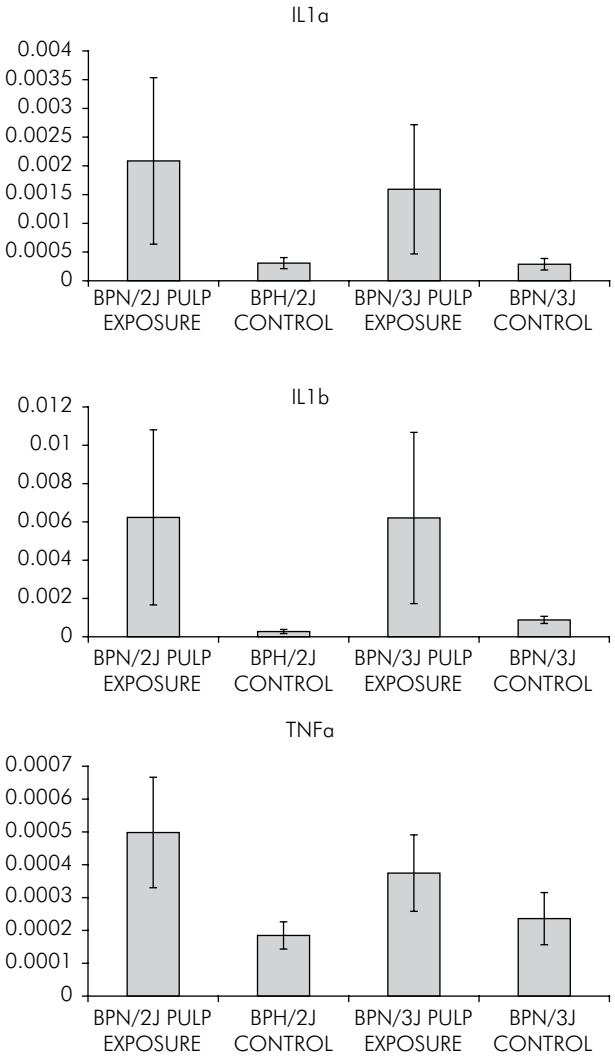

Figure 3. Averages of IL1α, IL1β and TNFα expressions. The value is in picograms of cytokines per milligram of periapical tissue. n = 5 for each group for each cytokine. No statistically significant difference was found.

Discussion

Although there is a high prevalence of hypertension in patients hospitalized for periapical abscesses[23] and there are indications of the negative influence of hypertension in periapical lesions, our results did not show a clear relationship between this health condition and periapical lesion phenotype. The periapical lesion size and cytokines expressions from a periapical lesion were similarly independent of the systemic condition.

The hypertensive group presented almost twice the number of differentiated osteoclast compared with normotensive group. Since the final outcome of periapical lesions and periodontitis is bone destruction, the effect of metabolic disorders on bone needs to be considered. The osteoclasts are responsible for bone resorption and angiotensin II may be the link. Hypertension is mediated by angiotensin II, the molecule responsible for activating osteoclasts due to up-regulated RANKL expression in osteoblasts.[25] In other words, angiotensin II induces the expression of RANKL through the receptor activators of NF-κB ligands in osteoblasts, leading to the activation of osteoclasts,[25] which are responsible for bone fracture, osteoporosis and also bone destruction in endodontics. In a ligature-induced periodontitis model and increased RANKL/OPG ratio toward a more osteoclastic condition was observed in the SHR rats versus normotensive controls.[20,21] Hypertension may also negatively affect bone mineral density due to abnormal metabolism of 1,25-dihydroxyvitamin D, a key regulator of calcium homeostasis and bone metabolism, intestinal calcium transport, and angiotensin II-mediated osteoclast activation.[25,26]

Pulp inflammation can soon spread in apical direction and so an immune-inflammatory response occurs through the action of immune cells resulting in abscess formation, which is an acute phase.[27] After that, a lymphocytic infiltration will happen as a defense mechanism against systemic spread of bacteria and/or bacterial byproducts to other sites in the body, which is the granuloma phase.[27]

In our study, the protocol used was doing pulp exposure and sacrificing the mice after 21 days. Some studies reported that in this period we have the granuloma phase,[28] and also the immune/inflammatory response and the systemic and local bone metabolism can be responsible for the higher prevalence of chronic apical periodontitis in hypertensive patients when compared to normotensive ones.[16]

The immune system is not a primary cause of hypertension, but it is a secondary factor following initiation of pre-hypertension, which is mainly caused by genetics and lifestyle, exhibiting a modest elevation of blood pressure about 135 to 140 mmHg. Pre-hypertension and the resulting vascular injuries lead to production of damage-associated molecular patterns (DAMPs), neoantigens, and immune regulatory mediators promoting immune and

inflammatory responses. Toll-like receptor 4 (TLR4) plays a fundamental role in pathogen recognition and activation of innate immunity, being the key pro-inflammatory signal in induction of hypertension target organ damage and periapical lesions.[5,29,30] So, it can be stated that hypertension can be considered an inflammatory vascular process[10] and this is why studies suggest a greater tendency to a chronic nature of lesions in hypertensive patients.[10]

In our study the expression of IL1α, IL1β and TNFα were observed from the periapical lesions, which shows the inflammatory response. In our study, we did not found statistically significant difference between expressions in the pulp-exposed group compared to the non-exposed one. Nevertheless, it was expected that because pulp exposure induces periapical lesion, inflammation and bone resorption would present together.

The results of the present study must be checked in different models once BPH/2J mice are genetically modified and the signs, symptoms and consequences of hypertension start to appear in older mice. However, to observe a standardized periapical lesion researchers usually use young adult mice. In our research, we used 7-week-old mice, which are adult mice, being not so young and not as old as necessary. So, we had a wide standard variation intragroup, as maybe some mice presented sequelae of hypertension while others did not.

Conclusion

Despite the periapical lesion size and cytokines expressions being similar for the different systemic conditions, the hypertension condition leads to higher osteoclasts differentiation, which could influence the endodontic treatment outcome in such a systemic condition.

Acknowledge

To FAPESP, process number 2013/09446-1.

References

1. Grossman L. Focal infection: are oral foci of infection related to systemic disease? Dent Clin North Am. 1960;(4):749-63.
2. American Association of Endodontists. Focal infection theory. Chicago: American Association of Endodontists; 2012.
3. Price SS, Funk AD, Shockey AK, Sharps GM, Crout RJ, Frere CL, et al. Promoting oral health as part of an interprofessional community-based women's health event. J Dent Educ. 2014;78(9):1294-300.
4. Armada-Dias L, Breda J, Provenzano JC, Breitenbach M, Rôças I, Gahyva SM, et al. Development of periradicular lesions in normal and diabetic rats. J Appl Oral Sci. 2006;14(5):371-5. doi:10.1590/S1678-77572006000500013
5. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, et al. Inflammation, immunity, and hypertension. Hypertension. 2011;57(2):132-40. doi:10.1161/HYPERTENSIONAHA.110.163576
6. Yoneda M, Naka S, Nakano K, Wada K, Endo H, Mawatari H, et al. Involvement of a periodontal pathogen, Porphyromonas gingivalis on the pathogenesis of non-alcoholic fatty liver disease. BMC [Internet]. 2012 [cited 2016 Apr 08];12:16. Available from: http://bmcgastroenterol.biomedcentral.com/articles/10.1186/1471-230X-12-16. doi:10.1186/1471-230X-12-16
7. Dregan A, Charlton J, Chowienczyk P, Gulliford MC. Chronic inflammatory disorders and risk of type 2 diabetes mellitus, coronary heart disease, and stroke: a population-based cohort study. Circulation. 2014;130(10):837-44. doi:10.1161/CIRCULATIONAHA.114.009990
8. Mancia G, Fagard R, Narkiewicz K, Redán J, Zanchetti A, Böhm M, et al.; ESH/ESC Task Force for the Management of Arterial Hypertension. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. J Hypertens. 2013;31(10):1925-38. doi:10.1097/HJH.0b013e328364ca4c
9. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014;311(5):507-20. doi:10.1001/jama.2013.284427
10. McMaster WG, Kirabo A, Madhur MS, Harrison DG. Inflammation, immunity, and hypertensive end-organ damage. Circ Res. 2015;116(6):1022-33. doi:10.1161/CIRCRESAHA.116.303697
11. Chong AJ, Shimamoto A, Hampton CR, Takayama H, Spring DJ, Rothnie CL, et al. Toll-like receptor 4 mediates ischemia/reperfusion injury of the heart. J Thorac Cardiovasc Surg. 2004;128(2):170-9. doi:10.1016/j.jtcvs.2003.11.036

12. Seaberg EC, Muñoz A, Lu M, Detels R, Margolick JB, Riddler SA, et al.; Multicenter AIDS Cohort Study. Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003. AIDS. 2005;19(9):953-60. doi:10.1097/01.aids.0000171410.76607.f8
13. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. J Exp Med. 2007;204(10):2449-60. doi:10.1084/jem.20070657
14. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension. 2001;38(3):399-403. doi:10.1161/01.HYP.38.3.399
15. Tinsley JH, South S, Chiasson VL, Mitchell BM. Interleukin-10 reduces inflammation, endothelial dysfunction, and blood pressure in hypertensive pregnant rats. Am J Physiol Regul Integr Comp Physiol. 2010;298(3):R713-9. doi:10.1152/ajpregu.00712.2009
16. Segura-Egea JJ, Jimenez-Moreno E, Calvo-Monroy C, Ríos-Santos JV, Velasco-Ortega E, Sánchez-Domínguez B, et al. Hypertension and dental periapical condition. J Endod. 2010;36(11):1800-4. doi:10.1016/j.joen.2010.08.004
17. Bonato CF, do-Amaral CCF, Belini L, Salzedas LMP, Oliveira SHP. Hypertension favors the inflammatory process in rats with experimentally induced periodontitis. J Periodontal Res. 2012;47(6):783-92. doi:10.1111/j.1600-0765.2012.01496.x
18. Elias GP, Santos OAM, Sassaki KT, Delbem ACB, Antoniali C. Dental mineralization and salivary activity are reduced in offspring of spontaneously hypertensive rats (SHR). J Appl Oral Sci. 2006;14(4):253-9. doi:10.1590/S1678-77572006000400008
19. Inoue T, Moriya A, Goto K, Tanaka T, Inazu M. What is the difference of bone growth in SHR and SD rats? Clin Exp Pharmacol Physiol Suppl. 1995;22(1):S242-3. doi:10.1111/j.1440-1681.1995.tb02900.x
20. Bastos MF, Brilhante FV, Bezerra JP, Silva CA, Duarte PM. Trabecular bone area and bone healing in spontaneously hypertensive rats: a histometric study. Braz Oral Res. 2010;24(2):170-6. doi:10.1590/S1806-83242010000200008
21. Bastos MF, Brilhante FV, Gonçalves TED, Pires AG, Napimoga MH, Marques MR, et al. Hypertension may affect tooth-supporting alveolar bone quality: a study in rats. J Periodontol. 2010;81(7):1075-83. doi:10.1902/jop.2010.090705
22. Zhang YF, Wang YX, Griffith JF, Kwong WK, Ma HT, Qin L, et al. Proximal femur bone marrow blood perfusion indices are reduced in hypertensive rats: a dynamic contrast-enhanced MRI study. J Magn Reson Imaging. 2009;30(5):1139-44. doi:10.1002/jmri.21954
23. Allareddy V, Lin CY, Shah A, Lee MK, Nalliah R, Elangovan S, et al. Outcomes in Patients Hospitalized for Periapical Abscess in the United States. J Am Dent Assoc. 2010;141(9):1107-16. doi:10.14219/jada.archive.2010.0341
24. Mindiola MJ, Mickel AK, Sami C, Jones JJ, Lalumandier JA, Nelson SS. Endodontic treatment in an American Indian population: a 10-year retrospective study. J Endod. 2006;32(9):828-32. doi:10.1016/j.joen.2006.03.007
25. Shimizu H, Nakagami H, Osako MK, Hanayama R, Kunugiza Y, Kizawa T, et al. Angiotensin II accelerates osteoporosis by activating osteoclasts. FASEB J. 2008;22(7):2465-75. doi:10.1096/fj.07-098954
26. Lucas PA, Brown RC, Drüeke T, Lacour B, Metz JA, McCarron DA. Abnormal vitamin D metabolism, intestinal calcium transport, and bone calcium status in the spontaneously hypertensive rat compared with its genetic control. J Clin Invest. 1986;78(1):221-7. doi:10.1172/JCI112555
27. Cintra LTA, Samuel RO, Facundo ACS, Prieto AKC, Sumida DH, Bomfim SRM, et al. Relationships between oral infections and blood glucose concentrations or HbA1c levels in normal and diabetic rats. Int Endod J. 2014;47(3):228-37. doi:10.1111/iej.12136
28. Silva RAB, Ferreira PDF, De Rossi A, Nelson-Filho P, Silva LAB. Toll-like receptor 2 knockout mice showed increased periapical lesion size and osteoclast number. J Endod. 2012;38(6):803-13. doi:10.1016/j.joen.2012.03.017
29. Simino J, Rao DC, Freedman BI. Novel findings and future directions on the genetics of hypertension. Curr Opin Nephrol Hypertens. 2012;21(5):500-7. doi:10.1097/MNH.0b013e328354e78f
30. Schiffrin EL. Immune mechanisms in hypertension and vascular injury. Clin Sci (Lond). 2014;126(4):267-74. doi:10.1042/CS20130407