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Jacobs Journal of Physiology

Research Article

Influence of Periodontal β_1 IgG on The Antioxidant Enzyme System in Rat Atria Myocardium

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Received: 10-09-2015

Accepted: 11-20-2015

Published: 12-11-2015

Abstract

In this paper we demonstrate that circulating antibodies from serum of periodontitis patients presenting atria β_1 adrenoceptors (AR) induce changes upon antioxidant superoxide dismutase (SOD) and catalase (CAT) enzymes and the release of nitrites during early hypoxia. We also demonstrate the existence of reactivity against the second extracellular loop of human myocardial β_1 AR by means of the ELISA, using the β_1 synthetic peptide with an amino acid sequence identical to that of the second loop of human myocardial β_1 AR as a coating antigen. These autoantibodies in the serum of chronic periodontitis patients have two complementary results, i.e., a decrement of SOD's activity and an increment of CAT's activity. These phenomena are accompanied by a further one; namely, the stimulation of the generation of nitrites by rat atria myocardium during hypoxia. The effect of the presence of isoproterenol (ISO) resembles those observed in the presence of autoantibodies. The actions of β_1 IgG and ISO are blunted by atenolol, L-NMMA and β_1 synthetic peptide, respectively. Normal IgG (n IgG) is without effect in our study system. These results indicate that the presence of β_1 IgG in atria myocardium modifies the activities of SOD and CAT during early hypoxia; a modification which is accompanied by an increment of the nitrate levels. This increment may, in turn, be the cause of an increment of the levels of reactive oxygen species (ROS) and may be one of the factors that subsequently trigger radioprotection or myocardial injury, leading to irreversible cellular and tissue damage.

Keywords: Myocardium; Hypoxia; Normoxia; Nitrites; Antioxidant Enzymes

Introduction

To introduce our study let's recall some facts: the release of catecholamine activates the β_1 adrenoceptor during myocardial ischemia injury when this is abundantly expressed on cardiomyocytes. Increased reactive oxygen species (ROS) and myocardial apoptosis are thought to be mediated by the β_1 adrenoceptor in vitro [1] and in vivo [2]. When this adrenoceptor is stimulated by β_1 IgG in cardiac atria it appears through a canonical cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA) pathway [3,4] The β_1 adrenoceptor's antagonists can preserve the membrane's phospholipids, by scavenging free radicals and reducing the lipid peroxidation [5]. This being the case, seeking for β_1 adrenoceptor's antagonists based on a target receptor and fully understanding the protective mechanisms are necessary for the treatment of myocardial ischemia injury.

In this connection, it is important to note, that hypoxia and associated metabolic disorders are the key pathogenetic factors of all severe complications in extreme conditions of various origins. In line with the discovery of the major role of nitric oxide as a factor involved in the relaxation of blood vessels and as a universal regulator of many biochemical processes in the body, it is recently of interest to study changes in the content of nitric oxide in blood and organs of

animals under low oxygen conditions [6].

Periodontal diseases are multi-factorial [7, 8]. When it comes to patient related variables, the stress or local hyper activation of the autonomic adrenergic system are sought to be co-factors to the prevalence of the disease and its progression [9]. Periodontitis is linked to systemic illnesses, such as cardiovascular diseases and stroke, as well. Increasing evidence also indicates that periodontal disease is a risk factor for coronary disease, by way of the correlated dysfunction of the endothelial cells, whose growth is induced by either periodontopathic bacteria or their products, or by inflammatory mediators derived from infected periodontal tissue [10-12]. These findings raise core questions about the effect of other non-thrombotic factors triggering functional alterations in the myocardium, thus inducing the "remodelling phenomenon" of the heart.

The antioxidant enzyme system plays animportant role in the cell defense against ROS-mediatedcell damage. This system consists of three enzymes:superoxidedis- mutase (SOD;EC1.15.1.1), catalase (CAT ;EC1.11.1.16), andperoxidase, of which glutathione peroxidase (GPx;EC 1.11.1.9)is the most common in mammalian cells[14]. SOD is responsiblefor the elimination of cytotoxic active oxygen by catalyzing the dismutation of the super- oxideradical to oxygen and hydrogenperoxid[15]. The rearea variety of Reactive oxygenspecies (ROSs), which include hydroxy radicals, superoxide anion,hydro- genperoxide, and nitricoxide, are produced byoxygen exposure onormal cells. ROSs are very transient species due to their high chemical reactivities, which result in lipid peroxidation as well as in the oxidation of DNA and proteins[13]

There area variety of revelatory in this sense is that reactive oxygen species (ROS), which include hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide, are produced by oxygen exposure to normal cells [13]. ROS are very transient species due to their high chemical relativities, and their presence in cells lead to lipid peroxidation as well as to the oxidation of DNA and proteins [13]. The antioxidant enzyme system plays an important role in the cell defence against ROS-mediated cell damage. This system consists of three enzymes: superoxide dismutase (SOD), catalase (CAT), and peroxidase, being glutathione peroxidase the most common in mammalian cells [14]. SOD is responsible for the elimination of cytotoxic active oxygen by catalyzing the dismutation of the superoxide radical to oxygen and hydrogen peroxide [15]. On the other hand, there is a variety of CAT's mainly existing in the peroxisome, which do not only react to hydrogen peroxide by activating it's decomposition into water and oxygen, but also react to hydrogen donors [16]. The present study thus seeks to clarify the activities of antioxidant enzymes in the isolated rat atria myocardium against short-term hypoxia. To this aim, we examine the activities and the pharmacological mechanisms of the enzymes, SOD and CAT in the presence of β_1 IgG from serum of chronic periodontitis patients. We also detect the nitrates and study the atria adrenergic system by screening sera of patients with periodontitis against β_1 synthetic peptide with an amino acid sequence identical to the second extracellular loop of human heart's β_1 adrenoceptors. Another aim of our study is to analyze the action of the β_1 adrenoceptor agonist, isoproterenol (ISO).

Methods

Patients

The study group consisted of 24 adult patients; specifically, 22 male and 2 female, with chronic periodontitis (CP), who were attending the Periodontology Clinic of the metropolitan area of Buenos Aires. These patients were mean aged 40 years old, and ranged from 32 to 50 years old. Healthy subjects were used as controls, among them, 16 male and 4 female. The control group ranged from 30 to 46 years old, that is, they were mean aged 38. At least six sites with ongoing periodontal disease were required to be considered for the study. These sites included the following characteristic clinical signs of CP: loss of clinical attachment, loss of horizontal or/and angular alveolar bone, periodontal pocket formation and gingival inflammation; more precisely, alveolar bone loss > 2 mm and a pocket depth > 5 mm with bleeding and attachment loss > 3 mm. In the healthy subjects (control group), the probing depth was < 3 mm and the attachment loss was < 2 mm. Non of the subjects (CP patients and/or controls) had systemic illnesses or were smokers. They had not gone through any periodontal treatment, or any antibiotic or anti-inflammatory based treatments within the 5 months and 3 weeks prior to the study, respectively. All of the patients consented to participate in the study and the investigation was conducted according to the tenets of the Declaration of Helsinki of 1975 as revised in 2000.

Human Sera and IgG Purification

Sera and the corresponding IgG were obtained from patients with CP and healthy individuals (control). Six millilitres of blood was obtained by venipuncture and allowed to clot at room temperature. The serum was separated by centrifugation at 2000 g and stored at -20ºC until used in assays. The IgG was obtained by precipitation with 50% ammonium sulphate, followed by 3 washes and re-precipitation with 33% ammonium sulphate. The resulting precipitate was submitted to chromatography equilibrated with 10 mM phosphate buffer (pH 8). The eluted peaks were concentrated by ultra filtration of 10 mg protein/ml. Control immune electrophoresis with goat anti-human total serum and goat non-specific anti-human IgG showed only one precipitation line.

Enzyme Linked Immunosorbant Assay (ELISA)

Fifty millilitre of peptide solution (20 µg/ml) in 0.1 M Na_2CO_3

buffer (pH 9.6) was used to coat microtiter plates at 4°C overnight. The coating antigen used was the β_1 synthetic peptide ($5x10^{-5}$ M). After blocking the wells with 2% bovine serum albumin in PBS for 1 hour at 37ºC, 1/30 dilution of sera from periodontitis patients and healthy individuals were added in duplicate and allowed to react with the peptide for 2 hours at 37°C. After thoroughly washing the wells with 0.05% Tween 20 in PBS, 100 µl of 1:6000 goat anti-human IgG alkaline phosphate conjugated antibodies were added and incubated for 1 hour at 37ºC; p-nitrophenylphosphate (1 mg/ml) was added to the substrate after extensive washing; the reaction was stopped after 30 min. Optical density (OD) was measured at 405 nm with an ELISA reader. Non-antigen-paired wells with a non-specific peptide and wells with no primary antiserum were used as a negative control. The results of each sample were expressed as a mean ± standard error of duplicate values.

Animals and Atria Preparation

Adult male Wistar strain rats (250-300 g) were used. The animals housed in standard environmental conditions were fed with a commercial pellet diet and water ad libitum. The experimental protocol followed the Guide to The Care and Use of Experimental Animals (DHEW Publication, NIH 80-23). Rats were killed by decapitation after being previously anesthetized with a mixture of ketamine and xilazine (50 and 5 mg/Kg^{-1} respectively). The atria was carefully dissected from the ventricles and immersed in a tissue bath containing a Krebs Ringer Bicarbonate (KRB) solution gassed in oxygen with 5% CO_2 and maintained at pH 7.4 and 37°C. The KRB solution was composed as described previously (Sterin-Borda et al. 1976).

Nitrate And Nitrites Assay

The rat atria samples were mixed with an equal volume of 100 µl of Griess reagent (Ingredient A: 0.1% naphthalene diamine dihydrochloride at a final concentration of 5 mmol/l; Ingredient B: 1% sulfanilamide at a final concentration of 5 mmol/l in orthophosphoric acid) in a 96 well microtiter plate (NUNC, Roskilde, Denmark). Nitric oxide concentrations were determined by a nitrate/nitrite colorimetric assay kit (Cayman Chemical Co, Ann Arbor, MI, USA) and measured spectrophotometrically at 540 nm, using a micro plate reader (Reader Model 230S; Organon Teknika, Boxtel, The Netherlands) according to [17]. The nitrate and nitrites values were expressed as µM/ml.

Biochemical Analysis of Antioxidant Enzymes

For analysis of the activity of SOD the atria were homogenized at 10% w/v in PBS pH 7.2 and centrifuged for 10 min with 800 g at 4°C. The supernatant was separated, sonicated for 30s and centrifuged at 20000 g /10 min [18]. Then, the supernatant was used for the analysis. The activity of the SOD enzyme was determined using the Superoxide Dismutase Assay Kit (Cayman Chemical Co, Ann Arbor, MI, USA). The reactions were followed using a Beckman DU-68 spectrophotometer (Beckman Instruments, Fullerton, CA) and their specific activity was expressed as U/ml/mg protein. For analysis of CATs' activity, the atria were homogenized at 10% w/v in a phosphate buffer (KH_2PO_4 50 mM and K_2HPO_4 50 mM 1:1.5 (v/v) pH 7.4. The homogenate was centrifuged at 1,500 g/10 min and the supernatant used to assay CATs' activity was measured in a medium containing a 50 mM phosphate buffer, a pH 7.0 supernatant and 100 mM H_2O_2. The catalysis of H_2O_2, which was observed spectrophotometrically, was shown by a decrease in absorbance at 240 nm (Cayman Chemical Co, Ann Arbor, MI, USA) and its specific activity was expressed as nmol/min/ml/mg protein. The protein's concentration was measured using Folin's phenol reagent, as described elsewhere [19]; bovine serum albumin was used as standard. The readings of the Beckman DU-68 spectrophotometer (Beckman Instruments, Fullerton, CA) were taken at 660 nm.

Experimental Protocol

The isolated atria underwent a 50 min stabilization period under basal conditions, during which they were equilibrated in buffer gassed with 95% O_2 and 5% CO_2 (37ºC, pH 7.4; pO_2 > 600 mmHg). Such a stabilization process was followed by a 50 min hypoxia as the atria were equilibrated in buffer gassed with 95% N_2 and 5% CO_2 (37ºC, pH 7.4; pO_2 > 100 mmHg). The adrenergic agonist [isoproterenol (ISO), $1x10^{-7}$ M] and the antibody (β_1 IgG $1x10^{-8}$ M) were added 10 min before the beginning of the hypoxic period. The total duration of this experimental design was 120 min. Afterwards samples were processed in order to analyse the antioxidant enzymes (SOD and CAT) biochemical and estimate the nitrites' production. All this protocol was done in atria subjected to normoxia and hypoxia, as described above. In the blocking experiments, atenolol ($1×10^{-7}$ M, a β_1-specific adrenergic antagonist), synthetic β_1-adrenergic peptide ($5×10^{-5}$ M) and nitric oxide synthases inhibitor (NG-monomethyl-L-arginine, L-NMMA) $1×10^{-4}$ M were used. Normal IgG was used as control (n IgG). All inhibitors were added at the beginning of the stabilization period (0 min).

Drugs

Stock solutions of isoproterenol (ISO), atenolol and NG-monomethyl-L-arginine, (L-NMMA) (Sigma Chemical Company, St. Louis, MO, USA) were freshly prepared before each experiment in its respective specific buffers. The β_1 synthetic peptide sequence, corresponding to the second extracellular loop of the human β_1 adrenoceptor was HWWRA ESDEA RRCYN DPKCC DFVTN RC.

Statistical analysis

A Student's t-test for unpaired values was used to determine the levels of significance. Analysis of variance (ANOVA) and a post hoc test (Dennett's method and Student-Newman-Keuls test) were employed when pair-wise multiple comparison procedures were necessary. The differences between means were considered significant at a $P < 0.05$.

Results

To demonstrate the presence of serum IgG directed against atria of the β_1 adrenoceptor (β_1 IgG) we preformed an ELISA, using a β_1 synthetic peptide corresponding to the amino acid sequence of the second extracellular loop of the human β_1 adrenoceptor as a coating antigen.

Figure 1A and 1B show the optical density values in a concentration-response curve. The immune reactivity (optical density values) of serum and IgG from chronic periodontitis patients are significantly higher than those from normal IgG individuals ($p<0.001$). The Scatterogram of Figure 1C shows the optical density values of every one of the 24 periodontitis patients and the 20 healthy individuals. The optical density values obtained with the reactive autoantibodies are always more than two standard deviations higher than those of healthy subjects.

All of these results demonstrate the presence of circulating autoantibodies in the serum of chronic periodontitis patients, which interact with atria β_1 adrenoceptors. It is important to know that the amino acid sequence of rodent and human β_1 adrenoceptors peptides have strong homology [20]. So, we study the β_1- adrenoceptors -mediated effect of auto-antibodies from patients with periodontitis on rat cardiac tissue excluding any interspecies reaction.

Sera autoantibodies (β_1 IgG) from chronic periodontitis patients induce a significant stimulation of SOD's activity and a decrement of CATs' activity ($p<0.001$) in isolated rat atria myocardium both during hypoxia and normoxia experimental conditions, as shown in Figure 2A and 2B.

It is important to note, that whereas the activities of both antioxidant enzymes in atria are identical in either experimental conditions; namely, normoxia and hypoxia, basal values during hypoxia are significantly lower than those in normoxia ($p<0.001$).

These results could be interpreted as meaning that the modification of the activity of antioxidant enzymes in the presence of β_1 IgG is responsible for changes upon the levels of reactive oxygen species (ROS) that, in turn, favour the ischemia of the myocardium.

To determine that the stimulation of SOD and the inhibition of CAT enzymes triggered by β_1 IgG of chronic periodontitis patients on rat atria myocardium during hypoxia are dependent on the atria β_1 adrenoceptor's activation and on the nitric oxide synthases' (NOS) activity, we used a β_1 antagonistic adrenoceptor and an specific inhibitor of NOS. Figure 3A and 3B show the inhibition of the atria β_1 adrenoceptor by atenolol ($1x10^{-7}$ M) and the inhibition of NOS by L-NMMA ($1x10^{-4}$ M) as preventing both the action of SOD and CAT enzymes during hypoxia.

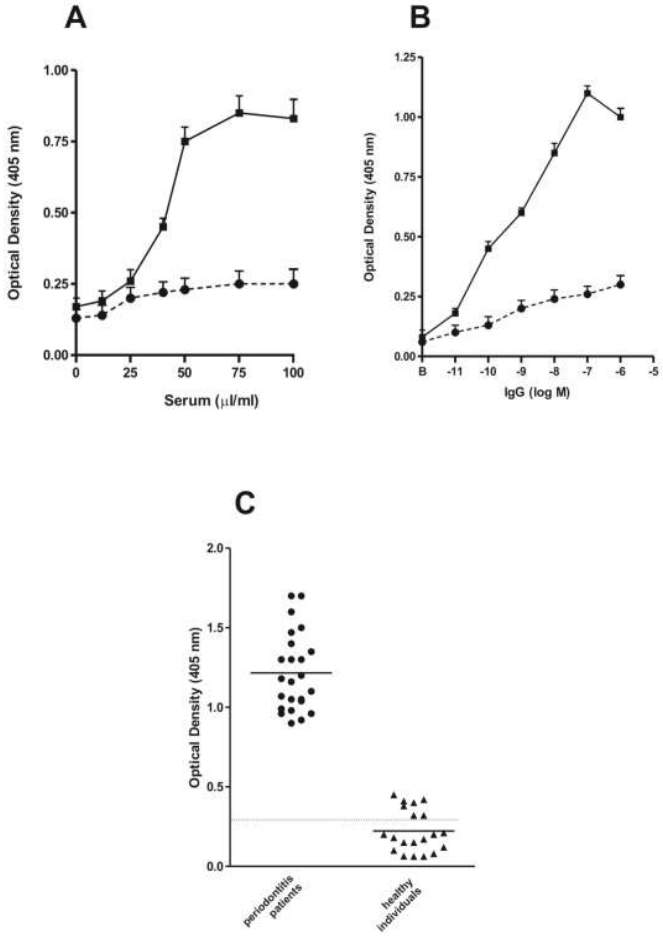

Figure 1. Immunoreactivity of serum and circulating IgG autoantibodies (β1 IgG). In detail, A: Concentration-response curves of standardized pool of serum from chronic periodontitis patients (.) and standardized pool of serum from healthy subjects (·). B: Concentration-response curves of standardized pool of β1 IgG from chronic periodontitis patients (.) and standardized pool of normal IgG from healthy subjects (·). C: Scatterogram showing immunoreactivity of individual optical density values for each serum sample (1:30 dilution) from 24 chronic periodontitis patients compared to 20 healthy subjects (control). The horizontal lines in C are mean values ($p<0.0001$) related to the comportment of periodontitis patients versus healthy subjects. Values represent the mean ± SEM of 24 patients and 20 healthy individuals performed by duplicate.

A similar result was observed in A549 cells and rat lungs [21]. Also, the biological activity of β_1 IgG on atria is absorbed by the β_1 synthetic peptide ($5x10^{-5}$ M), the autoantibody is without action in our system. In normoxia these inhibitors provoke the same results (see Figure 3A and 3B).

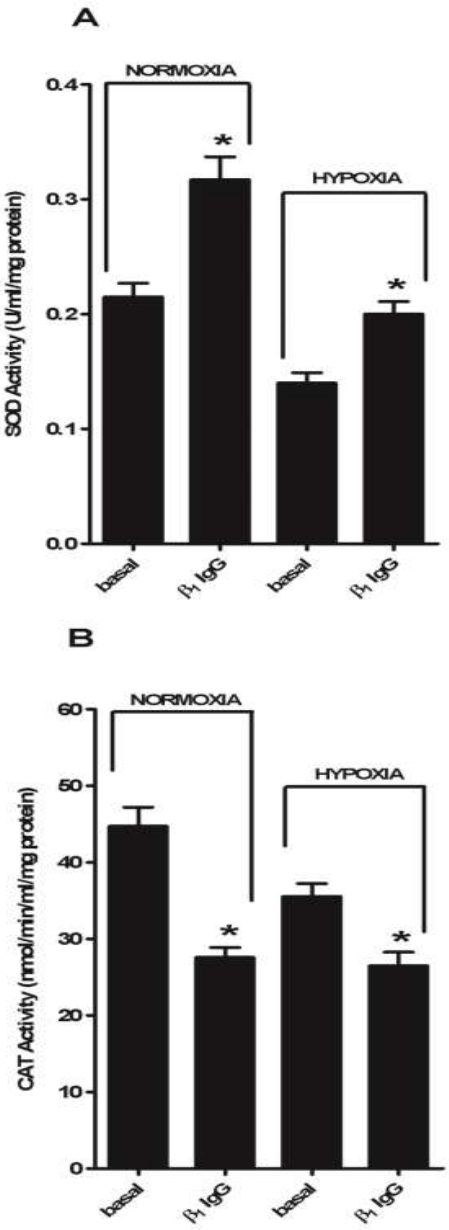

Figure 2. SOD's and CAT's activities in rat atria myocardium. In detail, A: β_1 IgG ($1x10^{-8}$ M) inducing significant increment of SOD's activity expressed as U/ml/mg protein when the autoantibody was incubated for 2 hours with rat atria in normoxia and hypoxia ($p<0.001$ for β_1 IgG versus basal values). B: β_1 IgG ($1x10^{-8}$ M) inducing significant decrement of CATs' activity expressed as nmol/min/ml/mg protein when the autoantibody was incubated for 2 hours with rat atria in normoxia and hypoxia ($p<0.001$ for β_1 IgG versus basal values). Values represent the mean ± SEM of 8 experiments in each case performed by duplicate.

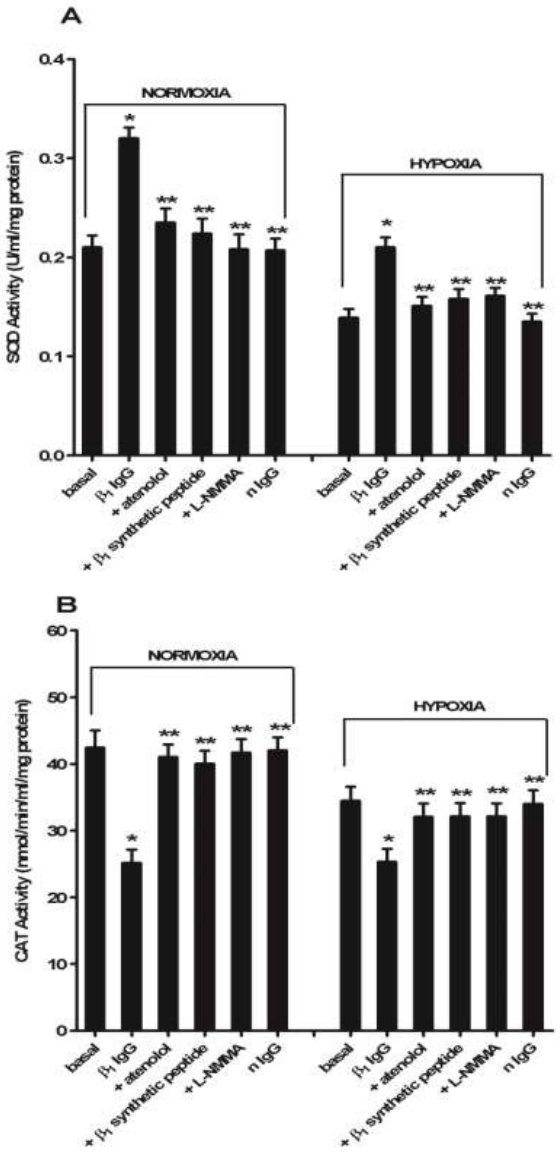

Figure 3: Pharmacological mechanism of SOD's and CATs' activities in rat atria myocardium, as follows: A: SOD activity determination (U/ml/mg protein) in rat atria incubated for 2 hours with β_1 IgG ($1x10^{-8}$ M) alone or in the presence of atenolol ($1x10^{-7}$ M), β1 synthetic peptide ($5x10^{-5}$ M) or L-NMMA ($1x10^{-4}$ M) during normoxia and hypoxia; Normal IgG (n IgG) from sera of healthy individuals was also shown as control. (*$p<0.001$ versus basal values and **$p<0.001$ versus β1 IgG's values). B: CAT activity determination (nmol/min/ml/mg protein) in rat atria incubated for 2 hours with β_1 IgG ($1x10^{-8}$ M) alone or in the presence of atenolol ($1x10^{-7}$ M), β1 synthetic peptide ($5x10^{-5}$ M) or L-NMMA ($1x10^{-4}$ M) during normoxia and hypoxia; Normal IgG (n IgG) from sera of healthy individuals was also shown as control. (*$p<0.001$ versus basal values and **$p<0.001$ versus β_1 IgG's values). Values represent the mean ± SEM of 6 experiments in each case performed by duplicate.

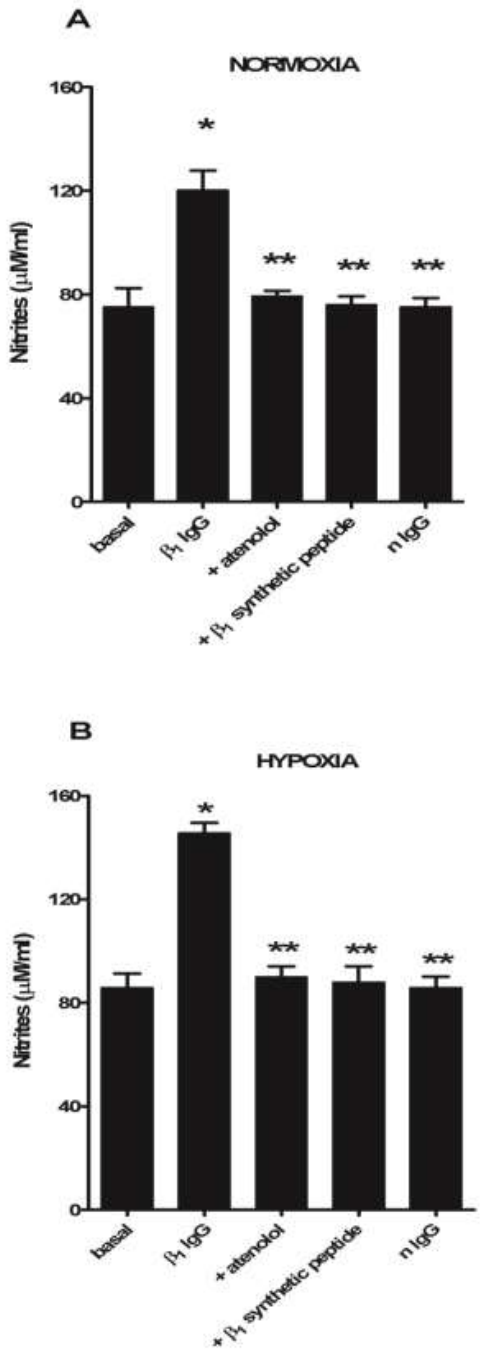

Figure 4: Nitrates' production in rat atria: A: Effect of β_1 IgG ($1x10^{-8}$ M) alone or in the presence of atenolol ($1x10^{-7}$ M) or β_1 synthetic peptide ($5x10^{-5}$ M) on the production of nitrites (µM/ml) during normoxia. Normal IgG (n IgG) from sera of healthy individuals is tested as control (*p<0.001 versus basal values and **p<0.001 versus β_1 IgG values). B: Effect of β1 IgG ($1x10^{-8}$ M) alone or in the presence of atenolol ($1x10^{-7}$ M) or β1 synthetic peptide ($5x10^{-5}$ M) on the production of nitrites (µM/ml) during hypoxia. Normal IgG (n IgG) from sera of healthy individuals is tested as control (*p<0.001 versus basal values and **p<0.001 versus β_1 IgG values). Values represent the mean ± SEM of 9 experiments in each case performed by duplicate.

To proof the influence of β_1 IgG on the variation of SOD's and CATs' activities during hypoxia and the blunting of L-NMMA's antibody's action on antioxidant activities, the production of nitrates in rat atria myocardium are measured with the following results: nitrates are significantly increased in value in hypoxia in comparison to normoxia ($p<0.001$) (Figure 4A and 4B). The same Figures showed that both, atenolol ($1x10^{-7}$ M) and the β_1 synthetic peptide (5x10-5 M), block the stimulatory actions of β_1 IgG.

These results could indicate that the increment of nitric oxide as an early proinflammatory substance modulates the hypoxia detriment, favouring, in part, the normal behaviour of the cardiac function. The β_1 adrenoceptor agonist, ISO ($1x10^{-7}$ M), can itself induce the same results upon the antioxidant enzymes' activities in the course of hypoxia and normoxia conditions as β_1 IgG. That is to say, the β1 adrenoceptor antagonistic agent (atenolol, $1x10^{-7}$ M), the NOS inhibitor (L-NMMA, $1x10^{-4}$ M) and the β_1 synthetic peptide ($5x10^{-5}$ M) blunt the action of ISO-β_1 IgG (Table 1). Instead, normal IgG ($1x10^{-8}$ M) from healthy subjects is without any effect.

Discussion

In this study we examined the presence of the biological activity of β_1 IgG in the sera of chronic periodontitis patients, modulating nitrites in the course of short-term hypoxia on rat isolated atria myocardium. We have done this, comparing the influence of β_1 IgG in rat atria under hypoxia and normoxia experimental conditions.

From the point of view of the biochemical analysis of the antioxidant enzymes (SOD and CAT) in atria in the presence of β_1 IgG, the result of this comparison is the enhancement of SOD and a reduction in CATs' activities. Seen from the point of view of their intrinsic mechanism, the result is an imbalance in the activity of both cardiac SOD and CATs triggered by the autoantibody (β_1 IgG), which may be conducive to excessive amounts of ROS's formation. Seen together, there results can be summarised saying that, increased SOD's and decreased CATs' activities in the cardiac atria (membrane and cytosolic) alter the degradation of O_2- to H_2O_2 and the detoxification of H_2O_2 into H_2O, during cardiac hypoxia equally. In addition, the β_1 adrenoceptor agonist ISO elicited in rat atria myocardium via the β_1 cardiac adrenoceptor's activation induces the generation of ROS [22]. However, the modification of SOD's and CATs' activities, and most probably, both the formation of ROS by the action of β_1 IgG upon atria myocardium and its binding to the β_1 adrenoceptor subtype, may be one step in a pathway to protect heart function against the emerging condition [23] occurring during brief cardiac hypoxia [24- 26].

Table 1. Influence of isoproterenol (ISO) upon rat atria during normoxia and hypoxia alone or in the presence of inhibitors.

Additions and experimental conditions	**SOD Activity** (U/ml/mg protein)	**CAT Activity** (nmol/min/ml/mg protein**)**	**Nitrites** (μM/ml)
Basal normoxia	0.210 ± 0.011	43 ± 2	65 ± 6
ISO normoxia	0.282 ± 0.019*	26 ± 2*	130 ± 9*
ISO +atenolol normoxia	0.214 ± 0.012*	39 ± 3*	74 ± 7*
ISO + L-NMMA normoxia	0.215 ± 0.013*	39 ± 6*	70 ± 4*
Basal hypoxia	0.145 ± 0.009	35 ± 3	86 ± 7
ISO hypoxia	0.182 ± 0.004*	26 ± 2*	152 ± 9*
ISO +atenolol hypoxia	0.156 ± 0.002*	31 ± 3*	95 ± 6*
ISO + L-NMMA hypoxia	0.160 ± 0.004*	28 ± 3*	90 ± 5*

Values are mean ± SEM of five experiments in each group performed in duplicate. Enzyme activities were measured in rat atria after incubation during 2 hours in presence of ISO ($1x10^{-7}$M) with or without inhibitors, i.e. Atenolol $1x10^{-7}$M (β_1 adrenergic antagonist) and L-NMMA $1x10^{-4}$M in normoxia and hypoxia experimental conditions. The mean value used to compare basal normoxia, basal hypoxia, and atenolol and L-NMMA in comparison with ISO alone in normoxia and hypoxia is *p<0.001.

In support of this interpretation, it should be noted that the nitrite-mediated cardioprotection [27, 28] has been determined. Here we demonstrate the β_1 IgG action upon atria myocardium provoke an increment of nitrites during hypoxia. The results related to this finding may be important in view of explaining the increment of S-nitrosylated proteins identified during hypoxia as able to reverse intracellular reductans as well as SOD's enzymes [29]. The outcome is a dynamic process of S-nitrosylation/de-nitrosylation reactions, which seem to be key to heart regulation [30].

Further support to these findings is given by the fact that ISO in heart is dependent on the generation of ROS, so that its pharmacological action on rat atria β_1 adrenoceptor may induce pre and post conditioning protection [31] Moreover, there is an alteration in the antioxidant enzyme activities of SOD and CAT during the action of β_1 IgG and ISO on rat atria myocardium in the course of hypoxia, which is impaired by atenolol and β_1 synthetic peptide. These facts corroborate the statement pharmacologically: the alterations of the atria adrenergic system are implicated in this phenomenon.

Against this background, the stimulation of the β_1 adrenergic receptor, which increases ROS' production and myocardial apoptosis in myocardial ischemia, can be confirmed to be mediated by the β_1 adrenoceptor subtype in vitro [1] and in vivo [2] through an increased production of cAMP dependent protein kinase A (PKA) pathway [3]. This strengthens the idea that the β_1 adrenergic antagonist exerts a protective mechanism in myocardium injury [21]. It supports the fact that L-NMMA, a specific NOS inhibitor, provokes an inhibition of β_1 IgG's action on SOD's and CAT's enzymes during hypoxia on rat atria, indicating that nitric oxide (NO) may have antioxidant properties as was reported previously [32] The facts that NO moduling cell function [33] results from the activation of soluble guanylate cyclase to form cGMP and NO nitrosylated target proteins are involved in several processes of pro-oxidation and anti-oxidation [34]All of our results may be explained by comparison with previously published data obtained by means of a cardiomyocyte-restricted mouse model with an over expression of SOD [35] and a model with inducible gene transfer [36] i.e. where levels of ROS are attenuated. Moreover, increased level of NO availability is produced in response to hypoxia, leading to myocardium protection [35]. This finding is further backed by a previous report [37] demonstrating that NO and ROS participate in the immune signal and have the ability to kill pathogens and their enhancement in the downstream of chronic periodontitis patients. This makes them into complex modulators acting in an inflammatory process worsened by the lesion of β1 IgG with an alteration of SOD's and CATs' activities and the generation of large amounts of nitrates.

In addition, it can be now said that it is possible that there are also, specific oxidation events that modify intracellular signals at the levels of the β_1 adrenoceptor on atria myocardium in chronic periodontitis disease. This specific oxidation leads to the alterations of the cardiac β_1 adrenoceptor, which could be thus seen as a cause of an adrenergic dysfunction in the course of periodontitis disease.

Conclusion

The present results showed the presence of β_1 IgG in the sera of periodontitis patients, the β_1 AR biological activity of the autoantibody, the β_1 IgG capacity to bind and activate atria β_1 AR and the increment and decrement of the specific antioxidant activities of SOD and CATs upon atria myocardium with increased levels of nitrates during short-term hypoxia, were determined in periodontitis disease. This phenomena is a defensive reaction to the increment of the amount of ROS in the heart, which, in some cases, triggers cardioprotection, and yet in others, myocardium injury and consequent irreversible cellular and tissue damage.

References

1. Communal C, Singh K, Sawyer DB, Colucci WS. Opposing effects of [beta]1- and [beta]2-adrenergic receptors on cardiac myocyte apoptosis: role of a pertussis toxin-sensitive G protein. Circulation. 1999, 100(22): 2210-2212.

2. Sabbah HN, Sharov VG, Gupta RC, Todor A, Singh V et al. Chronic therapy with metoprolol attenuates cardiomyocyte apoptosis in dogs with heart failure. J Am Coll Cardiol. 2000, 36(5): 1698-1705.

3. Jane-wit D, Altuntas CZ, Johnson JM, Yong S, Wickley PJ, Clark P et al. Beta 1-adrenergic receptor autoantibodies mediate dilated cardiomyopathy by agonistically inducing cardiomyocyte apoptosis. Circulation. 2007, 116(4): 399-410.

4. Segovia M, Reina S, Borda E, Sterin-Borda L. Autoantibodies to the β(1)-Adrenoceptor from Patients with Periodontitis as a Risk Factor for Cardiac Dysfunction. Dentistry. 2011:791393. doi: 10.5402/2011/791393.

5. Kawai K, Qin F, Shite J, Mao W, Fukuoka S et al. Importance of antioxidant and antiapoptotic effects of beta-receptor blockers in heart failure therapy. Am J Physiol Heart Circ Physiol. 2004, 287(3):H1003-H1012.

6. Kuropteva ZV, Reutov VP, Baider LM, Belaya OL, Krushinskii AL et al. Effect of hypoxia on nitric oxide formation in animal heart tissues. Biochem Biophys. 2011, 441(4):261-264.

7. Oliver RC, Brown IJ, Löe H. Periodontal disease in the United State population. J Periodontol. 1998, 69(2): 269-278.

8. Beck JD, Koch GG, Zambon JJ, Genco RJ, Tudor GE. Evaluation of oral bacteria as risk indicators for periodontitis in older adults. J Periodontol. 1992, 63(2): 93-99.

9. Bonchard P, Boutouyrie P, Mattout C, Bourgeois D. Risk assessment for severe clinical attachment loss in an adult population. J Periodontol. 2006, 77(3): 479-489.

10. Ramseier CA, Kinney JS, Herr AE, Braun T, Sugai JV et al. Chronic therapy with metoprolol attenuates cardiomyocyte apoptosis in dogs with heart failure. J Am Coll Cardiol. 2000, 36(5): 1698-1705.

11. Nakajima T, Yamazaki K. Periodontal disease risk of atherosclerotic coronary heart disease. Odontology. 2009, 97: 84-91.

12. Amitage GC. Periodontal infections and cardiovascular disease-how strong is the association? Oral Dis. 2000, 6(6): 335-350.

13. Matès JM, Perez-Gomez C, Nuñez de Castro I. Antioxidant enzymes and human diseases. Clin Biochem. 1999, 32(8): 595-603.

14. Matès JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000, 153(1-3): 83-104.

15. Kurobe N, Kato K. Sensitive enzyme immunoassay for rat Mn-superoxide dismutase: tissue distribution and developmental profiles in the rat central nervous tissue, liver, and kidney. Biomed Res. 1991, 12(2): 97-103.

16. Morita M, Kudo H, Doi Y, Hirano T, Ikemura K et al. Enhanced immunocytochemical expression of antioxidant enzymes in rat submandibular gland after normobaric oxygenation. Anat Rec. 2002, 268(4): 371-380.

17. Green LC, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N et al. Nitrate biosynthesis in man. Proc Natl Acad Sci USA. 1981, 78(12): 7764-7768.

18. Jung K, Henke W. Developmental changes of antioxidants enzymes in kidney and liver from rats. Free Radic Biol Med. 1996, 20(4): 613-617.

19. Simoes A, Nicolau J, de Souza DN, Ferreira LS, de Paula Eduardo C et al. Effect of defocused infrared diode laser on salivary flow rate and some salivary parameters of rats. Clin Oral Invest. 2008, 12(1): 25-30.

20. Jasper JR, Link RE, Chruscinski AJ, Kobika BK, Berstein B. Primary structure of the mouse β1 adrenergic receptor gen. Biochem Biophys Acta. 1993, 1178(3): 307-309.

21. Wang J, Jiang C, Alattar M, Hu X, Ma D et al. Oxidative injury induced by cadmium sulfide nanoparticles in A549 cells and rat lungs. Inhal Toxicol. 2015, 27(12): 649-658.

22. Salie R, Moolman JA, Lochner A. The mechanism of beta-adrenergic preconditioning: roles for adenosine and ROS during triggering and mediation. Basic Res Cardiol. 2012, 107(5): 281-285.

23. Garlid AO, Jaburek M, Jacobs JP, Garlid KD. Mitochondrial reactive oxygen species: which ROS signals cardioprotection? Am J Physiol Heart Circ Physiol. 2013, 305(7): H960-H968.

24. Cohen MV, Yang XM, Downey JM. Acidosis, oxygen, and interference with mitochondrial permeability transition pore formation in the early minutes of reperfusion are critical to postconditioning's success. Basic Res Cardiol. 2008, 103(5): 464-471.

25. Liu Y, Yang XM, Iliodromitis EK, Kremastinos DT, Dost T et al. Redox signaling at reperfusion is required for protection from ischemic preconditioning but not from a direct PKC activator. Basic Res Cardiol. 2008, 103(1): 54-59.

26. Penna C, Rastaldo R, Mancardi D, Raimondo S, Cappello S et al. Post-conditioning induced cardioprotection requires signalling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K+ channel and protein kinase C activation. Basic Res Cardiol. 2006, 101(2): 180-189.

27. Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA et al. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. J Exp Med. 2007. 204(9): 2089-2102.

28. Perlman DH, Bauer SM, Ashrafian H, Bryan NS, Garcia-Saura MF et al. Mechanistic insights into nitrite-induced cardioprotection using an integrated metabolomic/proteomic approach. Circ Res. 2009, 104(6): 796-804.

29. Sun J, Murphy E. Protein S-nitrosylation and cardioprotection. Circ Res. 2010, 106(2): 285-296.

30. Beigi F, Gonzalez DR, Minhas KM, Sun QA, Foster MW et al. Dynamic denitrosylation via S-nitrosoglutathione reductase regulates cardiovascular function. Proc Natl Acad Sci USA. 2012, 109(11): 4314-4319.

31. Forbes RA, Steenbergen C, Murphy E. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. Circ Res. 2001, 88(8): 802-809.

32. Williams-Pritchard G, Knight M, Hoe LS, Headrick JP, Peart

JN. Essential role of EGFR in cardioprotection and signaling responses to A1 adenosine receptors and ischemic preconditioning. Am J Physiol Heart Circ Physiol. 2011, 300(6): H2161-H2168.

33. Tullio F, Angotti C, Perrelli MG, Penna C, Pagliaro P. Redox balance and cardioprotection. Basic Res Cardiol. 2013, 108(6): 392-416.

34. Pagliaro P, Moro F, Tullio F, Perrelli MG, Penna C. Cardioprotective pathways during reperfusion: focus on redox signaling and other modalities of cell signaling. Antioxid Redox Signal. 2011, 14(5): 833-850.

35. Skyschally A, Schulz R, Gres P, Korth HG, Heusch G. Attenuation of ischemic preconditioning in pigs by scavenging of free oxyradicals with ascorbic acid. Am J Physiol Heart Circ Physiol. 2003, 284(2): H698-H703.

36. Li Q, Guo Y, Wu WJ, Ou Q, Zhu X et al. Gene transfer as a strategy to achieve permanent cardioprotection I: rAAV-mediated gene therapy with inducible nitric oxide synthase limits infarct size 1 year later without adverse functional consequences. Basic Res Cardiol. 2011, 106(6): 1355-1366.

37. Wink DA, Hines HB, Cheng RY, Switzer CH, Flores-Santana W et al. Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol. 2011, 89(6): 873-891.