Journal of Complementary Medicine Research, ISSN: 2146-8397 Vol. 14, No. 4, 2023 (pp. 29-34)

RESEARCH ARTICLE WWW.JOCMR.COM

The role of hyperbaric oxygen therapy combined with antioxidant C and E vitamins on AT1 and AT2 angiotensin II receptors in a murine acute myocardial infarction model

Nallely Acevedo-Villavicencio[1], Maria C. Castillo-Hernandez[2*], Alexandre Kormanovski-Kovzova[3], Icela Palma-Lara[4], Rodrigo Romero[5], Pedro J. Curi-Curi[6], Aaron Dominguez-Lopez[7], Ruth M. Lopez-Mayorga[8], Gustavo Guevara-Balcazar[9]

[1,2,3,4,5,6,7,8,9]*Seccion de estudios de Posgrado e Investigacion, Escuela Superior de Medicina, Instituto Politecnico Nacional*

ABSTRACT

Renin-angiotensin system is involved in the regulation and development of several cardiovascular diseases. AT1 and AT2 angiotensin II receptors participate in cardiovascular remodeling because the expression of these receptors and their ratios under different cardiac pathological conditions may be important in terms of myocardial function and structure.
Hyperbaric oxygenation therapy (HBO) combined with antioxidant vitamins (C and E) may be beneficial in myocardial ischemia, but the mechanisms that cause these effects are not entirely clear. This study aims to examine whether the concomitant use of these therapies results in the inhibition of the expression of AT1 receptors and increases the expression of AT2 receptors. A model of acute myocardial infarction (AMI) in Wistar rats was used to determine if exposition from 1 to 3 sessions of HBO with concomitant administration of vitamins C and E has an effect in the expression of AT1 and AT2 receptors of the heart. The obtained results suggest that after administration of 3 sessions of HBO and Vitamins C and E the expression of AT1 receptors significantly reduces while the expression of AT2 receptor increases dramatically, so we can state that this combined therapy is beneficial for patients with AMI.

Corresponding Author e-mail: castillohernandezmc@gmail.com

How to cite this article: Villavicencio A N, Hernandez C C M, Kovzova K A, Lara P I, Romero R, Curi-Curi J P, Lopez D A, Lopez-Mayorga M R, Guevara-Balcazar G (2023), The role of hyperbaric oxygen therapy combined with antioxidant C and E vitamins on AT1 and AT2 angiotensin II receptors in a murine acute myocardial infarction model. Journal of Complementary Medicine Research, Vol. 14, No. 4, 2023 (pp. 29-34)

INTRODUCTION

Acute myocardial infarction (AMI) is a frequent cause of mortality and disability all around the world. This condition leads to cardiac myocytes death due to an abrupt imbalance between blood oxygen supply and cell demand (1). It is well known that renin-angiotensin system (RAS) plays a main role in the pathophysiology of cardiovascular diseases such as AMI (2), due to the effects of angiotensin II at the AT1 and AT2 receptors. Regardless of the hemodynamic changes that RAS produces, AT1 receptors induce hypertrophy and apoptosis in postinfarcted ventricular myocytes of the heart (3,4) and mediate vascular smooth muscle cell proliferation (5), while AT2 receptor activation inhibits cell proliferation and remodeling (6,7,8,9, 10,11). During ischemic cardiovascular injury, AT2 increases one day after an experimental myocardial infarction (12,13). The ratio of AT2 to AT1 receptor densities in the hypertrophied rat heart is also increased, so the expression of these receptors may be important in determining myocardial function and structure (14,15).

Actually, there are several reports of oxygen hyperbaric therapy, which is defined by the Hyperbaric Medical Society (UHMS), as an intervention in which an individual breathes 100% oxygen at 1.5 to 2 atmosphere absolute (ATA). (https://www.uhms.org/resources/hbo-indications.html). Hyperbaric oxygenation (HBO_2) promotes cell replication, collagen formation, and mechanisms of homeostasis, such as active membrane sodium–potassium transport. Additionally, HBO_2 inhibits induction of the leukocyte adhesion to endothelium, diminishes tissue damage, enhances leukocyte motility, improves microcirculation, and stimulates neo angiogenesis (16). Therefore, it can be proposed that such effects of HBO2 may be beneficial in the postinfarcted heart.

KEYWORDS:
Renin-angiotensin system, cardiovascular injury, oxygen therapy.

ARTICLE HISTORY:
Received: Apr 18, 2023
Accepted: May 20, 2023
Published: Jun 21, 2023

DOI:
10.5455/jcmr.2023.14.04.06

It has been suggested that increased intake of various antioxidant agents such as vitamins C and E reduce the incidence rates of cardiovascular diseases, cancer, and other adverse outcomes (17). Vitamin C has a protective role in the development of atherosclerotic heart disease by inhibiting low-density lipoprotein oxidation (18,19,20,21,22). Vitamin E or α-tocopherol is a peroxyl radical scavenger that promotes the integrity of long-chain polyunsaturated fatty acids at the membrane of cells, and thus, maintains their bioactivity (21,22). The effect of vitamin E has been studied in an experimental myocardial infarction and the results suggest that prolongs survival in animals after AMI, reducing oxidative stress, arrhythmia and heart dysfunction (19).The interaction between vitamin C and vitamin E radicals can take place in liposomal membrane systems (23, 24), and it has been stated that combined treatment with this vitamins suppressed neutrophil-mediated free radical production and lowered blood lipid peroxidation in patients with AMI (19,25).

Based on their beneficial effects during ischemic cardiovascular injury, the aim of this study was to determine the role of combined therapy between hyperbaric oxygenation and antioxidant vitamins C and E on angiotensin II AT1 and AT2 receptors in a murine myocardial infarction model.

MATERIALS AND METHODS

An experimental, prospective, longitudinal, and analytic study was designed in order to determine the role of HBO2 and antioxidant vitamins C and E in an AMI murine model. Male Wistar rats weighing 300 ± 50 g were used and kept in cages at room temperature, exposed to a cycle of 12 hours of light for 12 hours of darkness, maintained with water and feed *ad libitum*.

Eight study groups were conformed considering 5 randomly selected rats each one, as described below:

a. Without hyperbaric oxygenation (healthy; myocardial infarction; myocardial infarction + C and E vitamins; sham)

b. With hyperbaric oxygenation (healthy; myocardial infarction; myocardial infarction + C and E vitamins; sham)

In order to perform the murine myocardial infarction model in the groups of rats that were randomized to AMI, a technique of occlusion of the proximal anterior descending coronary artery was used. The rats were anesthetized with Xylazine (8 mg/kg) and Ketamine (100 mg/kg body weight). A thoracic trichotomy was performed and under asepsis and antisepsis conditions, the orotracheal intubation was performed with 16G catheter which was connected to a Harvard rodent ventilator 683 (60 ventilations/minute). Subsequently a longitudinal total sternotomy was performed followed by an anterior pericardiotomy to expose the heart. Once identified, the anterior descending coronary artery was ligated with a 5-0 nylon suture, cardiac hemostasis was corroborated, thoracic cavity was closed, the rats were placed in an artificial warmth area and were monitored to detect post-surgical alterations opportunely. After a period of 10 to 20 minutes, the rats were extubated and then administered with buprenorphine (0.2 mg / kg subcutaneously/24hr) for pain control. It must be noted that the rats that were randomized at the group called SHAM, a simulated surgery following the same procedure described above was performed, without ligation of the anterior descending coronary artery.

Electrocardiograms were performed in the presurgical, immediate postsurgical period and after the corresponding days of treatment. The animals were anesthetized with Xylazine at a dose of 8 mg / kg and Ketamine at a dose of 100 mg / kg body weight, an electrocardiogram (Contec) was used, and it was calibrated at a speed of 50 mm / s and a voltage of 20 mm / mV. Once anesthetized, the extremities were cleaned with 70% alcohol, conduction gel was placed and the electrodes were placed and the electrocardiographic data corresponding to aVF, aVL, aVR, Dl, Dll, Dlll, were taken. V1, V3 and V6 to assess the electrical conduction, corroborate the infarction in the corresponding groups, detect cardiac arrhythmias, determine the cardiac rhythm and calculate the heart rate.

At the same time that the rats were anesthetized for taking the EKG, the blood pressure was indirectly measured in the tail of the rat by using the Harvard Bioscience equipment, indirect rat tail blood pressure system. The pressure transducer was placed on the tail of the animals in order to detect the pulse of the caudal artery and therefore determine systolic blood pressure.

The rats selected for the hyperbaric oxygen therapy groups, HBO_2 was administered in an experimental chamber at two atmospheres of pressure for one hour, using one session per day for 3 days. The pressurization was carried out in a 15 minutes period: one hour of isopression to 2 ATA. After the exposure time, the depressurizing was performed in the next 15 minutes, the animals were removed from the chamber and placed in their respective cage.

The groups of rats to be treated with the antioxidant therapy were dosed with D-alpha-Tocopherol acetate (Vitamin E-gelcaps at 25mg/kg/d) and ascorbic acid at a dose of 20 mg/kg/d were administered by orogastric route, after the first 24 postoperative hours for 3 consecutive days. Simultaneously, HBO_2 therapy was administrated for the corresponding groups using the same technique already described.

Finally, the rats of all the groups, and the ones that were treated with HBO_2 after 3 sessions, were slaughtered by decapitation with previous anesthesia using sodium pentobarbital at a dose of 60 mg/kg intraperitoneally. Once anesthetized, a total thoracotomy was performed to remove the heart, which was posteriorly stored at REVCO in a -70°C of temperature. Finally, an analysis of the heart tissue modifications with western blot transfer technique was performed.

The expression of AT1R and AT2R was determinate by Western blotting. For Western Blotting, the heart was homogenized in the buffer containing (in mM) Tris 50nM, EDTA, Triton 1%, 1mM 0, NaCl 150mM, cocktail of protease inhibitors (pancreas extract and pronase inhibitor, thermolysin, chymotrypsin and papain inhibitor) (Roche Diagnostics). Homogenates proteins were determined using the Bradford assay kit (Bio Rad Protein Assay; Bio-Rad Laboratories) to each 30 µL of dilute extract or PBS (three replicates) in microplate. Polyacrylamide gels were used for electrophoresis with 30 mg of protein and transferred to polyvinylidene difluoride membrane (Hybond-P, Healthcare, GE, Amersham, UK).

Membranes were blocked with 6% of nonfat milk in 20 mM Tris buffer pH 7.4, 0.1% Tween 20 (TBS-T) for 2 hours at room temperature. The blots were incubated with primary polyclonal antibodies for the AT1 (Cat. SC-31181, Santa Cruz Biotechnology, Santa Cruz, CA) and AT2 (Cat. SC-7420, Santa Cruz Biotechnology, Santa Cruz, CA) receptors overnight at 4 ºC in a 1:100 dilution for membranes. Secondary antibody donkey anti-goat IgG HRP (Cat. SC-2020, Santa Cruz Biotechnology,

Santa Cruz, CA) was incubated for three hours at room temperature in a dilution of 1:1500.

Then, for the reveal, the membranes were incubated with the chemiluminescent substrate using luminol (western Blotting Luminol Reagent (sc-2040) from Santa Cruz biotechnology) following the manufacturer's instructions. After that procedure, they were placed in the amplification chamber for 15 min in direct contact with light sensitive film (Amershamhyperfilm ECL from GE healthcare limited). Subsequently the films were submerged in revealing liquid (professional Kodak d-19) for 30 seconds and in fixative, allowed to dry and labeled appropriately for future reading and analysis.

The intensity of the bands detection was measured by scanning the bands with a scanner (HP Scanjet G3110), and densitometry was performed using Image Analysis Software (BioRad). The presence ofproteins was normalized with reference protein beta-actin andquantified by densitometry with Image J 1.44 p program (National Institutes of Health, Bethesda, MD).

Statistical analysis

Data was expressed as the mean ± SEM. Comparisons among the groups of data were carried out using the one-way ANOVA followed by Tukey's post hoc test. Significance was accepted at $p < 0.05$.

RESULTS

Results of this study are reported in terms of functional and molecular parameters. Electrocardiogram, blood pressure, and heart rate are the functional variables considered for the analysis, and Western Blot reveals the modifications found in the heart tissue of the rats.

Electrocardiogram

In order to corroborate AMI in the corresponding groups and calculate the heart rate, electrocardiograms (EKG) were performed. A more homogeneous pattern can be observed in the complexes at the EKG of the rats with a 24-hr infarction with treatment, favorably compared with the aberrant conduction of the infarcted heart without treatment. In addition, the treated group showed a significant lower heart rate, which can be translated into a less cardiac work, and a decrease in oxygen requirement in order to preserve the cardiac tissue hibernating.

Figure 1 shows EKG in control rats (A), myocardial infarct (B), myocardial infarct at 3 days (C), and myocardial infarct at 3 days with treatment (D).The electrocardiograms of the infarcted heart at 3 days of evolution, showed an improvement in electrical conduction of the heart, which may reflect an enhancement in the potentially fatal sequelae of AMI, meliorating the quality of life of those who suffer this disease.

Heart rate

In Figure 2 it can be observed the heart rate taken from the electrostatic devices in a group without infarction and with the different treatments. The rhythm is unusual and has an average frequency of 336 bpm in the control rats. Interestingly, heart rate tends to decrease with the different treatments, being significantly noteworthy in the group where a combined treatment of HBO and vitamins C and E has been received, effect that depends on the exposure time.

On the other hand, in the myocardial infarction group, left anterior descending coronary artery ligation causes a significant increase in heart rate at the postoperative period (Figure 2). Interestingly, after days of evolution, in the groups without treatment, a considerable decrease in this parameter can be appreciated, which can be translated into data sequelae of AMI, in particular, heart failure.

However, in the groups that combined treatment of vitamin C and E and hyperbaric oxygenation, a heart rate recovery is observed, which is more markedly in the group at 3 days of treatment. This fact traduces a preservation of cardiac function and could potentially diminish the life-threatening sequelae of AMI (Figure 2).

Blood pressure

Compared with the group of healthy rats, it was found that this parameter decreased in the groups with combined treatment (Figure 3). However, this decrease in blood pressure, rather than considered not to be hypotension, at the postinfarction period could potentially become beneficial as will be mentioned later.

In the SHAM group, blood pressure behaved similarly to healthy rats according to the different days of treatment, presenting a slight decrease in blood pressure in the immediate postoperative period, which may be related to the same surgery, manipulation of the pericardial structures, bleeding, and even the anesthetic effect. This alteration reverted at 24 hours of evolution. (Figure 3)

In the group of rats with AMI, it can be observed (Figure 3) that at the immediate postoperative period blood pressure increases significantly modifying the cardiac physiology of the experimental model from the first postinfarction minutes. Subsequently, it can be observed that in groups of 1 and 3 days without treatment, the arterial pressure remained high persistently. Regarding the treatments, we can observe that the synergic treatment of vitamin C and E with hyperbaric oxygenation provides a significant improvement in blood pressure presenting a tendency to normalization, which can translate into a limitation of myocardial tissue damage in the post-infarction period,

Western blot

Figure 4 shows how monotherapy with OHB in an infarcted heart dramatically decreases the expression of the AT1 receptor, an effect that is enhanced by the concomitant administration of vitamins C and E. Interestingly, we can observe that the administration of vitamins itself induce an increase in the expression of the AT2 receptor (figure 5). Similar results are obtained in the group treated with HBO alone. However, this cardioprotective finding is enhanced with the administration of both therapies, demonstrating an important synergistic effect.

DISCUSSION

Statistics indicate that, at the last decades, cardiovascular diseases have been increasing in such an important way that currently occupy the first place of global morbidity and mortality (26). Although there are clinical guidelines for management of patients with AMI (27), research for innovative adjuvant therapeutic options may improve the quality of life of patients with this pathology. Although there are few reports, HBO therapy has begun to be studied because of its beneficial effects in several pathologies. Until now, there is a lack of literature evidence regarding cardiovascular pathologies, hence the importance of the present work.

Evaluation of the modification in the expression of AT1 and AT2 receptors, which have a great importance in cardiovascular diseases, (28) may clarify which are the ways that can be silencing or stimulating after sessions of HBO and the administration of vitamin C and E. Our results indicate that HBO significantly modifies the expression of both AT1 and AT2 receptors, so offering monotherapy is a viable therapeutic option for patients with AMI. These findings corroborate the ones that have been carried out by other members and collaborators of our work team, in several lines of research parallel to this work (17).

As an interesting aspect, we can point out that vitamins C and E, antioxidant substances per excellence that were simultaneously administered in this study, have been used in many AMI experimental models to reduce the area of infarction, decrease cardiac arrhythmias after infarction, and stabilize ROS (19,20,21,29) in the process of cardiac tissue necrosis, allowing to limit the sequelae that characterizes the natural history of this disease. This important effects in the reduction of the infarction zone, were corroborated in our study, because angiotensin AT1 receptors are not statistically modified by the administration of monotherapy with vitamins C and E.

However, the AT2 receptors do show significant differences, which may justify their beneficial effect on the patients who suffer AMI. Interestingly, this study allowed us to realize that when applying both therapies there is a synergy between its beneficial effects, decreasing the harmful effects of the AT1 receptor and increasing the cardioprotective effects of the AT2 receptor. In addition to being a viable and accessible therapy for any patient, this evidence has not been described in the literature (7,9, 10). It is necessary to develop more researching protocols in order to provide continuous knowledge that validate the use of hyperbaric oxygen therapy concomitantly with vitamins C and E.

ACKNOWLEDGEMENT

The current study was supported by the SIP project (Escuela Superior de Medicina, IPN) and COFAA.

Ethical standars

All animals were used in accordance with the ethical standards established by the IPN High School of Medicine and comply with the requirements determined in the subject NOM-062-ZOO-1999 Technical specifications for the production, care and use of animals laboratory, SAGARPA, and the Guide for the care and use of laboratory animals, National Research Council.

Funding

The study was supported by grant SIP given by Instituto Politecnico Nacional.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The manuscript has not been published previously.

Authorship contributions

1.Nallely Acevedo-Villavicencio : development, research
2.Maria C. Castillo-Hernandez: development, research and writing of the paper
3.Alexandre Kormanovski-Kovzova: oxidant and antioxidant studies
4.Icela Palma-Lara: Histopathological studies
5.Rodrigo Romero: WB and Histopathological studies
6.Pedro J. Curi-Curi: development, research and writing of the paper
7.Aaron Dominguez-Lopez: hyperbaric oxygen therapy metodology
8.Ruth M. Lopez-Mayorga: cardiovascular studies
9.Gustavo Guevara-Balcazar: Development, analisis, writing and direction of the proyect

REFERENCES

1. Zwaan C., Daemen M.J.A.P. and HermensW.Th. Mechanisms of cell death in acute myocardial infarction: pathophysiological implications for treatment. Neth Heart J. 2001;9(1):30-44.
2. Pacurari M, Kafoury R., Tchownwou P.B., Ndebele K. The Renin-Angiotensin-Aldosterone System in Vascular Inflammation and Remodeling. International Journal of Inflammation. 2014; 2014: 689360. http://dx.doi.org/10.1155/2014/689360
3. Yu Liu , Annarosa Leri, Baosheng Li , Xiaowei Wang , Wei Cheng , Jan Kajstura , *et al*. Angiotensin II Stimulation In Vitro Induces Hypertrophy of Normal and Postinfarcted Ventricular Myocytes. Circulation Research. 1998; 82:1145-1159. https://doi.org/10.1161/01.RES.82.11.1145
4. Bodh I.J. Apoptosis after reperfused myocardial infarction: Role of angiotensin II. Experimental and Clinical Cardiology. 2004;9(4):219-228.
5. Xu J., Carretero O.A., Lin C.X., Cavasin M.A., Shesely E.G., Yang J.J., *et al*. Role of cardiac overexpression of ANG II in the regulation of cardiac function and remodeling post myocardial infarction. Am J Physiol Heart Circ Physiol. 2007;293:H1900-H1907. doi:10.1152/ajpheart.00379.2007.
6. M Stoll, U M Steckelings, M Paul, S P Bottari, R Metzger, and T Unger .The angiotensin AT2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. Clin Invest. 1995;95(2):651-657. https://doi.org/10.1172/JCI117710.
7. Tsuzuki S, Matoba T, Eguchi S, Inagami T. Angiotensin II type 2 receptor inhibits cell proliferation and activates tyrosine phosphatase. Hypertension. 1996 Nov;28(5): 916-8.doi: 10.1161/01.hyp.28.5.916.
8. Paradis P., Dali-Youcef N., Paradis F.W., Thibault G., Nemer M. Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. Proc Natl Acad Sci USA. 2000;97(2): 931-6.doi: 10.1073/pnas.97.2.931.
9. Touyz R.M., Schiffrin E.L. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev. 2000;52(4):639-72.
10. Takashi Naito, Li-Jun Ma, Haichun Yang, YiqinZuo, YiweiTang, *et al*. Angiotensin type 2 receptor actions contribute to angiotensin type 1 receptor blocker effects on kidney fibrosis. Am J Physiol

Renal Physiol. 2010 Mar; 298(3): F683–F691. doi: 10.1152/ajprenal.00503.2009
11. Melaku L. Angiotensin-converting enzyme 2 and its potential protective effect upon heart. Archives of Medicine and Health Sciences. 2018;6(2): 238-246.doi: 10.4103/amhs.amhs_44_17
12. Kaschina E, Namsolleck P, Unger T. AT2 receptors in cardiovascular and renal diseases. Pharmacol Res. 2017;125(Pt A):39-47https://doi.org/10.1016/j.phrs.2017.07.008.
13. P.K. Mehta, K.K. Griendling. Angiotensin II cell signaling physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol. 2007;292:C82-C97doi: 10.1152/ajpcell.00287.2006.
14. Matsubara H, Kanasaki M, Murasawa S, Tsukaguchi Y, Nio Y, Inada M. Differential gene expression and regulation of angiotensin II receptor subtypes in rat cardiac fibroblasts and cardiomyocytes in culture.J Clin Invest. 1994;93(4): 1592-601.doi: 10.1172/JCI117139.
15. Billet S, Aguilar F, Baudry C, Clauser E. Role of angiotensin II AT1receptor activation in cardiovascular diseases. Kidn Intern. 2008; 74:1379–1384.doi: 10.1038/ki.2008.358.
16. Ryan Choudhury. Hypoxia and hyperbaric oxygen therapy: a review. International Journal of General Medicine. 2018;11: 431-442.doi: 10.2147/IJGM.S172460.
17. Herbaczyńska-Cedro K. Supplementarion with vitamins C and E suppresses leukocytes oxygen free radical production in patients with myocardial infarctation. Eur Heart J. 1995;16(8):1044–9.doi: 10.1093/oxfordjournals.eurheartj.a061045.
18. Rodrigo R, Hasson D, Prieto JC, Dussaillant G, Ramos C, León L, *et al*. The effectiveness of antioxidant vitamins C and E in reducing myocardial infarct size in patients subjected to percutaneous coronary angioplasty (PREVEC Trial): study protocol for a pilot randomized double-blind controlled trial. Trials. 2014;15: 192.doi: 10.1186/1745-6215-15-192
19. Shite J, Qin F, Mao W, Kawai H, Stevens SY, Liang CS. Antioxidant vitamins attenuate oxidative stress and cardiac dysfunction in tachycardia-induced cardiomyopathy. J Am Coll Cardiol. 2001;38(6):1734–40.doi: 10.1016/s0735-1097(01)01596-0.
20. Traber MG. Vitamin E Regulatory Mechanisms. Annu Rev Nutr. 2007; 27:347–62. 85. doi: 10.1146/annurev.nutr.27.061406.093819.
21. Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radic Biol Med [Internet]. Elsevier Inc.; 2011;51(5):1000–13. http://dx.doi.org/10.1016/j.freeradbiomed.2011.05.017
22. 86.
23. De Mello WC, Frohlich ED. On the local cardiac renin angiotensin system. Basic and clinical implications. Peptides. Elsevier Inc.; 2011;32(8):1774–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21729730
24. Niki E. Action of ascorbic acid as a scavenger and stable oxygen radicals. Am J Clin Nutr. 1991; 54:1119S – 1124S. doi: 10.1093/ajcn/54.6.1119s.
25. Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among vitamin C, vitamin E, and 13-carotene13. Am J Clin nutr. 1995; 62:1322–6.doi: 10.1093/ajcn/62.6.1322S.
26. Singh RB, Niaz MA, Rastogi SS, Rastogi S. Usefulness of antioxidantvitamins in suspected acutemyocardial infarction (the Indian experiment of infarct survival-3). Am J Cardiol. 1996;77(4): 232-6.doi: 10.1016/s0002-9149(97)89384-8.
27. FAO, WHO. Vitamin and mineral requirements in human nutrition Second edition. World Heal Organ [Internet]. 1998;1–20. Available from: www.who.org
28. Sinz E. Soporte Vital Cardiovascular Avanzado. American Heart Association; 2011.
29. Diem T. DINH, Albert G. FRAUMAN, Colin I. JOHNSTON MEF. Angiotensin receptors: distribution, signaling and function. Clin Sci. 2001;100(5):481–92.
30. Nam CM, Oh KW, Lee KH, Jee SH, Cho SY, Shim WH, *et al*. Vitamin C intake and risk of ischemic heart disease in a population with a high prevalence of smoking. J Am Coll Nutr. 2003;22(0731-5724):372–8.doi: 10.1080/07315724.2003.10719320.

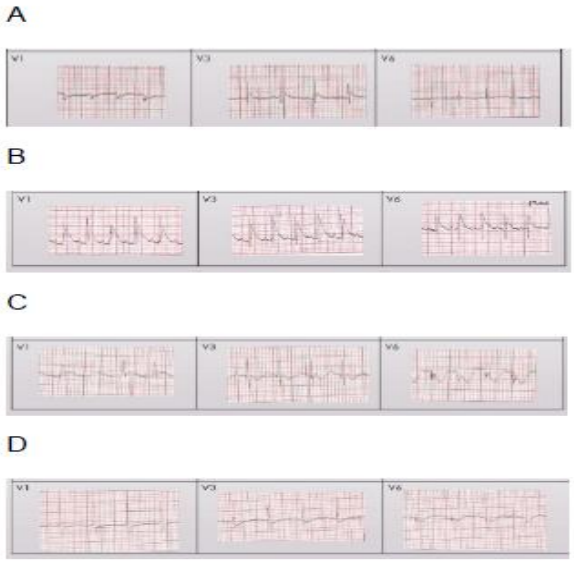

Figure 1: Electrocardiogram in control rats (a), myocardial infarct (b), myocardial infarct 3days (c) and myocardial infarct 3days with treatment (d). n=5, *p>0.05 +EEM.

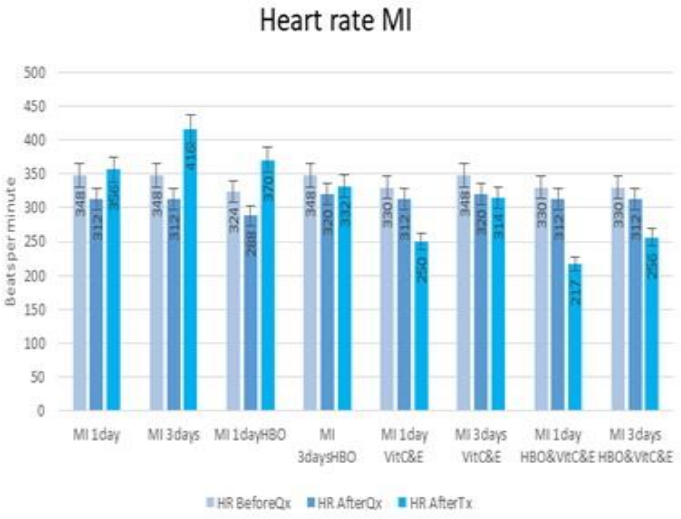

Figure 2: Heart rate in rats with and without myocardial infarct with hyperbaric oxygen and vitamin C and E. n=5, *p>0.05 +EEM.

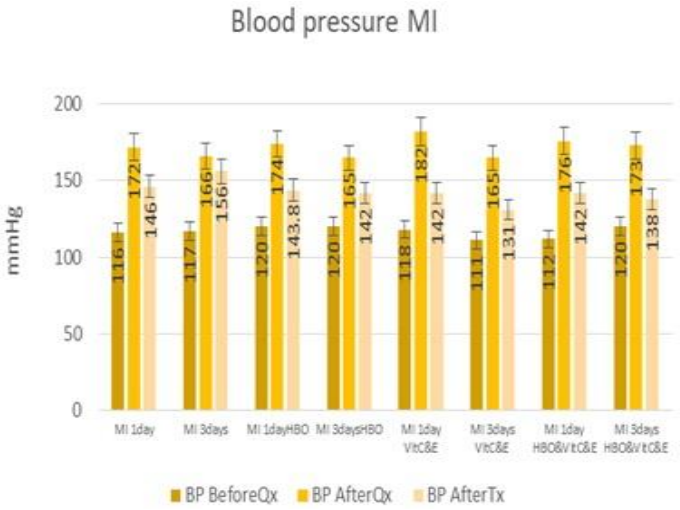

Figure 3: Blood pressure in rats with and without myocardial infarct with hyperbaric oxygen and vitamin C and E. n=5, *p>0.05 +EEM.

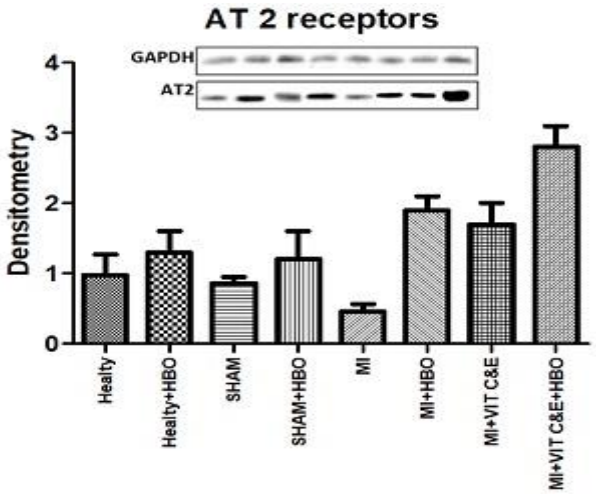

Figure 5: Expression of At2 receptor in a myocarial infarct in rats with hyperbaric oygen therapy and vitamin C and E

*p>0.05 +EEM.

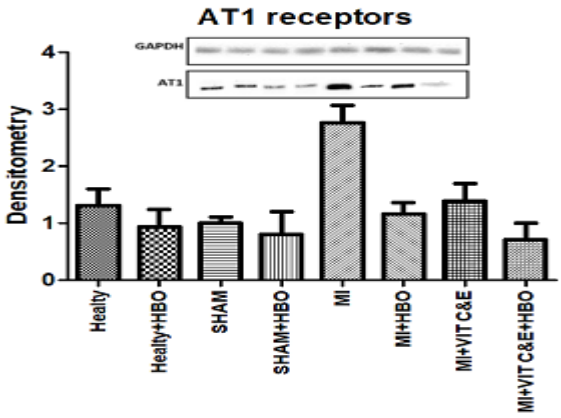

Figure 4: Expression of At1 receptor in a myocardial infarct in rats with hyperbaric oxygen therapy and vitamin C and E treatment. n=5, *p>0.05 +EEM.